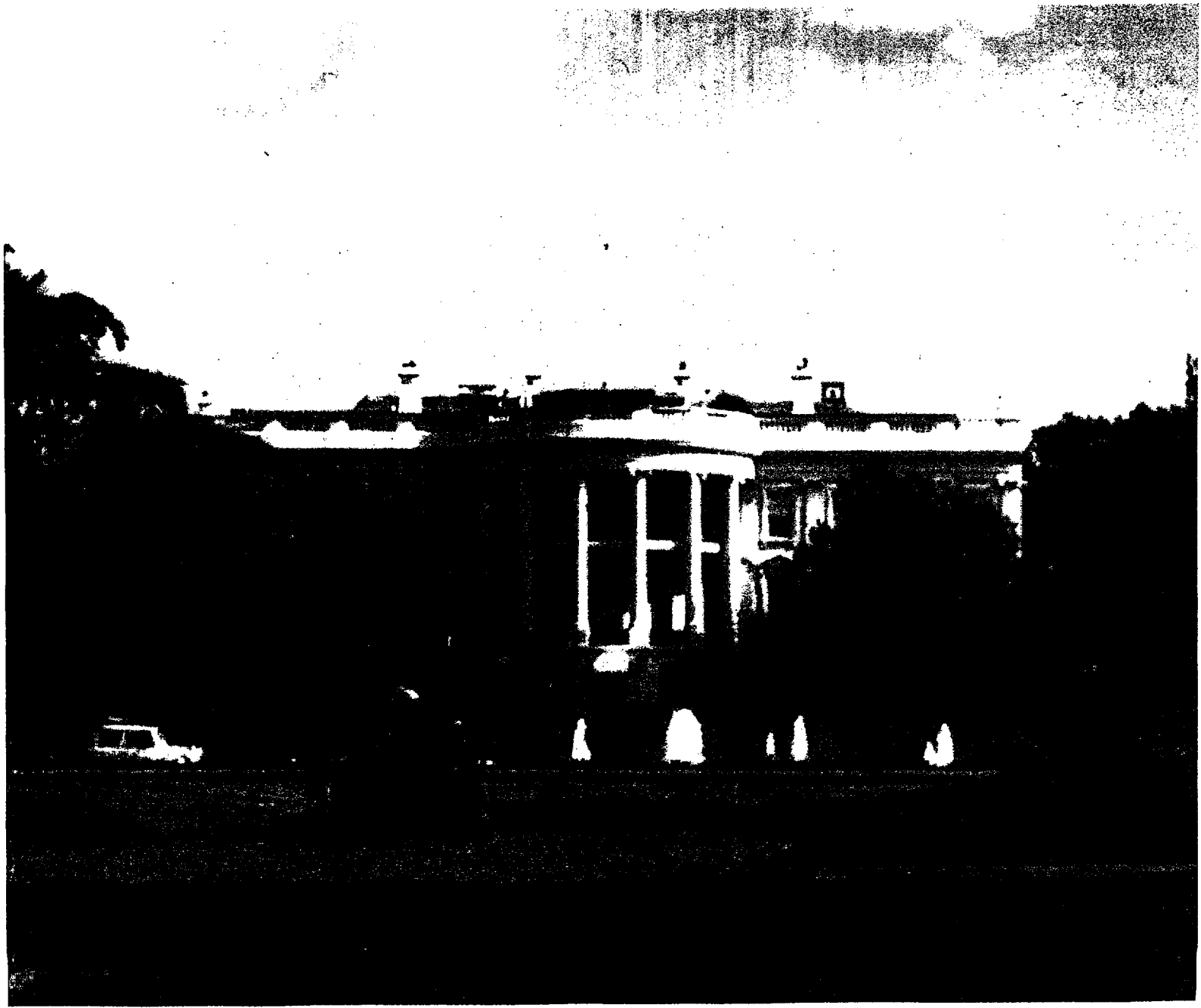
United States
Environmental Protection
Agency

Office of Research and
Development
Washington, DC 20460

EPA/625/R-92/013
Revised October 1999
http://www.epa.gov/ORD/NRMRL

EPA Environmental Regulations and Technology

Control of Pathogens and Vector Attraction in Sewage Sludge



EPA/625/R-92-013
Revised October 1999

Environmental Regulations and Technology

Control of Pathogens and Vector Attraction in Sewage Sludge

(Including Domestic Septage)
Under 40 CFR Part 503

This guidance was prepared by

U.S. Environmental Protection Agency
Office of Research and Development
National Risk Management Research Laboratory
Center for Environmental Research Information
Cincinnati, OH 45268

Printed on Recycled Paper

Notice

This report has been reviewed by the U.S. Environmental Protection Agency and approved for publication. The process alternatives, trade names, or commercial products are only examples and are not endorsed or recommended by the U.S. Environmental Protection Agency. Other alternatives may exist or may be developed.

Foreword

This guidance document was produced by the U.S. Environmental Protection Agency's (EPA) Pathogen Equivalency Committee (PEC) whose members include Robert Bastian, Bob Brobst, John Cicmanec, G. Shay Fout, Hugh McKinnon, Mark Meckes, Frank Schaefer, Stephen Schaub, and James E. (Jim) Smith, Jr. The contributions of Jim Smith, who was instrumental in the administration, organization, and direction of this project; and of Bob Brobst, Mark Meckes, and Robert Bastian along with Greg B. Kester of the State of Wisconsin, who provided significant comments and guidance, are especially appreciated. Eliot Epstein and Nerissa Wu of E&A Environmental Consultants, Inc., in Canton, Massachusetts prepared the document with information and comments from the PEC and from EPA and State sludge coordinators and private contributors including Joseph B. Farrell, a consultant, and Robert Reimers of Tulane University. The assistance of reviewers including John Colletti, Madolyn Dominy, Lauren Fondahl, Alia Roufaeal, and John Walker of EPA; Jeffrey G. Faust of Bio Gro; Jose Pearce of the State of North Carolina; Bob Southworth, a consultant; and all other contributors, too numerous to name, is very much appreciated.

The following individuals assisted by updating guidance for analytical procedures: Appendix F: Sample Preparation for Fecal Coliform Tests and Salmonella sp. Analysis - Mark Meckes; Appendix H: Method for the Recovery and Assay of Enteroviruses from Sewage Sludge - Shay Fout; Appendix I: Analytical Method for Viable Helminth Ova - Frank Schaefer; Appendix J: Composting: Basic Concepts Related to Pathogens and Vector Attraction - Eliot Epstein of E&A Environmental Consultants and Bob Brobst of EPA's Region 8 Office in Denver, CO.

COVER PHOTOGRAPH: Application of sewage sludge compost to the White House lawn.

Contents

Chapter 1 Introduction 1
1.1 What is Sewage Sludge? 1
1.2 U.S. Regulation of Treated Sewage Sludge (Biosolids) 4
1.3 Implementation Guidance 4
1.4 Definitions 5
1.5 Pathogen Equivalency Committee 6
1.6 What's in this Document? 6

Chapter 2 Sewage Sludge Pathogens 8
2.1 What are Pathogens 8
2.2 Pathogens in Sewage Sludge 8
2.3 General Information on Pathogens.. 10
2.4 Protecting Public Health-The Part 503 11
2.5 Frequently Asked Questions 16

Chapter 3 Overview of Part 503 Subpart D Requirements, Their Applicability, and Related Requirements 20
3.1 Introduction 20
3.2 Pathogen Reduction Requirements 20
3.3 Vector Attraction Reduction (VAR) Requirements [503.33] 21
3.4 Applicability of the Requirements [503.15 and 503.251 21
3.5 Frequency of Monitoring 22
3.6 Sampling Stockpiled or Remixed Biosolids 22
3.7 Record Keeping Requirements [503.17 and 503.27] 23
3.8 Reporting Requirements for Sewage Sludge [503.18 and 503.281 23
3.9 Permits and Direct Enforceability [503.3] 25

Chapter 4 Class A Pathogen Requirements
4.1 Introduction 26
4.2 Vector Attraction Reduction to Occur With or After Class A Pathogen Reduction [503.32(a)(2)] 28
4.3 Monitoring of Fecal Coliform or *Salmonella* sp. to Detect Growth of Bacterial Pathogens [503.32(a)(3)-(8)] 27
4.4 Alternative 1: Thermally Treated Sewage Sludge [503.32(a)(3)] 28
4.5 Alternative 2: Sewage Sludge Treated in a High pH-High Temperature Process (Alkaline Treatment) [503.32(a)(4)] 30
4.6 Alternative 3: Sewage Sludge Treated in Other Processes [503.32(a)(5)] 31
4.7 Alternative 4: Sewage Sludge Treated in Unknown Processes [503.32(a)(6) 32
4.8 Alternative 5: Use of PFRP [503.32(a)(7)] 32
4.9 Alternative 6: Use of a Process Equivalent to PFRP [503.32(a)(8)] 33
4.10 Frequency of Testing 33

Chapter 5 Class B Pathogen Requirements and Requirements for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site 36
5.1 Introduction 36
5.2 Sewage Sludge Alternative 1: Monitoring of Fecal Coliform [503.32(b)(2)] 36
5.3 Sewage Sludge Alternative 2: Use of a Process to Significantly Reduce Pathogens [503.32(b)(3)]. 37

Contents (continued)

5.4 Sewage Sludge Alternative 3: Use of Processes Equivalent to PSRP [503.32(b)(4)] 38
5.5 Site Restrictions for Land Application of Biosolids [503.32(b)(5)] 38
5.6 Domestic Septage [503.32(c)] 41

Chapter 6 Processes to Significantly Reduce Pathogens (PSRPs) 43
6.1 Introduction 43
6.2 Aerobic Digestion.. 43
6.3 Anaerobic Digestion 45
6.4 Air Drying 46
6.5 Cornposting.. 47
6.6 Lime Stabilization.. 48
6.7 Equivalent Processes

Chapter 7 Processes to Further Reduce Pathogens (PFRPs) 51
7.1 Introduction 51
7.2 Composting.. 51
7.3 Heat Drying 53
7.4 Heat Treatment 54
7.5 Thermophilic Aerobic Digestion 54
7.6 Beta Ray and Gamma Ray Radiation 55
7.7 Pasteurization 55
7.8 Equivalent Processes 56

Chapter 8 Requirements for Reducing Vector Attraction.. 58
8.1 Introduction 58
8.2 Option 1: Reduction in Volatile Solids Content [503.32(b)(l)] 58
8.3 Option 2: Additional Digestion of Anaerobically Digested Sewage Sludge [503.32(b)(2)] 60
8.4 Option 3: Additional Digestion of Aerobically Digested Sewage Sludge [503.32(b)(3)]. 60
8.5 Option 4: Specific Oxygen Uptake Rate (SOUR) for Aerobically Digested Sewage Sludge [503.32(b)(4)] 60
8.6 Option 5: Aerobic Processes at Greater than 40°C [503.32(b)(5)] 61
8.7 Option 6: Addition of Alkali [503.32(b)(6)] 61
8.8 Option 7: Moisture Reduction of Sewage Sludge Containing No Unstabilized Solids.. 62
8.9 Option 8: Moisture Reduction of Sewage Sludge Containing Unstabilized Solids [503.32(b)(8)] 62
8.10 Option 9: Injection [503.32(b)(9)] 62
8.11 Option 10: Incorporation of Sewage Sludge into the Soil [503.32(b)(10)] 63
8.12 Option 11: Covering Sewage Sludge [503.32(b)(11)] 63
8.13 Option 12: Raising the pH of Domestic Sludge [503.32(b)(12)] 63
8.14 Number of Samples and Timing 63
8.15 Vector Attraction Reduction Equivalency 63

Chapter 9 Sampling Procedures and Analytical Methods 65
9.1 Introduction 65
9.2 Laboratory Selection 65
9.3 Safety Precautions 65
9.4 Requirements for Sampling Equipment and Containers 66
9.5 Sampling Frequency and Number of Samples Collected 67
9.6 Sampling Free-Flowing Sewage Sludges.. 68
9.7 Sampling Thick Sewage Sludges 69
9.8 Sampling Dry Sewage Sludges 69

Contents (continued)

9.9 Control of Temperature, pH, and Oxygenation After Sample Collection Samples for Microbial Tests 70
9.10 Sample Cornpositing and Size Reduction 71
9.11 Packaging and Shipment 72
9.12 Documentation 73
9.13 Analytical Methods 73
9.14 Quality Assurance 74

Chapter 10 Meeting the Quantitative Requirements of the Regulation 76
10.1 Introduction 76
10.2 Process Conditions 76
10.3 Schedule and Duration of Monitoring Events 77
10.4 Comparison of Feed Sludge and Sludge Product Samples 79
10.5 The Effect of Sludge Processing Additives on Monitoring 79
10.6 Collecting Representative Samples 80
10.7 Regulatory Objectives and Number of Samples that Should be Tested 81

Chapter 11 Role of EPA's Pathogen Equivalency Committee in Providing Guidance Under Part 503 90
11 .1 Introduction 90
11.2 Overview of the PEC's Equivalency Recommendation Process 92
11.3 Basis for PEC Equivalency Recommendations 92
11.4 Guidance on Demonstrating Equivalency for PEC Recommendations 97
11.5 Guidance on Application for Equivalency Recommendations 98
11.6 Pathogen Equivalency Committee Recommendations 100
11.7 Current Issues 100

Chapter 12 References and Additional Resources 103

Appendices
A EPA Regional and State Sludge Coordinators, Map of EPA Regions, and listing of EPA Pathogen Equivalency Members 107
B Subpart D of the Part 503 Regulation.. 116
C Determination of Volatile Solids Reduction by Digestion 121
D Guidance on Three Vector Attraction Reduction Tests 143
E Determination of Residence Time for Anaerobic and Aerobic Digestion 149
F Sample Preparation for Fecal Coliform tests and *Salmonella* sp. Analysis 153
G Kenner and Clark (1974) Analytical Method for *Salmonella* sp. Bacteria 157
H Method for the Recovery and Assay of Enteroviruses from Sewage Sludge 158
I Analytical Method for Viable Helminth Ova 174
J The Biosolids Composting Process 181

Acknowledgements

This guidance document was produced by the U.S. Environmental Protection Agency (EPA's) Pathogen Equivalency Committee (PEC) whose members include Robert Bastian, Bob Brobst, John Cicmanec, G. Shay Fout, Hugh McKinnon, Mark Meckes, Frank Schaefer, Stephen Schaub, and James E.(Jim) Smith, Jr. The contributions of Jim Smith, who was instrumental in the administration, organization, and direction of this project; and of Bob Brobst, Mark Meckes, and Robert Bastian along with Greg B. Kester of the State of Wisconsin, who provided significant comments and guidance, are especially appreciated. Eliot Epstein and Nerissa Wu of E&A Environmental Consultants, Inc. in Canton, Massachusetts prepared the document with information and comments from the PEC and from EPA and State sludge coordinators and private contributors including Joseph B. Farrell, a consultant, and Robert Reimers of Tulane University. The assistance of reviewers including John Colletti, Madolyn Dominy, Lauren Fondahl, Alia Roufaeal, and John Walker of EPA; Jeffrey G. Faust of Bio Gro; Joe Pearce of the State of North Carolina; Bob Southworth, a consultant; and all other contributors, too numerous to name, is very much appreciated.

The following individuals assisted by updating guidance for analytical procedures: Appendix F: Sample Preparation for Fecal Coliform Tests and Salmonella sp. Analysis - Mark Meckes; Appendix H: Method for the Recovery and Assay of Enteroviruses from Sewage Sludge - Shay Fout; Appendix I: Analytical Method for Viable Helminth Ova - Frank Schaefer; Appendix J: Cornposting: Basic Concepts Related to Pathogens and Vector Attraction - Eliot Epstein of E&A Environmental Consultants and Bob Brobst of EPA's Region 8 Office in Denver, CO.

Chapter 1
Introduction

1.1 What Is Sewage Sludge?

Sewage sludge -the residue generated during treatment of domestic sewage (Figure l-l) - is often used as an organic soil conditioner and partial fertilizer in the United States and many other countries. It is applied to agricultural land (pastures and cropland), disturbed areas (mined lands, construction sites, etc.), plant nurseries, forests, recreational areas (parks, golf courses, etc.), cemeteries, highway and airport runway medians, and home lawns and gardens (see photographs, page 2-3). Certain treatment works (POTWs) own or have access to land dedicated solely to disposal of sewage sludge, a practice referred to as surface disposal. The U.S. Environmental Protection Agency (EPA), the primary federal agency responsible for sewage sludge management, encourages the beneficial use of sewage sludge through land application (Figure 1-2), after it has been appropriately treated for its intended use. In 1995 it was found that 54% of sewage sludge generated in the United States was land applied (Bastian, 1997).

Sewage sludge has beneficial plant nutrients and soil conditioning properties; however, it may also contain pathogenic bacteria, viruses, protozoa, parasites, and other microorganisms that can cause disease. Land application and surface disposal of untreated sewage sludge create a potential for human exposure to these organisms through direct and indirect contact. To protect public health from these organisms and from the pollutants that some sewage sludge contains, many countries now regulate the use and disposal of sewage sludge.

"Se wage Sludge" v. 'Biosolids"

Throughout the wastewater and sewage sludge industry, the term "sewage sludge" has largely been replaced by the term "biosolids." "Biosolids" specifically refers to sewage sludge that has undergone treatment and meets federal and state standards for beneficial use. The distinction between untreated sewage sludge and biosolids that have undergone processing and analysis will be made throughout this document.

What is Beneficial Use?

For the purposes of this document, land application is considered to be beneficial use. The document specifically deals with land application and the issues related to the pathogen and vector attraction reduction requirements for

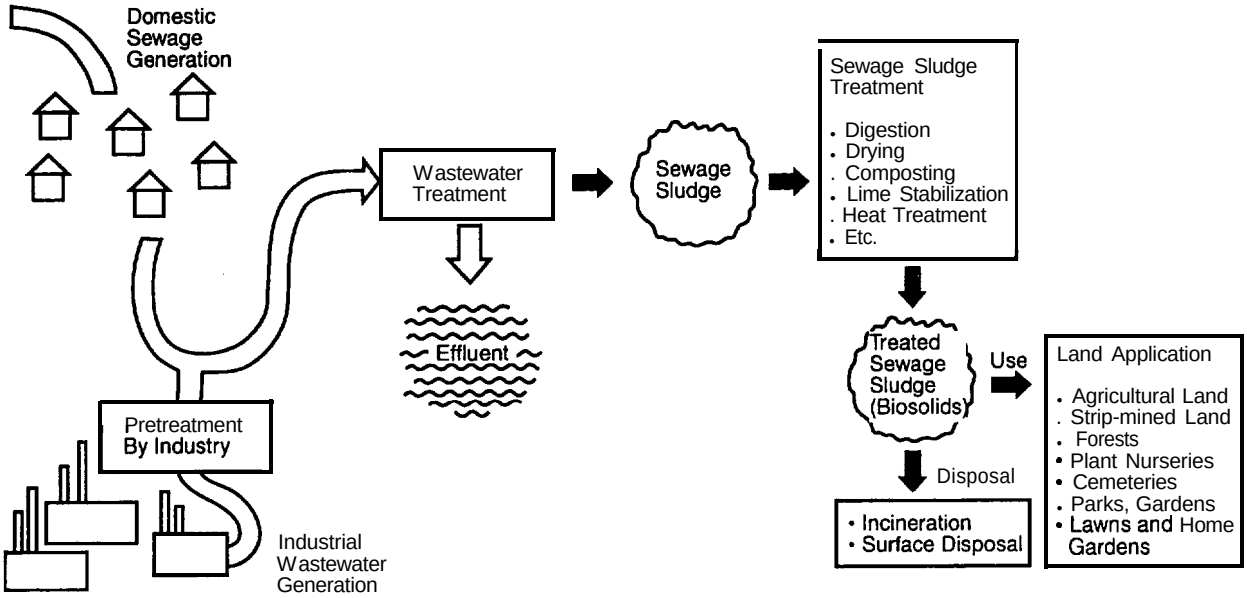
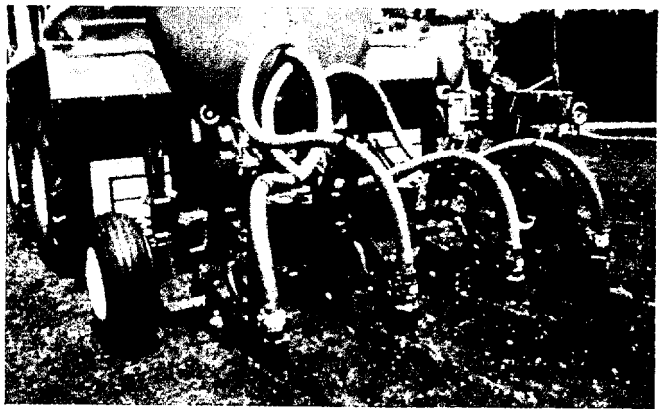


Figure l-l. Generation, treatment, use, and disposal of sewage sludge.

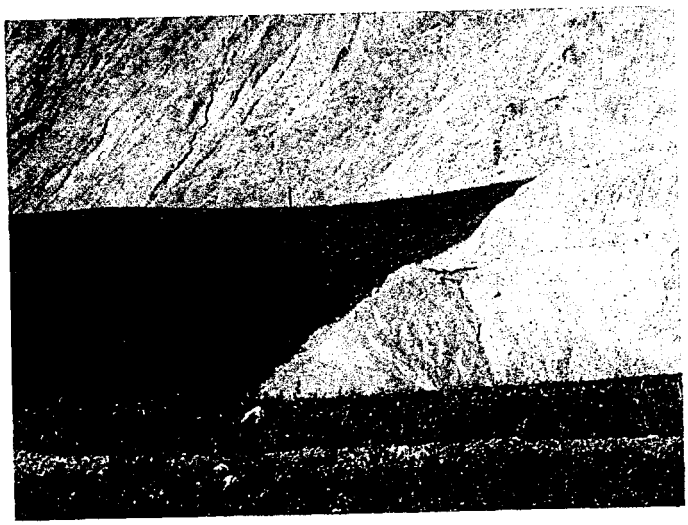
Highway median strip in Illionis after land application of dried sludges. (Photo courtesy of Metropolitan Water Reclamation District of Greater Chicago)

Flower beds amended with sludge compost in Tulsa, Oklahoma. (Photo courtesy of City of Tulsa, Oklahoma)



Injection of liquid sludge Into sod.

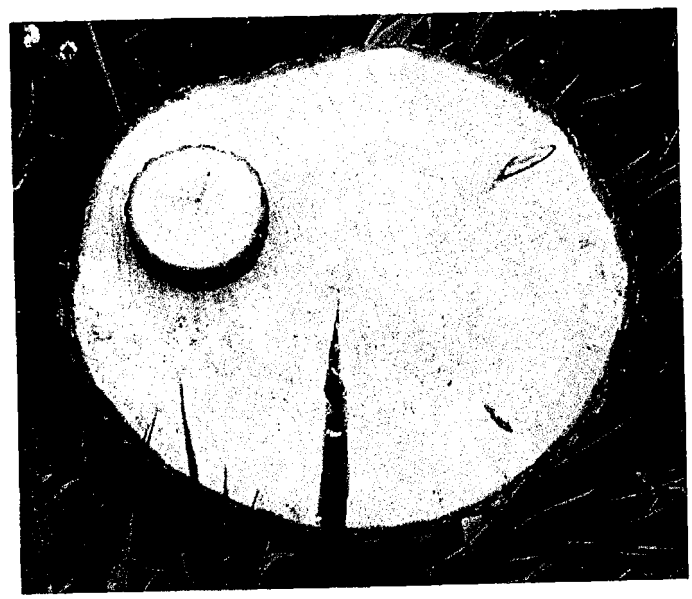
Oat field showing sludge-treated (right) and untreated (left) areas. (Photo courtesy of City of Tulsa, Oklahoma)



Reclaimed mine spoil land.

Corn grown on sludge-treated soil (right) and untreated soil (left).

Mine spoil land sludge treatment. Note lush vegetative cover on reclaimed soil which will support grazing. (Photo courtesy of City of Tulsa, Oklahoma)



Cross-sectlon of Douglas fir tree showing how sludge application increases tree growth. Note increased size of outer rings indicating more rapid growth after sludge applicatlon. (Photo courtesy **of** Metro Silvigrow)

'The U.S. Environmental Protection Agency (EPA) will actively promote those municipal sludge management practices that provide for the beneficiai'use of sewage sludge while maintaining or improving environmental quality and protecting human health. To implement this policy, EPA will continue to issue regulations that protect public health and other environmental values. The Agency will require states to establish and maintain programs to ensure that local governments utilize sewage sludge management techniques that are consistent with federal and state regulations and guidelines. Local communities will remain responsible for choosing" among alternative programs; for planning, coristructing, and operating facilities to meet their needs: and for ensuring the continuing availability of adequate and acceptable disposal or use capacity.

Figure 1-2. **EPA** policy on **sewage** sludge management. Source: EPA, 1984.

land applied biosolids. For more information on the pathogen and vector attraction reduction requirements for the surface disposal of biosolids, please refer to Section 503.25 of the regulation.

1.2 U.S. Regulation of Treated Sewage Sludge (Biosolids)

In the United States, the use and disposal of treated sewage sludge (biosolids), including domestic septage, are regulated under 40 CFR Part 503[1]. This regulation, promulgated on February 19, 1993, was issued under the authority of the Clean Water Act (CWA) as amended in 1977 and the 1976 Resource Conservation and Recovery Act (RCRA). For most sewage sludge[2], the new regulation replaces 40 CFR 257, the original regulation governing the use and disposal of sewage sludge, which has been in effect since 1979.

The EPA policy shown below was developed in response to specific language in the CWA and RCRA federal policy statements in order to facilitate and encourage the beneficial reuse of sewage sludge (U.S. EPA, 1984).

Protection of Public Health and the Environment

Subpart D of the Part 503 regulation protects public health and the environment through requirements designed to reduce the potential for contact with the disease-bearing microorganisms (pathogens) in sewage sludge applied to the land or placed on a surface disposal site. These requirements are divided into:

- Requirements designed to control and reduce pathogens in treated sewage sludge (biosolids)
- Requirements designed to reduce the ability of the treated sewage sludge (biosolids) to attract vectors (insects and other living organisms that can transport biosolids pathogens away from the land application or surface disposal site)

Subpart D includes both performance and technology based requirements. It is designed to provide a more flexible approach than the approach in the Part 257, which required sewage sludge to be treated by specific listed or approved treatment technologies. Under Part 503, treatment works may continue to use the same processes they used under Part 257, but they now also have the freedom to modify conditions and combine processes with each other, as long as the applicable Part 503 requirements are met.

Environmental Effects of Pathogens in Sewage Sludge

Because of concern over the effect of pathogens from biosolids on animal health (certain human pathogens can cross species lines and infect animals, particularly warm blooded animals) the 503 regulations require that sewage sludge undergo pathogen treatment prior to land application. For sewage sludge subject to Class Be pathogen treatment site restrictions are also required. While relatively little research has been conducted on specific inter-species crossover to wildlife, more information is available for grazing animals which are more likely to have a greater exposure to biosolids than wildlife. Available information on the impact of biosolids pathogens on grazing animals suggests that the Part 503 Subpart D requirements for pathogen control (which include restrictions on grazing) protect grazing animals (EPA, 1992). References regarding the impact of biosolids application on both wild and domestic animals are included at the end of this chapter.

1.3 Implementation Guidance

This document is not regulatory in nature. A complete copy of Subpart D of the Part 503 Regulation appears in Appendix B. This document is only intended to serves as a guide to pathogen and vector attraction reduction for anyone who is involved with the treatment of sewage sludge for land application. This includes:

- Owners and operators of domestic sewage treatment works
- Developers or marketers of sewage sludge treatment processes
- Groups that distribute and market biosolids products
- Individuals involved in applying biosolids to land
- Regional, state, and local government officials responsible for implementing and enforcing the Part 503 Subpart D regulation

[1]Because domestic septage is a form of sewage sludge, any use of the term "sewage sludge" in this document includes domestic septage.
[2]Sewage sludge generated at an industrial facility during the treatment of domestic sewage commingled with industrial waslewater in an industrial wastewater treatment facility is still covered under 40 CFR Part 257 if the sewage slude is applied to the land.

- Consultants to these groups
- Anyone interested in understanding the federal requirements concerning pathogens in sewage sludge

This guide was previously released in 1993. The updates and amendments to this document are a product of comments and suggestions from the regulatory and sewage sludge management community. This edition of the document includes clarification of many of the sampling and monitoring issues and reflects the increased understanding of analytical issues. There are additional operational guidelines and examples of how a variety of facilities have complied with the Part 503 requirements. Some of the notable additions to this edition include:

- Clarification of Class A processes
- More specific guidelines for the operation of composting facilities
- More information on site restrictions including permit conditions which may apply to specific crops
- Recommendation for the use of the Kenner and Clark methodology for Salmonella sp . bacteria analysis
- Guidelines for retesting biosolids products that have been stored or remixed
- More information on public health and pathogens
- More information on sampling and monitoring protocols
- Updates on the Pathogen Equivalency Committee and approved processes

Other publications related to pathogen or vector attraction issues include the "Technical Support Document for Reduction of Pathogens and Vector Attraction in Sewage Sludge" (U.S. EPA, 1992) and "Part 503 Implementation Guidance" (U.S. EPA, 1995). Although the federal regulation under 40 CFR Part 503 includes restrictions for pollutant concentrations and application rates, this document is intended to clarify pathogen and vector related requirements and does not discuss pollutant limits.

This document does not discuss the general requirements and management practices which must be followed for land application of all biosolids except in the case of "exceptional quality" biosolids which have met certain pollutant limits and pathogen and vector attraction reduction requirements. In addition to meeting the regulation set forth in this document, bulk biosolids application must be conducted in accordance with agronomic rates, and biosolids appliers must ensure that applied biosolids are not applied within 10 meters of any water body, do not enter surface waters or wetlands without the approval of the appropriate permitting authority, and do not adversely affect endangered or threatened species or their habitats.

It should be noted that the Part 503 regulation and the sampling and monitoring requirements outlined in the regulation were developed as minimum requirements. EPA supports the beneficial use of treated sewage sludge (biosolids) and encourages facility operators and generators of biosolids products to develop sampling and monitoring plans that go beyond the minimum regulatory requirements as needed to ensure consistent product quality.

For most states, the authority for implementing the Part 503 regulation currently remains with the Regional EPA offices. A guide to EPA offices and relevant contacts can be found in Appendix A.

1.4 Definitions

The sections of this document that discuss specific regulatory requirements utilize the same terminology used throughout the Part 503 regulation in order to maintain consistency between the regulation and this guidance document. However, in some parts of this document, particularly in sections which discuss operational parameters and other issues related to biosolids management, terms which are not formally defined by the regulations are used. The following glossary has been provided in order to prevent confusion about the intent and jurisdiction of the Part 503 regulation.

Applier - The applier is the individual or party who land applies treated sewage sludge (biosolids). This may include farmers, municipalities, and private enterprises that land apply or their contractors.

Biosolids - Sewage sludge that has been treated and meets state and federal standards for land application.

Control - Some of the regulatory requirements make a distinction based on whether the biosolids preparer (see below) has "control" over the material. A preparer loses control over material when it is sold or given away. Until that point, the material is still within the control of the preparer even if the treatment process has ended and the material is in storage on or off-site.

Detectable Limits - Minimum concentration at which an analyte can be measured. The detectable limit for any given analyte varies depending on the lab methodology used and the volume of material analyzed. As such, detectable limits may fluctuate. Throughout this document, the term "detectable limit" refers to the limits as they are defined in the allowable lab methodologies outlined in the Appendices.

Exceptional Quality (EQ) Biosolids - The term "EQ" is not used in the Part 503 regulation, but it has become a useful description for regulators and biosolids preparers when referring to biosolids that meet the pollutant concentration limits of Table 3 of Section 503.13, Class A pathogen reduction, and one of the first eight treatment processes for meeting vector attraction reduction standards. Biosolids that fall into this category are not subject to the Part 503 general requirements and management practices for land application.

Preparer - The person(s) who generate biosolids from the treatment of domestic sewage in a treatment works or change the quality of the sewage sludge received from the generator. This includes facilities that derive a material from sewage sludge prior to land application of the material which could include wastewater treatment facilities, composting or other sewage sludge processing operations, and soil blenders who handle non-EQ biosolids materials. A soil blender who takes EQ biosolids and mixes them with other (non-sewage sludge) materials for land application is not a preparer. However, a soil blender that takes non-EQ biosolids and mixes it with other materials for land application is a preparer.

Product - This may include materials such as composted, heat-dried, lime stabilized, alkaline stabilized, or otherwise processed biosolids which have met the requirements of the Part 503. The term "product" is sometimes used in this document in discussions regarding material distribution. The term "sludge derived material" is used in the Part 503 to refer to these materials.

Sewage Sludge - The solid, semi-solid, or liquid residue generated during the treatment of municipal sewage in a treatment works. The term "biosolids" refers to sewage sludge which has undergone treatment and meets state and federal requirements for land application. The distinction between untreated sewage sludge and treated biosolids is made throughout this document.

1.5 Pathogen Equivalency Committee

The Pathogen Equivalency Committee (PEC) is made up of U.S. EPA experts who review pathogen and vector attraction reduction issues and make recommendations to the appropriate permitting authority. The primary role of the PEC is to review proposals for Processes to Significantly Reduce Pathogens (PSRP) and Processes to Further Reduce Pathogens (PFRP) equivalency determinations and to offer guidance on the issues associated with pathogen and vector attraction reduction.

More information on the PEC and the process of applying for equivalency is presented in Chapter 11.

1.6 What's in this Document?

Chapter 2 of this document provides basic information about pathogens and describes why pathogen control is required to protect public health and the environment, and Chapters 3 through 5 discuss the current federal requirements under Subpart D of Part 503. Chapters 6 and 7 review the different PFRP and PSRP processes, and Chapter 8 discusses vector attraction reduction issues. Chapters 9 and 10 summarize sampling and analysis protocols used to meet the quantitative requirements of Part 503. Chapter 11 outlines the process for applying for equivalency and discusses the kind of support EPA's Pathogen Equivalency Committee can provide to permitting authorities. Chapter 12 lists general references and additional resources related to biosolids use; specific references related to particular topics are also included at the end of each chapter.

The Appendices provide additional information on:

- Determination of volatile solids and residence time for digestion
- Sample preparation and analytical methods for meeting the Part 503 pathogen reduction requirements
- Tests for demonstrating vector attraction reduction
- Additional references on pathogen research and technical background to regulations

Appendix A lists EPA and state sewage sludge coordinators, and Appendix B contains Subpart D of the Part 503 regulation.

References and Additional Resources

Alberici, T.M., W.E. Sopper, G.L. Storm, and R.H. Yahner. 1989. Trace metals in soil, vegetation, and voles from mine land treated with sewage sludge. Journal of Environmental Quality 18:115-1 20.

Anderson, T.J., and G.W. Barrett. 1982. Effects of dried sewage sludge on meadow vole (Microtus pennsylvanicus) populations in two grassland communities. Journal of Applied Ecology 19:759-772.

Bastian, R.K. 1997. The biosolids (sludge) treatment, beneficial use, and disposal situation in the USA. European Water Pollution Control Journal, Vol 7, No. 2, 62-79.

Danron, B.L., H.R. Wilson, M.F. Hall, W.L. Johnson, O.Osuna, R.L. Suber, and G.T. Edds. 1982. Effects of feeding dried municipal sludge to broiler type chicks and laying hens. Polut. Sci. 61 :1073-1081.

Hegstrom, L.J. and S.D. West. Heavy metal accumulation in small mammals following sewage sludge application to forests. Journal of Environmental Quality 18:345.

Keinholz, E.W. 1980. Effects of toxic chemicals present in sewage sludge on animal health. P 153-l 71 in Sludge - Health risks of land application. Ann Arbor Science Publishers.

Keinholz, E.W., G.M. Ward, D.E. Johnson, J. Baxter, G. Braude, and G. Stern. 1979. Metropolitan Denver sludge fed to feedlot steers. J. Anim. Sci. 48:735-741.

National Research Council. 1996. Use of reclaimed water and sludge in food crop production. Washington, D.C.

U.S. EPA/USDA/FDA. 1981. Land Application of Municipal Sewage Sludge for the Production of Fruits and Vegetables; A Statement of Federal Policy and Guidance. SW-905. U.S. EPA, Office of Solid Waste, Washington, D.C. 21 pp.

U.S. EPA. 1984. EPA policy on municipal sludge management. Federal Register, 49(114):24358-24359. June 12, 1984.

U.S. EPA. 1991. Interagency Policy on beneficial use of municipal sewage sludge on federal land. Federal Register, 56(138):33186-33188. July 19, 1991.

U.S. EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No.: PB89-136618. Springfield, VA: National Technical Information Service.

U.S. EPA. 1995. Part 503 implementation guidance. EPA 833-R-95-001. Washington, D.C.

WEF/U.S. EPA. 1997. Biosolids: A short explanation and discussion. In Biosolids Fact Sheet Project.

Chapter 2
Sewage Sludge Pathogens

2.1 What are Pathogens?

A pathogen is an organism or substance capable of causing disease. The Part 503 regulation only discusses pathogenic organisms, and throughout this document, "pathogen" refers only to living organisms, except where specified. Pathogens infect humans through several different pathways including ingestion, inhalation, and dermal contact. The infective dose, or the number of a pathogenic organism to which a human must be exposed to become infected, varies depending on the organism and on the health status of the exposed individual.

Pathogens that propagate in the enteric or urinary systems of humans and are discharged in feces or urine pose the greatest risk to public health with regard to the use and disposal of sewage sludge. Pathogens are also found in the urinary and enteric systems of other animals and may propagate in non-enteric settings. However, because this document is concerned with the regulation of sewage sludge, this chapter focuses on the pathogens most commonly found in the human enteric system.

2.2 Pathogens in Sewage Sludge

What pathogens can be found in sewage sludge?

The four major types of human pathogenic (disease-causing) organisms (bacteria, viruses, protozoa, and helminths) all may be present in domestic sewage. The actual species and quantity of pathogens present in the domestic sewage from a particular municipality (and the sewage sludge produced when treating the domestic sewage) depend on the health status of the local community and may vary substantially at different times. The level of pathogens present in treated sewage sludge (biosolids) also depends on the reductions achieved by the wastewater and sewage sludge treatment processes.

The pathogens in domestic sewage are primarily associated with insoluble solids. Primary wastewater treatment processes concentrate these solids into **sewage** sludge, so untreated or raw primary sewage sludges have higher quantities of pathogens than the incoming wastewater. Biological wastewater treatment processes such as lagoons, trickling filters, and activated sludge treatment may substantially reduce the number of pathogens in the wastewater (EPA, 1989). These processes may also reduce the number of pathogens in sewage sludge by creating adverse conditions for pathogen survival.

Nevertheless, the resulting biological sewage sludges may still contain sufficient levels of pathogens to pose a public health and environmental concern. Part 503 Regulation thus requires sewage sludge to be treated by a Class A pathogen treatment process or a Class B process with site restrictions. These requirements prevent disease transmission. Table 2-l lists some principal pathogens of concern that may be present in wastewater and sewage sludge. These organisms and other pathogens can cause infection or disease if humans and animals are exposed to sufficient levels of the organisms or pathogens. The levels, called infectious doses, vary for each pathogen and each host.

As mentioned in Chapter 1, one concern is the potential effect of some human pathogens on animals. Enteric viruses can cross species lines, and animal life, particularly warm blooded animals, can be affected if they are exposed to some of the pathogens found in sewage sludge. Domestic animals are protected by site restrictions which limit grazing on sludge amended land.

How could exposure to these pathogens occur?

If improperly treated sewage sludge was illegally applied to land or placed on a surface disposal site, humans and animals could be exposed to pathogens directly by coming into contact with the sewage sludge, or indirectly by consuming drinking water or food contaminated by sewage sludge pathogens. Insects, birds, rodents, and even farm workers could contribute to these exposure routes by transporting sewage sludge and sewage sludge pathogens away from the site. Potential routes of exposure include:

Direct Contact

- Touching the sewage sludge.
- Walking through an area - such as a field, forest, or reclamation area - shortly after sewage sludge application.
- Handling soil from fields where sewage sludge has been applied.

Table 2-1. Principal Pathogens of Concern in domestic Sewage and Sewage Sludge

Organism	Disease/Symptoms
Bacteria	
Salmonella sp.	Salmonellosis (food poisoning), typhoid fever
Shigella sp.	Bacillary dysentery
Yersinia sp.	Acute gastroenteritis (including diarrhea, abdominal pain)
Vibrio cholerae	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli (pathogenic strains)	Gastroenteritis
Enteric Viruses	
Hepatitis A virus	Infectious hepatitis
Norwalk and Norwalk-like viruses	Epidemic gastroenteritis with severe diarrhea
Rotaviruses	Acute gastroenteritis with severe diarrhea
Enteroviruses	
Polioviruses	Poliomyelitis
Coxsackieviruses	Meningitis, pneumonia, hepatitis, fever, cold-like symptoms, etc.
Echoviruses	Meningitis, paralysis, encephalitis, fever, cold-like symptoms, diarrhea, etc.
Heovirus	Respiratory infections, gastroenteritis
Astroviruses	Epidemic gastroenteritis
Caliciviruses	Epidemic gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis
Entamoeba histolytica	Acute enteritis
Giardia *lamblia*	Giardiasis (including diarrhea, abdominal cramps, weight loss)
Balantidium coli	Diarrhea and dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth Worms	
Ascaris lumbricoides	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness
Ascaris suum	May produce symptoms such as coughing, chest pain, and fever
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms
Taenia saginata	Nervousness, insommia, anorexia, abdominal pain, digestive disturbances
Taenia *solium*	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Necator americanus	Hookworm disease
Hymenolepis nana	Taeniasis

Source: Kowal (1985) and EPA (1989).

- Inhaling microbes that become airborne (via aerosols, dust, etc.) during sewage sludge spreading or by strong winds, plowing, or cultivating the soil after application.

Indirect Contact

- Consumption of pathogen-contaminated crops grown on sewage sludge-amended soil or of other food products that have been contaminated by contact with these crops or field workers, etc.
- Consumption of pathogen-contaminated milk or other food products from animals contaminated by grazing in pastures or fed crops grown on sewage sludge-amended fields.
- Ingestion of drinking water or recreational waters contaminated by runoff from nearby land application sites or by organisms from sewage sludge migrating into ground-water aquifers.
- Consumption of inadequately cooked or uncooked pathogen-contaminated fish from water contaminated by runoff from a nearby sewage sludge application site.
- Contact with sewage sludge or pathogens transported away from the land application or surface disposal site by rodents, insects, or other vectors, including grazing animals or pets.

The purpose of the Part 503 regulation is to place barriers in the pathway of exposure either by reducing the number of pathogens in the treated sewage sludge (biosolids) to below detectable limits, in the case.of Class A treatment, or, in the case of Class B treatment, by preventing direct or indirect contact with any pathogens possibly present in the biosolids.

Each potential pathway has been studied to determine how the potential for public health risk can be alleviated. The references listed at the end of this chapter include some of the technical writings which summarize the research on which the Part 503 regulation is based.

For example, the potential for public health impacts via inhalation of airborne pathogens was examined. Pathogens may become airborne via the spray of liquid biosolids from a splash plate or high-pressure hose, or in fine particulate dissemination as dewatered biosolids are applied or incorporated. While high-pressure spray applications may result in some aerosolization of pathogens, this type of equipment is generally used on large, remote sites such as forests, where the impact on the public is minimal. Fine particulates created by the application of dewatered biosolids or the incorporation of biosolids into soil may cause very localized fine particulate/dusty conditions, but particles in dewatered biosolids are too large to travel far, and the fine patticulates do not spread beyond the immediate area. The activity of applying and incorporating biosolids may create dusty conditions. However, the biosolids are moist materials and do not add to the dusty conditions, and by the time biosolids have dried sufficiently to create fine particulates, the pathogens have been reduced (Yeager & Ward, 1981)

The study of each pathway and the potential for public health risk resulted in site restrictions that are protective of public health and the environment and that must be followed when Class B biosolids are land applied. While the site restrictions provided in the Part 503 rule are sufficient to protect the public from health impacts, workers exposed to Class B biosolids might benefit from several additional precautions. For example, dust masks should be worn for the spreading of dry materials, and workers should wash

their hands carefully after working with sewage sludge or biosolids. Other recommended practices for workers handling biosolids or sewage sludge include:

- Wash hands before eating, drinking, smoking or using the restroom.
- Use gloves when touching biosolids or sewage sludge or surfaces exposed to biosolids or sewage sludge.
- Remove excess sewage sludge or biosolids from shoes prior to entering an enclosed vehicle.
- Keep wounds covered with clean, dry bandages.
- If contact with biosolids or sewage sludge occurs, wash contact area thoroughly with soap and water.

Table 2-2 shows the various pathways of exposure and how the process requirements and site restrictions of the Part 503 regulation protect public health for each pathway.

Table 2-2. Pathways of Exposure and Applicable Site Restrictions (Class B Biosolids Only)

Pathways	Part 503 Required Site Restriction
Handling soil from fields where sewage sludge has been applied	No publis access* to application sites until at least 1 year after Class B biosolids application.
Handling soil or food from home gardens where sewage sludge has been applied	Class B biosolids may not be applied on home gardens.
Inhaling dust"	No public access to application sites until at least 1 year after Class B biosolids application.
Walking through fields where sewage sludge has been applied"	No public access to fields until at least 1 year after Class B biosolids application.
Consumption of crops from fields on which sewage sludge has been applied	Site restrictions which prevent the harvesting of crops until environmental attenuation has taken place.
Consumption of milk or animal products from animals grazed on fields where sewage sludge has been applied	No animal grazing for 30 days after Class B biosolids have been applied.
Ingestion of water contaminated by runoff from fields where sewage sludge has been applied from biosolids amended land from affecting surface water.	Class B biosolids may not be applied within 10 meters of any waters in order to prevent runoff
Ingestion of inadequately cooked fish from water contaminated by runoff from fields where sewage sludge has been applied affecting surface water.	Class B biosolids may not be applied with 10 meters of any waters in order to prevent runoff from biosolids amended land from
Contact with vectors which have been in contact with sewage sludge	All land applied biosolids must meet one of the Vector Attraction Reduction options (see Chapter 8).

'Public access restrictions do not apply to farm workers. If there is low probability of public exposure to an application site, the public access restrictions apply for only 30 days. However, application sites which are likely to be accessed by the public, such as ballfields, are subject to 1 year public access restric tions.
"Agricultural land is private property and not considered to have a high potential for public access. Nonetheless, public access restrictions still are applied.

2.3 General Information on Pathogens

The EPA has attempted, through this and other documents, to provide the public with a broad understanding of the risk assessment and scientific basis of the Part 503 regulation. The regulation is based on the results of extensive research and experience with land application of treated sewage sludge (biosolids). However, as for all regulations, proper interpretation and implementation of the regulation are the most important aspects of protecting public health and the environment.

Biosolids preparers should have a basic knowledge of microbiology so that they can:

- Understand the goals of the Part 503 regulation and what is expected to meet the requirements
- Address questions regarding pathogens and the protection of public health and the environment
- Design appropriate testing/sampling programs to meet the Part 503 requirements
- Make informed decisions about laboratory and analytical methodology selection

This section outlines some of the generic issues of pathogen testing and quantification. References related to these issues are listed at the end of this chapter as well as in Chapter 12. Other chapters discuss sampling and sample preservation as well as meeting the Part 503 requirements in more detail.

Survivability of Pathogens

Wastewater generally contains significantly high concentrations of pathogens which may enter the wastewater system from industries, hospitals, and infected individuals. The wastewater treatment process tends to remove pathogens from the treated wastewater, thereby concentrating the pathogens in the sewage sludge. Like any other living organisms, pathogens thrive only under certain conditions. Outside of these set conditions, survivability decreases. Each pathogen species has different tolerance to different conditions; pathogen reduction requirements are therefore based on the need to reduce all pathogenic populations. Some of the factors which influence the survival of pathogens include pH, temperature, competition from other microorganisms, sunlight, contact with host organisms, proper nutrients, and moisture level.

The various Class A and Class B pathogen reduction processes as well as the site restrictions for the land appli-

cation of Class B biosolids are based on research regarding the survivability of pathogens under specific treatment conditions. Table 2-3 shows a comparison of the survival of bacteria, viruses, and parasites in different sewage sludge treatments. Table 2-4 shows the survival time of various pathogens on soil or plant surfaces after land application of biosolids.

Iden tification of Pathogens

Some of the pathogens of concern that appear in domestic sewage and sewage sludge are shown in the photographs on pages 12 and 13. These include ascarids (*Ascaris lumbricoides* and *Toxacara*), whipworms *(Trichuris* sp.), tapeworms (*Hymenolepis* sp. and *Taenia* sp.), amoeba (*Entamoeba* coli), and giardia *(Giardia lamblia*). As shown in these photographs, several color staining procedures are needed to identify the organisms and the different structures within the organisms. The photograph of *Giardia lamblia* depicts specimens stained with Lugol's iodine solution, showing two nuclei, a median body, and axonemes in each. In addition, scientists use a blue filter when photographing the pathogenic organisms through a microscope. This filter is necessary to show the natural color of the organisms.

What Units are Used to Measure Pathogens?

Density of microorganisms in Part 503 is defined as num*ber of microorganisms per unit mass of total so/ids (dry weight).* Ordinarily, microorganism densities are determined as number per 100 milliliters of wastewater or sewage sludge. While the use of units of volume is sensible for wastewater, it is less sensible for sewage sludge. Many microorganisms in sewage sludge are associated with the solid phase. When sewage sludge is diluted, thickened, or filtered, the number of microorganisms per unit volume changes markedly, whereas the number per unit mass of solids remains almost constant. This argues for reporting their densities as the number present per unit mass of solids, which requires that sewage sludge solids content always be determined when measuring microorganism densities.

A second reason for reporting densities per unit mass of total solids is that biosolids application to the land is typically measured and controlled in units of mass of dry solids per unit area of land. If pathogen densities are measured as numbers per unit mass of total solids, the rate of pathogen application to the land is directly proportional to the mass of dry biosolids applied.

Table 2-3. Summary of the Effects of Sewage Sludge Treatment on Pathogens (Log Reductions Shown)

PSRP Treatment	Bacteria	Viruses	Parasites (protozoa and helminths)
Anaerobic Digestion	0.5-4.0	0.5-2.0	0.5
Aerobic Digestion	054.0	0.5-2.0	0.5
Composting (PSRP)	2.0-4.0	2.0-4.0	2.0-4.0
Air Drying	0.5-4.0	0.5-4.0	0.5-4.0
Lime Stabilization	0.5-4.0	4.0	0.5

'A l-log reduction (lo-fold) is equal to a 90% reduction. Class B processes are based on a 2-log reduction.

Table 2-4. Survival Times of Pathogens in Soil and on Plant Surfaces[a]

	Soil		Plants	
Pathogen	Absolute Maximum[a]	Common Maximum	Absolute Maximum[b]	Common Maximum
Bacteria	1 year	2 months	6 months	1 month
Viruses	1 year[c]	3 months	2 months	1 month
Protozoan cysts[d]	10 days	2 days	5 days	2 days
Helminth ova	7 years	2 years	5 months	1 month

[a]For survival rates, see Sorber and Moore (1986).
[b]Absolute maximum survival times are possible under unusual conditions such as consistently low temperatures or highly sheltered conditions (e.g., helminth ova below the soil in fallow fields) (Kowal, 1985).
[c]Little, if any, data are available on the survival times of Giardia cysts and Cryptosporidium oocysts.
Source: Kowal, 1985.

Different Methods for Counting Microorganisms

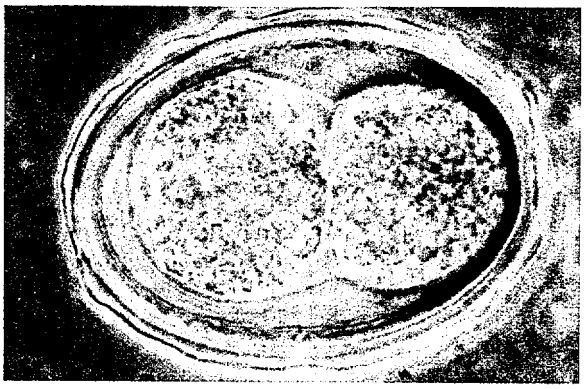
The methods and units used to count microorganisms vary depending on the type of microorganism. Viable helminth ova are observed and counted as individuals (numbers) under a microscope. Viruses are usually counted in plaque-forming units (PFU). Each PFU represents an infection zone where a single infectious virus has invaded and infected a layer of animal cells. For bacteria, the count is in colony-forming units (CFU) or most probable number (MPN). CFU is a count of colonies on an agar plate or filter disk. Because a colony might have originated from a clump of bacteria instead of an individual, the count is not necessarily a count of separate individuals. MPN is a statistical estimate of numbers in a sample. The sample is diluted at least once into tubes containing nutrient medium. The tubes are maintained under conditions favorable for bacterial growth. The original bacterial density in the sample is estimated based on the number of tubes that show growth and the level of dilution in those tubes.

Part 503 Density limits

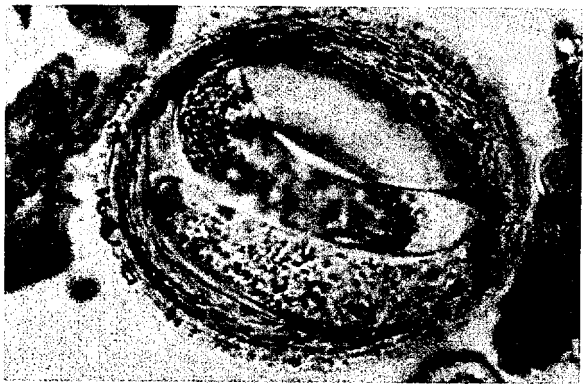
Under Part 503, the density limits for the pathogens are expressed as numbers of PFUs, CFUs, or MPNs per 4 grams dry weight sewage sludge. This terminology came about because most of the tests started with 100 ml of sewage sludge which typically contained 4 grams of sewage sludge solids. Also, expressing the limits on a "per gram" basis would have required the use of fractions (i.e., 0.25/g or 0.75/g). Density limits for fecal coliforms, the indicator organisms, however, are given on a "per gram" basis because these organisms are much more numerous than pathogens.

2.4 Protecting Public Health -The Part 503

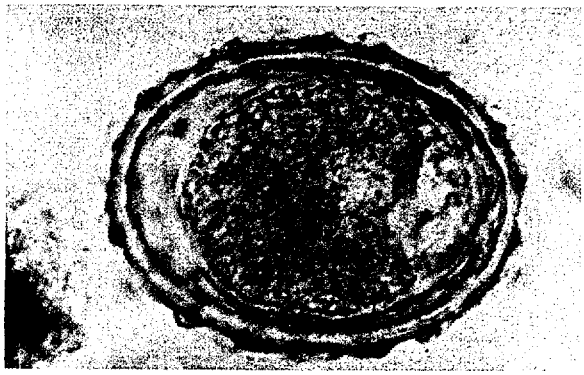
The Part 503 regulation protects public health by limiting the potential for public exposure to pathogens. This is



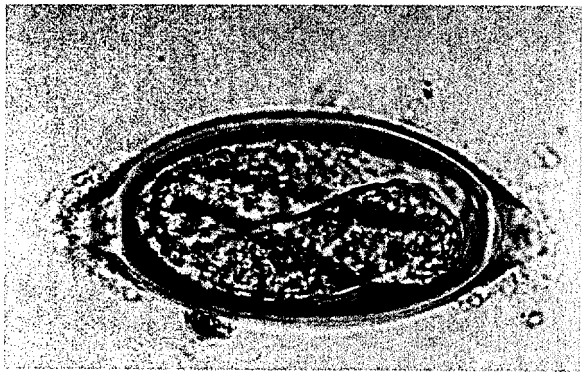
Ascaris lumbricoides **(or var. suum) eggs, 66 pm, from anaerobically digested sludge. Two-cell stage. (Photos on this page courtesy of Fox et al., 1981)**



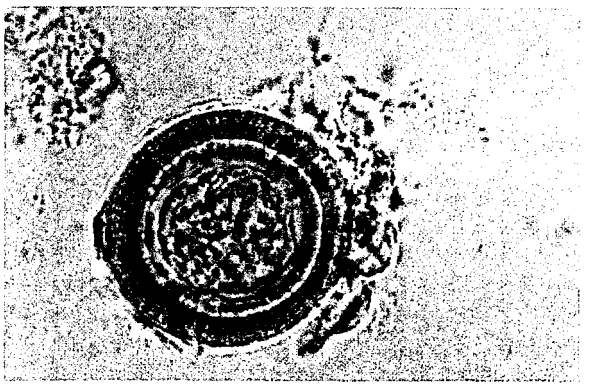
Toxocara **sp. egg, 90 µm, from raw sewage.**



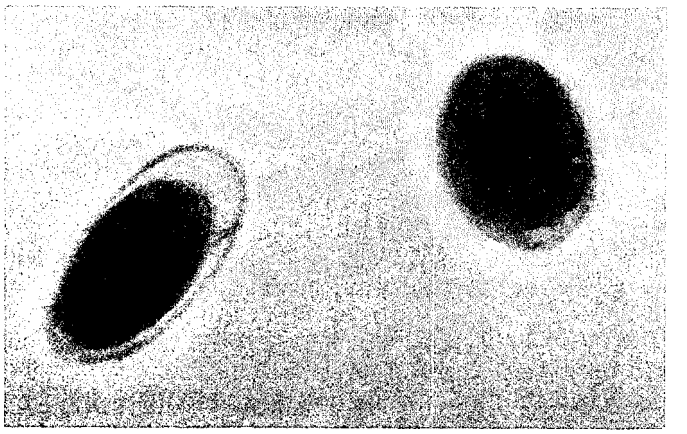
Ascaris lumbricoides **(or var. suum) eggs, 65 pm, from anaerobically digested sludge.**



Trichuris sp. **egg, 60 µm, from anaerobically digested sludge.**



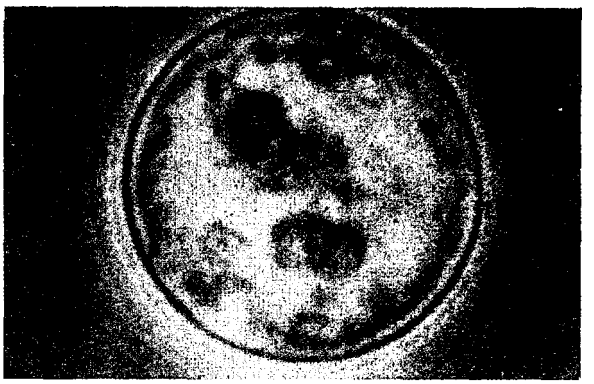
Taenia sp. ovum. (Photo courtesy of Fox et al., 1981)



***Giardla lamblia* cysts.** (Photo courtesy of Frank Schaefer, U.S. EPA, National Risk Management Research Laboratory, Cincinnati, Ohio)

***Hymenolepis* (tapeworm) ova.** (Photo courtesy of Fox et al., 1981)

Preparing compost for pathogen analysis. (Photo courtesy of U.S. Department of Agriculture, Beltsville, Maryland)



***Entamoeba coli* cysts, 15μm, from anaeroblcally digested sludge.** (Photo courtesy of Fox et al., 1981)

accomplished through treatment of the sewage sludge or through a combination of sewage sludge treatment and restrictions on the land application site that prevent exposure to the pathogens in the biosolids and allow time for the environment to reduce the pathogens to below detectable levels. The Part 503 vector attraction reduction requirements also help reduce the spread of pathogens by birds, insects, and other disease carriers (i.e. vectors) by requiring that all sewage sludge that is to be land applied undergo vector attraction reduction.

The Part 503 regulation also establishes the analytical protocol for pathogen analysis. More information on the quantification of pathogens and how pathogen reduction is measured is included in Chapter 10 and in the Appendices.

Reducing the Number of Pathogens

Pathogen reduction can be achieved by treating sewage sludge prior to use or disposal-and through environmental attenuation. Many sewage sludge treatment processes are available that use a variety of approaches to reduce pathogens and alter the sewage sludge so that it becomes a less effective medium for microbial growth and vector attraction (Table 2-5). Processes vary significantly in their effectiveness. For example, some processes (e.g. lime stabilization) may effectively reduce bacteria and viruses but have little or no effect on helminth eggs. The effectiveness of a particular process can also vary depending on the conditions under which it is operated. For example, the length of time and the temperature to which sewage sludge is heated is critical to the effectiveness of heat-based treatment processes.

Part 503 lists sewage sludge treatment technologies that are judged to produce biosolids with pathogens sufficiently reduced to protect public health and the environment. The regulation also allows the use of any other technologies that produce biosolids with adequately reduced pathogens as demonstrated through microbiological monitoring. The Part 503 establishes two classifications of biosolids based on the level of pathogen reduction the biosolids have undergone. Class A biosolids are treated to the point at which pathogens are no longer detectable. For Class B biosolids, a combination of treatment and site restrictions are designed to protect public health and environment.

Monitoring Indicator Species

Sewage sludge may contain numerous species of pathogenic organisms, and analyzing for each species is not practical. The microbiological requirements of the Part 503 are therefore based on the use of an indicator organism for the possible presence of pathological bacteria and both the representative and the hardiest of known species for viruses and helminths to represent the larger set of pathogenic organisms. The indicator and representative organ-

Table 2-5. General Approaches to Controlling Pathogens and Vector Attraction in Sewage Sludge

Approach	Effectiveness	Process Example9
Applicatioin of high temperatures (temperatures may be generated by chemical, biological, or physical processes).	Depends on time and temperature. Sufficient temperatures maintained for sufficiently long time periods can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Helminth ova are the most resistant to high temperatures.	Composting (using biological processes to generate heat). Heat drying and heat treatment (use physical processes to generate heat, e.g., hot gasses, heat exchangers) Pasteurization (physical heat, e.g., hot gases, heat exchangers). Aerobic digestion (biological heat)[b] Anaerobic digestion (physical heat)[b]
Application of radiation	Depends on dose. Sufficient doses can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Viruses are most resistant to radiation.	Gamma and high-energy electron beam radiation.
Application of chemica disinfectants	Substantially reduces bacteria and viruses and vector attraction. Probably reduces protozoan cysts. Does not effectively reduce helminth ova unless combined with heat.	Lime stabilization
Reduction of the sewage sludge's volatile organic content (the microbial food source).	Reduces bacteria. Reduces vector attraction.	Aerobic digestion Anaerobic digestion Composting[b]
Removal of moisture from the sludge	Reduces viruses and bacteria. Reduces vector attraction as long as the sewage sludge remains dry. Probably effective in destroying protozoan cysts. Does not effectively reduce helminth ova unless combined with other processes such as high temperature.	Air or heat drying

[a]See Chapters 6 and 7 for a description of these processes. Many processes use more than one approach to reduce pathogens.
[b]Effectiveness depends on design and operating conditions.

isms are ones that have been found to respond to treatment processes and environmental conditions in a manner similar to other organisms. Monitoring the levels of these organisms, therefore, provides information about the survival of the larger group.

For example, for helminth ova, tests are employed to determine their presence and viability. The only helminth ova viability that can be determined is that of *Ascaris* sp. *Ascaris* is the hardiest of known helminths; thus, if conditions are such that it cannot survive, it is not possible for other helminth species (*Toxacara*, *Trichuris*, and *Hymenolepis*) to survive.

For viruses, a test is available that simultaneously monitors for several enterovirus species (a subset of enteric viruses - see Table 2-1), which are presumed to be good representatives for other types of enteric viruses.

Salmonella sp. are bacteria of great concern as well as good representatives of reduction of other bacterial pathogens because they are typically present in higher densities than are other bacterial pathogens and are at least as hardy.

Fecal coliforms are enteric bacteria that are used as indicators of the likelihood of the presence of bacterial pathogens. Although fecal coliforms themselves are usually not harmful to humans, their presence indicates the presence of fecal waste which may contain pathogens. These bacteria are commonly used as indicators of the potential presence of pathogens in sewage sludges. They are abundant in human feces and therefore are always present in untreated sewage sludges. They are easily and inexpensively measured, and their densities decline in about the same proportion as enteric bacterial pathogens when exposed to the adverse conditions of sludge processing (EPA, 1992).

In the case of Class B biosolids, the microbiological limit for meeting Alternative 1 is 2 million MPN fecal coliforms per gram dry weight. Because untreated sewage sludge generally contains up to 100 million MPN fecal coliforms per gram dry weight, this limit assumes an approximate 2-log reduction in the fecal coliform population. Studies of anaerobic or aerobic digestion of sludges have shown that the corresponding reduction in the pathogen population will be significant and sufficient so that environmental attenuation can reduce pathogen levels to below detection limit within the time period of site restrictions (Farrell et al.1985; Martin et al. 1990).

For some processes, fecal coliforms may be an overly conservative indicator. Because bacteria may proliferate outside of a host, reintroduction of fecal coliforms into treated biosolids may result in their growth. Concentrations may exceed the Class A fecal coliform limit even though pathogens are not present. In these cases, because fecal coliforms themselves are not a concern, testing directly for *Salmonella* sp. as an indicator of pathogen survival is permissible. Another issue with fecal coliforms is that the tests for these bacteria may overestimate the number of coliforms from human species. This is of particular concern when additives such as wood chips or other bulking agents have been added to biosolids. (Meckes, 1995) In this case also, it is advisable to test directly for *Salmonella* sp.

It must however be noted that high counts of fecal coliforms may also indicate that a process is not being operated correctly. While a preparer may meet the regulatory requirements by testing for and meeting the regulatory limits for *Salmonella* sp., it is recommended that the pathogen reduction process be reviewed to determine at what point fecal coliforms are potentially not being reduced or are being reintroduced into treated biosolids, and ensure that process requirements are being fulfilled.

Regrowth of Bacteria

One of the primary concerns for biosolids preparers is regrowth of pathogenic bacteria. Some bacteria are unique among sewage sludge pathogens in their ability to multiply outside of a host. The processes outlined in the Part 503 regulation and in this document have been demonstrated to reduce pathogens, but even very small populations of certain bacteria can rapidly proliferate under the right conditions, for example, in sewage sludges in which the competitive bacterial populations have been essentially eliminated through treatment (see Section 4.3). Viruses, helminths, and protozoa cannot regrow outside their specific host organism(s). Once reduced by treatment, their populations do not increase. The Part 503 regulation contains specific requirements designed to ensure that regrowth of bacteria has not occurred prior to use or disposal.

Preventing Exposure

Exposure to pathogens in Class B biosolids is limited by restricting situations in which the public may inadvertently come into contact with biosolids and by limiting access to biosolids by vectors which may carry pathogens from the sewage sludge.

Site Restrictions

In the case of land application of Class B biosolids, site restrictions are sometimes required in order to protect public health and the environment. The potential pathways of exposure to Class B biosolids or to pathogens which may exist in Class B biosolids, are listed in Table 2.2 along with a description of how site restrictions impose barriers to exposure pathways. Site restrictions, discussed in detail in Chapter 5, place limits on crop harvesting, animal grazing, and public access on land where Class B biosolids have been applied.

The goal of site restrictions is to limit site activities such as harvesting and grazing until pathogens have been reduced by environmental conditions such as heat, sunlight, desiccation, and competition from other microorganisms. Table 2-3 summarizes the survival rates of four types of pathogenic organisms on soil and on plants. As shown,

helminths have the longest survival time; consequently, the duration of some of the site restrictions are based on helminth survival potential.

Vector Attraction Reduction

Insects, birds, rodents, and domestic animals may transport sewage sludge and pathogens from sewage sludge to humans. Vectors are attracted to sewage sludge as a food source, and the reduction of the attraction of vectors to sewage sludge to prevent the spread of pathogens is a focus of the Part 503 regulation. Vector attraction reduction can be accomplished in two ways; by treating the sewage sludge to the point at which vectors will no longer be attracted to the sewage sludge and by placing a barrier between the sewage sludge and vectors. The technological and management options for vector attraction reduction are discussed in Chapter 8.

2.5 Frequently Asked Questions

Because land application of biosolids has increased dramatically in the past several years, and because of some well publicized incidents of pathogen contamination (not necessarily related to biosolids), there have been many questions about the level to which public health is protected. Although it is not possible for every issue to be considered, the following section includes some of the questions which are most frequently asked. In addition, references are included at the end of this chapter and in Chapter 12.

Can biosolids carry the pathogen that causes mad cow disease?

It has been found that Bovine Spongiform Encephalopathy (BSE), or Mad Cow disease, is caused by a prion protein, or the resistant beta form of protein. The pathway for transmission is through the ingestion of tissue from infected animals. There has been no evidence that the BSE prion protein is shed in feces or urine. There have been no known cases of BSE in the United States, and the Food and Drug Administration (FDA) has taken various measures to prevent spread of the disease to or within the United States. For example, the primary route for infection, the use of animal carcasses in animal feed, is banned in this country. These measures have been effective, and BSE has not become a public health concern in the U.S. with regard to ingestion of beef or other exposure routes. Thus there should be no risk of BSE exposure from biosolids. (Tan, et al. 1999)

Is there any risk of HIV infection from biosolids?

The HIV virus is contracted through contact with blood or other body fluids of an infected individual. Feces and urine do not carry the HIV virus, but contaminated fluids may be discharged in minor amounts to the sewerage system. The conditions in the wastewater system are not favorable for the virus's survival. Separation from the host environment, dilution with water, chemicals from household and industrial sewer discharges, and the length of time from discharge to treatment all impede the survival of the virus (WEF/U.S. EPA Fact Sheet, 1997). HIV is seldom detected in wastewater, and the additional treatment that wastewater goes through, producing an effluent and sewage sludge which undergoes treatment to become Class A or B biosolids, makes it virtually impossible that biosolids would contain the HIV virus. (Lue-Hing, et al. 1999)

Wastewater treatment workers may come into contact with contaminated objects (bandages, condoms, etc.), but common sense hygiene practices already in place at wastewater treatment plants including the use of protective clothing and gloves greatly reduce the potential for exposure. The U.S. Department of Health and Human Services stated in 1990 that ". . .these workers (wastewater treatment workers) have no increased potential of becoming infected by blood borne infectious agents. Therefore, medical waste discarded to the sanitary sewer is not likely to present any additional public health effects to the wastewater workers or to the general public." (Johnson, et al. 1994)

What is a bioaerosol?

Bioaerosols are airborne water droplets containing microorganisms. These may include pathogenic microorganisms. Bioaerosols are a potential public health concern with regard to Class B biosolids because if pathogens are contained in the biosolids, they may become airborne and infect workers or the public through direct inhalation or through contact after settling on clothing or tools. It has been found that aerosolization of protozoa and helminths is unlikely, but bacteria or bacterial components (endotoxin) and viruses may become airborne and disperse from an application source depending on local meteorological and topographical conditions. However, Class B biosolids are rarely applied dry enough to become airborne; applying wet biosolids, particularly when the biosolids are incorporated or injected into the land, makes it highly unlikely that bioaerosols will be dispersed from land application.

The public access restrictions for land-applied Class B biosolids are based on the various pathways by which pathogens may impact public health. Site restrictions are adequate for the protection of public health, but site workers who are present during the application of Class B biosolids should follow standard hygiene precautions such as washing their hands after contacting biosolids and wearing dust masks if applying extremely dry material. More information on aerosolization of pathogens from land application can be found in the references following this chapter.

What is Aspergillus fumigatus?

Aspergillus fumigatus is a pathogenic fungus which is found in decaying organic matter such as sewage sludge, leaves, or wood. Because the fungus is heat resistant, and because sewage sludge composting facilities often use wood chips as a bulking agent, A. *fumigatus* has been associated with cornposting. Inhalation of *A. fumigatus* spores

may result in allergenic effects including irritation of the mucous membranes and asthma. However, *A. fumigatus* is a secondary, or opportunistic pathogen, and infection from *A. fumigafus* ("Aspergillosis") is limited to debilitated or immuno-compromised individuals. Studies of the health status of compost facility workers, the population most likely to be exposed to *Aspergillus fumigafus,* have not shown any negative health impacts (Millner, et al. 1994).

A. fumigafus is a ubiquitous fungus and has been found in homes, gardens, and offices at considerable levels. Numerous studies have been conducted to determine the level of the fungus in the areas surrounding active compost sites and compare this level to background concentrations of *Aspergillus fumigafus.* In general, it has been found that concentrations of *A. fumigafus* drop to background levels within 500-1000 feet of site activity. *A. fumigafus* is not covered in the Part 503.

There have been several incidents in which fruit has been contaminated with pathogens. Was this due to the land application of biosolids?

No. Pathogens such as *Salmonella* sp. and pathogenic strains of *E. coli* are typically associated with animal products (meat and eggs), but outbreaks have been known to occur as a result of vegetable or fruit contamination from the use of animal manures. Some of the well-publicized incidents include cases in which the consumption of fresh apple juice and cider resulted in widespread illness and the death of a child (Center for Disease Control, 1996). One case was found to be due to contamination from *E. coli* found in bovine feces, and the other was due to *Crypfosporidium* sp., also suspected to be from contact with animal manure. Other cases have involved the contamination of berries, melons, and alfalfa sprouts.

The Part 503 regulation applies only to the land application of biosolids. Education of field workers, regulation of working conditions, both domestically and abroad, and the use of animal manure products are beyond the scope of this document.

What is the fate of Giardia and Cryp tosporidium during Se wage Sludge Treatment?

Giardia lamblia and *Crypfosporidium parvum* are protozoan parasites that can infect the digestive tract of humans and other warm blooded animals. Semi-aquatic mammals can serve as hosts, transmitting the disease to humans who consume contaminated water. Domestic mammals (particularly ruminants) can serve as infective hosts and contaminate a drinking water supply. It is currently believed that at least 7% of the diarrhea1 cases in the United States are caused by *Crypfosporidium* sp.

West (1991) notes that human protozoan parasites such as *Crypfosporidium* sp. and Giardia sp. possess several traits which facilitate waterborne transmission. They can (1) be excreted in feces in large numbers during illness; (2) persist through conventional sewage treatment; (3) survive in an environmentally robust form or demonstrate resilience to inactivation while in aquatic environments; (4) be resistant to commonly used disinfectants in the treatment of drinking water; and (5) require low numbers to elicit infection in susceptible hosts consuming or exposed to contaminated water.

Stadterman et al. (1995) reported on an anaerobic digestion study which spiked *Crypfosporidium* sp. oocysts into the digester and then periodically removed samples to determine the die-off. They found that conventional anaerobic digestion produces about a 2-log removal or a better log reduction on this protozoan than it does on bacteria and viruses, but it does not reduce densities to the low values needed for Class A for this pathogen. The reported survival of some protozoa after anaerobic digestion at 35 °C is a cause for concern.

Jenkins et al. (1998) reported that ammonia inactivates these oocysts, depending on the concentration. High pH processes that increase the free ammonia concentration can inactivate these oocysts (although pH by itself does little).

A conservative conclusion from the limited research performed is that Class B processes can only be expected to reduce protozoan pathogens by about a factor of ten. The restrictions written into the regulation (access control, growing only certain crops, restrictions on root crops, etc.) are necessary to prevent exposure to these pathogens. The Class A processes reduce protozoa to below detectable limits.

References and Additional Resources

Ahmed, Anise U., and Darwin L. Sorensen. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Water Environment Research, Vol. 67, No.2:143-150.

Ault, Steven K., Michael Schott, 1993. Aspergillus, aspergillosis, and cornposting Operations in California, Technical Bulletin No. 1. California Integrated Waste Management Board.

Beuchat, Larry, and J.H. Ryu. 1997. Produce handling and processing practices. Emerging Infectious Diseases, Centers for Disease Control and Prevention. Vol 3, No. 4.

Casson, L.W., et al. 1992. HIV survivability in water. Water Environmental Research, Vol 64: 213-215.

Engineering News Record, August 13, 1987. No AIDS threat in sewage. Issue 47.

Farzadegan, Homayoon. 1991. Proceedings of a Symposium: Survival of HIV in environmental waters. Baltimore, MD. National Science Foundation and the Johns Hopkins University.

Farrell, J.B., G. Sternard, A.D. Venosa. 1985. Microbial destructions achieved by full-scale anaerobic digestion. Paper presented at Municipal Wastewater Sludge Disinfection Workshop, Kansas City, Mo. Water Pollution Control Federation October 1985.

Feldman, Kathleen, 1995. Sampling for airborne contaminants. BioCycle, August 1995 (84-86).

Gover, Nancy. 1993. "HIV in wastewater not a recognized threat, other pathogens can be." National Small Flows Clearinghouse Newsletter. July 1993.

Gupta, Phalguni. 1991. HIV survivability in wastewater. Proceedings of a Symposium: Survival of HIV in Environmental Waters. Baltimore, MD. National Science Foundation and the Johns Hopkins University.

Haines, John, 1995. Aspergillus in compost: Straw man or fatal flaw? BioCycle, April 1995 (32-35).

Harding, H.J., R.E. Thomas, D.E. Johnson, and C.A. Sorber. 1981. Aerosols generated by liquid sludge application to land. Report No. EPA-600/l-81 -028. Washington, DC: U.S. EPA, Office of Research and Development.

Haug, Roger T. 1993. The practical handbook of compost engineering. Lewis Publishers.

Hay, Johnathan C., 1996. Pathogen destruction and biosolids cornposting. BioCycle, Vol. 37 No.6:67-76.

Jenkins, M.B., D.D. Bowman, and W.C. Ghiorse. 1998. Inactivation of *Cryptosporidium parvum oocysts* by ammonia. Appl. Envir. Microbiol. 64, No.2, 784-788.

Johns Hopkins School of Hygiene and Public Health. 1991. HIV transmission in the environment: What are the risks to the public's health? Public Health News.

Johnson, R.W., Blatchley, E.R. III, and D.R. Mason. 1994. HIV and the blood borne pathogen regulation: Implications for the wastewater industry. Water Environment Research, Vol. 66: 684-691.

Kindzierski, W.B., R.E. Roberts, and N.J. Low. 1993. Health effects associated with wastewater treatment, disposal, and reuse. Water Environment Research, Vol. 65: 599-606.

Kowal, N.E. 1985. Health effects of land application of municipal sludge. Pub. No.: EPA/600/1-85/015. Research Triangle Park, NC: U.S. EPA Health Effects Research Laboratory.

Kowal, N.E. 1994. Pathogen risk assessment: Status and potential application in the development of Round II regulations. Proceedings of the June 19-20, 1994 Speciality Conference. The Management of Water and Wastewater Solids for the 21st Century: A Global Perspective. Water Environment Federation. Alexandria, VA.

Lue-Hing, Cecil, Leonard Casson, and Prakasam Tata. 1999. HIV in wastewater: present survivability and risk to wastewater treatment plant workers. Water Environment Federation monograph. Alexandria, VA.

Martin, J.H., Jr., H.F. Bastian, and G. Stern. 1990 Reduction of enteric microorganisms during aerobic sludge digestion. Wat. Res. 24(11):1377-l 385.

Meckes, Mark, E.W. Rice, C.H. Johnson, and S. Rock. 1995. Assessment of the Bacteriological Quality of Compost Science and Utilization. Vol. 3 No. 3:6-1 3.

Millner, P.D., S.A. Olenchock, E. Epstein, R. Rylander, J. Haines, J. Walker, B.L. Ooi, E. Horne, and M. Maritato. 1994. Bioaerosols associated with cornposting facilities. Compost Science and Utilization. 2(4):6-57.

Moore, B.E. 1993. Survival of human immunodeficiency virus (HIV), HIV-infected lymphocytes, and poliovirus in water. Applied and Environmental Microbiology. Vol. 59: 1437-l 443.

Morbidity and Mortality Weekly Report. 1996. Outbreak of E.Coli O157:H7 infections associated with drinking unpasteurized commercial apple juice. Centers for Disease Control and Prevention. Vol. 45, No. 44.

Obeng, L. 1985. Health aspects of water supply and sanitation. In Information and Training for Low-Cost Water Supply and Sanitation. Ed D. Trattles. World Bank. Washington, D.C.

Pell, Alice. 1997. Manure and microbes: Public and animal health problem? Journal of Diary Science 80:2673-2681.

Ponugoti, Prabhaker R., Mohamed F. Dahab, Rao Surampalli. 1997. Effects of different biosolids treatment systems on pathogen and pathogen indicator reduction. Water Environment Research, Vol. 69: 1195-l 206

Scheuerman, P.R., S.R. Farrah, and G. Bitton. 1991. Laboratory studies of virus survival during aerobic and anaerobic digestion of sewage sludge. Water Resources 25:241-245.

Smith, James E. and J. B. Farrell. 1996. Current and future disinfection - Federal Perspectives. Presented at Water Environment Federation 69th Annual Conference & Exposition.

Soares, Hugo M., Beatriz Cardenas, David Weir, and Michael S. Switzenbaum. 1995. Evaluating pathogen regrowth in biosolids compost. BioCycle, Vol. 36. No.6:70-76.

Sobsey, M.D., and P.A. Shields. 1987. Survival and transport of viruses in soils. Model studies pp. 155-l 77 in V.C. Rao and J.L. Melnick, eds. Human viruses in sediments, sludge, and soils. Boca Raton, FL: CRC Press

Sorber, C.A. ,B.E. Moore, D.E. Johnson, H.J. Harding, R.E. Thomas. 1984. Microbiological aerosols from the ap-

plication of liquid sludge to land. Journal WPCF Vol. 56, No.7:830-836.

Stadterman, A.M. Sninsky, J.L.Sykora and W. Jakubowski. 1995. Removal and inactivation of *Cryptosporidium* oocysts by activated sludge treatment and anaerobic digestion. Wat. Sci. Tech. 31, No. 5-6, 97-104

Tan, L., M.A. Williams, M.K. Khan, H.C. Champion, N.H. Nielsen. 1995. Risk of transmission of Bovine Spongiform Encephalopathy to humans in the United States. JAMA. 281, 24, 2330.

U.S. EPA. 1983. Enteric virus removal in wastewater treatment lagoon systems (Project Summary, EPA/600/S1-83-012)." Research Triangle Park, NC: U.S. EPA/Health Effects Research Laboratory.

U.S. EPA. 1985. Health effects of land application of municipal sludge (EPA/600/1-851015). Research Triangle Park, NC: U.S. EPA/Health Effects Research Laboratory.

U.S. EPA. 1986. Inactivation of enteric pathogens during aerobic digestion of wastewater sludge (Project Summary, EPA/600/SO-86/047). Cincinnati, OH: U.S. EPA/ Water Engineering Research Laboratory.

U.S. EPA. 1989. Technical support document for pathogen reduction in sewage sludge. NTIS No.: PB89-136618. Springfield, VA: National Technical Information Service.

U.S. EPA. 1991. Preliminary risk assessment for viruses in municipal sewage sludge applied to land (Project Summary, EPA/600/SR-92/064). Washington, DC: U.S. EPA/Office of Health & Environmental Assessment.

U.S.EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No.: PB93-11069. Springfield, VA: National Technical Information Service.

Ward, R.L., G.A. McFeters, and J.G. Yeager. 1984. Pathogens in sludge: Occurrence, inactivation, and potential for regrowth. Sandia National Laboratories, Albuquerque, NM. SAND83-0557, TTC-0428, UC-41. U.S. DOE Contract CEAC04-76DP00789.

WEF/U.S. EPA. 1997. Can Aids be transmitted by biosolids?" in WEF/U.S. EPA Biosolids Fact Sheet Project.

West, P. A., "Human pathogenic viruses and parasites: Emerging pathogens in the water cycle," Journal of Applied Bacteriology JABAA4, Vol.70, No. Supp, p 107S-114S, 1991.

Yanko, William A. 1988. Occurrence of pathogens in distribution and marketing municipal sludges. (EPA/600/1-87/014) County Sanitation District of Los Angeles and EPA/Health Effects Research Laboratory.

Yeager, J.G. and R.L Ward. 1981. Effects of moisture content on long-term survival and regrowth of bacteria in wastewater sludge. Appl. Environ. Microbiol. 41(5):1117-l 122.

Chapter 3
Overview of Part 503 Subpart D Requirements, Their Applicability, and Related Requirements

3.1 Introduction

The Subpart D (pathogen and vector attraction reduction) requirements of the 40 CFR Part 503 regulation apply to sewage sludge (both bulk sewage sludge and sewage sludge that is sold or given away in a bag or other container for application to the land) and domestic septage applied to the land or placed on a surface disposal site. The regulated community includes persons who generate or prepare sewage sludge for application to the land, as well as those who apply it to the land. Included is anyone who:

- Generates treated sewage sludge (biosolids) that is land applied or placed on a surface disposal site
- Derives a material from treated sewage sludge (biosolids)
- Applies biosolids to the land
- Owns or operates a surface disposal site

Sewage sludge cannot be applied to land or placed on a surface disposal site unless it has met, among other things, the two basic types of requirements in Subpart D:

- Requirements to ensure reduction of pathogens.
- Requirements to reduce the potential of the sewage sludge to attract vectors (rodents, birds, insects, and other organisms that can transport pathogens).

These two types of requirements are separated in Part 503 (they were combined in an earlier regulation, Part 257), which allows flexibility in how they are achieved. Compliance with the two types of requirements must be demonstrated separately. Therefore, demonstration that a requirement for reduced vector attraction has been met does not imply that a pathogen reduction requirement also has been met, and vice versa.

This chapter provides an overview of the Subpart D requirements, their applicability, and the requirements related to frequency of monitoring and recordkeeping. Where relevant, the titles of the sections in this chapter include the number of the Subpart D requirement discussed in the section. Chapters 4 through 8 provide detailed information on the pathogen and vector attraction reduction requirements.

Some of the pathogen and vector attraction reduction alternatives are suitable only for biosolids which have been processed by particular methods, such as by aerobic or anaerobic digestion or cornposting. Chapters 4 and 5 contain examples of how some facilities have met Part 503 requirements using appropriate pathogen and vector attraction reduction protocols, and Chapter 8 discusses each vector attraction option in detail.

3.2 Pathogen Reduction Requirements

Se wage Sludge [503.32(a) and (b)]

The pathogen reduction requirements for sewage sludge are divided into two categories: Class A and Class B. These requirements use a combination of technological and microbiological requirements to ensure reduction of pathogens.

The implicit goal of the Class A requirements is to reduce the pathogens in sewage sludge (including enteric viruses, pathogenic bacteria, and viable helminth ova) to below detectable levels, as defined in the 1992 regulation.

The implicit goal of the Class B requirements is to reduce pathogens in sewage sludge to levels that are unlikely to pose a threat to public health and the environment under the specific use conditions. For Class B biosolids that are applied to land, site use restrictions are imposed to minimize the potential for human or animal exposure to Class B biosolids for a period of time following land application and until environmental factors (e.g. sunlight, desiccation) have further reduced pathogens. Both Class A treatment of the sewage sludge which reduces pathogens to below detectable levels and the combination of Class B sewage sludge treatment and use restrictions on the land application site protect public health and the environment.

"Exceptional quality" (EQ) biosolids are biosolids which have met the Part 503 pollutant concentration limits (Table 3 of Section 503.13) as well as Class A pathogen reduction requirements and one of the first eight vector attraction reduction options listed in 503.33(b)(l) through (b)(8). EQ biosolids may be land applied without site restrictions.

Application of Class B biosolids must be conducted in compliance with site restrictions. Because it is not possible for regulators to follow the land application of biosolids applied on lawns and home gardens, Class B biosolids cannot be sold or given away in bags or other containers or applied on lawns and home gardens.

The testing requirements outlined throughout this document are minimum standards for compliance with the Part 503 rule. It should be pointed out that biosolids are properly distributed under the most recent test results. However, facilities which distribute biosolids between sampling events may wish to enhance their sampling programs to better ensure compliance with pathogen reduction requirements and to enhance public confidence in biosolids quality. More frequent testing should also enable the biosolids generators and preparers to better detect any changes in operations that might affect compliance and slow more rapid correction in any adverse changes. It should be noted that when additional determinations are made, even though they are in excess of Part 503 regulatory requirements, all these analytical results and records must be retained in the generator's, preparer's or land applier's files or reported to the regulatory authority depending on the classification of the operation or the regulatory authority's wishes.

Domestic Septage [503.32(c)]

As stated in Chapter 1, domestic septage is a form of sewage sludge. The requirements for domestic septage vary depending on how it is used or disposed. Domestic septage applied to a public contact site, lawn, or home garden must meet the same requirements as treated sewage sludge (biosolids) applied to these types of land (Class A requirements). Separate, less-complicated requirements for pathogen reduction apply to domestic septage applied to agricultural land, forests, or reclamation sites. These requirements include site restrictions to reduce the potential for human exposure to domestic septage and to allow for pH adjustment or environmental attenuation with site restrictions only on harvesting crops. No pathogen requirements apply if domestic septage is placed on a surface disposal site.

3.3 Vector Attraction Reduction (VAR) Requirements [503.33]

Subpart D provides 12 options to demonstrate vector attraction reduction. These are referred to in this document as Options 1 through 12. Table 8-2 summarizes these options, and Chapter 8 provides more detailed information on the options.

Reduction through Treatment

Options 1 through 8 apply to sewage sludge that has been treated in some way to reduce vector attraction (e.g., aerobic or anaerobic digestion, cornposting, alkali addition, drying). These options consist of either operating conditions or tests to demonstrate that vector attraction has been reduced in the treated sewage sludge. Option 12 is a requirement to demonstrate reduced vector attraction in domestic septage through elevated pH. This option applies only to domestic septage.

Reduction through Barriers

Options 9 through 11 are "barrier" methods. These options require the use of soil as a physical barrier (i.e., by injection, incorporation, or as cover) to prevent vectors from coming in contact with the land applied biosolids. They include injection of biosolids below the land surface, incorporation of biosolids into the soil, and placement of a cover over the biosolids. Options 9 through 11 apply to both biosolids and domestic septage. Option 11 may only be used at surface disposal sites.

Timing of Pathogen and Vector Attraction Reduction

In the case of Class A biosolids, pathogen reduction must take place before or at the same time as vector attraction reduction unless VAR Option 6, 7, or 8 is used. More information is provided in Section 4.2.

3.4 Applicability of the Requirements [503.15 and 503.251

The applicability of the pathogen and vector attraction reduction requirements is covered in 503.15 and 503.25. Tables 3-l to 3-3 summarize the applicability of the Subpart D requirements to sewage sludge and domestic septage.

Table 3-l. Subpart D Requirements for the Land Application of Bulk Biosolids[1]

	Applied to Agricultural Land, a Forest, a Public Contact Site[2], or a Reclamation Site[3]	Applied to a Lawn or Home Garden
Pathogen Requirements	Class A or Class B with site restrictions	Class A[4]
Vector Attraction Reduction Requirements	Options I-l 0[5]	Options 1-8[5,6]

[1] Bulk biosolids are biosolids that are not sold or given away in a bag or other container for application to the land.
[2] Public contact site is land with a high potential for contact by the public, e.g., public parks, ball fields, cemeteries, plant nurseries, turf farms, and golf courses.
[3] Reclamation site is drastically distrubed land (e.g., strip mine, construction site) that is reclaimed using biosolids.
[4] The regulation does not permit use of biosolids meeting Class B requirements on lawns or home gardens, because it would not be feasible under these circumstances to impose the site restrictions that are an integral part of the Class B requirements.
[5] See Chapter 8 for a description of these options.
[6] The two vector attraction reduction requirements that cannot be met when bulk biosolids are appliced to a lawn or a home garden are injection of the bulk biosolids below the land surface and incorporation of bulk biosolids into the soil. Implementation of these requiremtns for bulk biosolids applied to a lawn or a home garden would be difficult, if not impossible.

Table 3-2. Subpart D Requirements for Biosolids Sold or Given Away in a Bag or Other Container for Application to the Land

	Land Application
Pathogen Requirements	Class A[1]
Vector Attraction Reduction Requirements	Options 1-8[2]

'Class B biosolids cannot be sold or given away for use on home gardens or lawns because it is not feasible to impose the Class B site restrictions for these uses.
[2]Only the treatment-related options for vector attraction reduction apply to biosolids that are sold or given away in bags or other containers for application to the land, because of the barrier options, which are implemented at the site of application, would be impossible. See Chapter 8 for a description of these options.

Table 3-3. Supart D Requirements for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site[1] or Placed on a surface Disposal Site

	Application to Agricultural Land, a Forest, or a Reclamation Site[2]	Surface Disposal
Pathogen Reduction Requirements	Class B site restrictions only or a pH adjustment (pH ≥ 12 for 30 minutes) plus restrictions concerning crop harvesting	No pathogen requirements[3]
Vector Attraction Reduction Requirements	Options 9, 10, 12[4]	Optionis 9-l 2[4]

[1]For application to all other types of land, domestic septage must meet the same requirements as other forms of sewage sludge (see Tables 3-l and 3-2).
*Reclamation site is drastically disturbed land (e.g., strip mine, construction site) that is reclaimed using biosolids.
[3]There is no pathogen requirement for domestic septage placed on a surface disposal site because site restrictions for grazing of animals, public access, and crop growing are already imosed by the Part 503, Subpart C management practices to reduce exposure to pollutants in domestic septage placed on a surface disposal site.
[4]See Chapter 8 for a description of these options.

Table 3-4. Frequency of Monitoring for Land Application and Surface Disposal

Amount of Biosolids[1] (metric tons dry solids per 365-day period)	Minimum Frequency
Greater than zero but less than 290[2]	Once per year
Equal to or greater than 290 but less than 1,500[2]	Once per quarter (four times per year)
Equal to or greater than 1,500 but less than 1 5,000[2]	Once per 60 days (six times per year)
Equal to or greater than 15,000[2]	Once per month (12 times per year)

'Either the amount of bulk biosolids applied to the land, or the amount of sewage sludge received by a person who prepares biosolids that is sold or given away in a bag or other container for application to the land (dry weight basis), or the amount of biosolids (excluding domestic septage) placed on a surface disposal site.
[2]290 metric tons = 320 tons (approximately 0.9 tons/day for a year)
1,500 metric tons = 1,653 tons (approximately 4.5 tons/day for a year)
15,000 metric tons = 16,534 tons (approximately 45 tons/day for a year)

3.5 Frequency of Monitoring

Se wage Sludge [503.16(a) and 503.26(a)]

The Class A and Class B pathogen requirements and the vector attraction reduction Options 1 through 8 (the treatment related methods) all involve some form of monitoring. The minimum frequency of monitoring for these requirements is given in Part 503.16(a) for land application and Part 503.26(a) for surface disposal. The frequency depends on the amount of biosolids used or disposed annually (see Table 3-4). The larger the amount used or disposed, the more frequently monitoring is required.

In addition to monitoring frequency, a sampling plan should address the minimum number of samples per sampling event that are necessary to adequately represent biosolids quality. Both of these issues are addressed in Chapter 9.

As stated throughout this document, the monitoring requirements set forth in the Part 503 and this document are the minimum requirements. Persons or facilities that generate and distribute biosolids are encouraged to go beyond the minimum required programs as necessary.

Domestic Septage [503.16(b) and 503.26(b)]

One of the requirements that can be used for demonstrating both pathogen reduction and vector attraction reduction in domestic septage is to elevate pH to $12 for 30 minutes (see Sections 5.6 and 8.13). When this requirement is to be met, each container of domestic septage (e.g., each tank truckload) applied to the land or placed on a surface disposal site must be monitored for pH over 30 minutes.

3.6 Sampling Stockpiled or Remixed Biosolids

In many cases there are several steps of preparation before biosolids are actually used or distributed. For example, some products such as composted biosolids may be prepared and then mixed with other materials to create a soil blend. Other biosolids products may be prepared and then stored either on site or at a field until the material can be applied. In some cases, resampling and/or re-establishment of the biosolids quality may be necessary. Whether or not biosolids must undergo additional sampling or processing depends on the classification of the biosolids and on whether the biosolids remain in the control of the preparer or if they have been distributed or sold.

EQ Biosolids

If the biosolids are classified as exceptional quality (EQ) (see Section 3.2), they may be distributed for land application without site restriction. EQ is an industry term rather than a regulatory term. Land application of EQ biosolids is not regulated by the Part 503 once the biosolids leave the control of the biosolids preparer. Therefore, soil blenders or other (non-preparer) users who take EQ biosolids may store the biosolids or mix the EQ biosolids with other (non-sewage sludge) materials without resampling the product.

Conversely, if EQ biosolids remain within the control of the preparer, they are still considered biosolids and are still covered by the Part 503. Like all Class A products, they must undergo microbiological testing at the last possible point before being distributed. In addition, if the preparer mixes the EQ biosolids or otherwise changes the quality of the biosolids, the new biosolids product must again comply with pathogen reduction, vector attraction reduction, and microbiological requirements.

Non-EQ Class A Biosolids

Class A biosolids are not necessarily classified as EQ biosolids; if pollutant levels exceed the Table 3 limits or if one of the first eight vector attraction options has not been met, the Class A biosolids are not considered EQ. All Class A biosolids must undergo microbiological testing just before they are distributed, so testing for fecal coliforms or *Salmonella* sp. must take place after storage. In addition, if the preparer mixes the Class A biosolids with other materials or otherwise changes the quality of the biosolids, the new biosolids product must comply with pathogen reduction, vector attraction reduction, and microbiological requirements.

Non-EQ Class A biosolids must also be monitored after they are distributed. For example, if a Class A compost which does not meet one of the EQ pollutant limits is sold to a vendor who mixes the compost with soil, the soil blender becomes a biosolids preparer, and must therefore comply with all Part 503 regulations. The new biosolids product must comply with pathogen reduction, vector attraction reduction, and microbiological requirements.

Class B Biosolids

Class B biosolids can meet pathogen reduction requirements at any point; there is no requirement that Class B biosolids be tested just before distribution. Therefore, biosolids which have met the Class B pathogen reduction requirements can be stored on site without retesting. However, if the Class B biosolids are mixed with other materials or the quality of the biosolids are otherwise altered, the new biosolids product must meet pathogen reduction and vector attraction reduction requirements.

The same is true for Class B biosolids that are distributed and no longer under the control of the preparer. Stored Class B biosolids do not have to be retested for pathogen reduction, unless the quality of the biosolids is somehow altered through mixing or further processing.

3.7 Record Keeping Requirements [503.17 and 503.27]

Record keeping requirements are covered in Part 503.17 for land application and Part 503.27 for surface disposal. Records are required for both biosolids and domestic septage that are used or disposed. All records must be retained for 5 years except when the cumulative pollutant loading rates (CPLRs) in Subpart B (Land Application) of the Part 503 are used[1]. If CPLRs are used, records of pollutant loading at each site must be kept indefinitely. All records must be retained and made available to the regulatory authority upon request (see Section 3.8).

Land Application

Records must be kept to ensure that the biosolids meet the applicable pollutant limits, management practices*, one of the pathogen requirements, one of the vector attraction reduction requirements and, where applicable, the site restrictions associated with land application of Class B biosolids. When biosolids are applied to land, the person preparing the biosolids for land application and the person applying bulk biosolids must keep records[3,4]. The person applying biosolids that were sold or given away does not have to keep records.Table 3-5 summarizes the record keeping requirements for land application.

Surface Disposal

When biosolids are placed on a surface disposal site, the person preparing the biosolids and the owner/operator of the surface disposal site must keep records. In the case of domestic septage applied to agricultural land, forest, or a reclamation site or placed on a surface disposal site, the person applying the domestic septage and the owner/operator of the surface disposal site may be subject to pathogen record keeping requirements, depending on which vector attraction reduction option is met. Table 3-6 summarizes the pathogen-related record keeping requirements for surface disposal.

Certification Statement

In every case, record keeping involves signing a certification statement that the requirement has been met. Parts 503.17 and 503.27 of the regulation contain the required certification language.

3.8 Reporting Requirements for Sewage Sludge [503.18 and 503.28]

Reporting requirements for sewage sludge are found in Part 503.18 for land application and Part 503.28 for surface disposal. These requirements apply to Class I sludge management facilities[5] and to publicly owned treatment

---

[1]Cumulative pollutant loading rates are not related to pathogen control and therefore are not covered in this document.

[2]Pollutant limits and management practices are not related to the pathogen requirements and therefore are not covered in this document.

[3]Person as defined under Part 503.9 may be an individual, association, partnership, corporation, municipality, state or federal agency, or an agent or employee of a state or federal agency.

[4]When biosolids are prepared by one person, and another person who places it in a bag or other container for sale or give-away for application to the land changes the quality of that biosolids, both persons must keep the records required of preparers (see Table 3-5 and Section 3.6).

[5]A Class I sewage sludge management facility is any publicly owned treatment works (POTW) required to have an approved pretreatment program under 40 CFR 403.8(a) [including any POTW located in a state that has assumed local program responsibilities under 40 CFR 403.1 (e)] and any treatment works treating domestic sewage classified as a Class I sludge management facility by EPA or the state sludge management program because of the potential for its sewage sludge use or disposal practices to adversely affect public health and the environment.

Table **3-5.** Summary of Pathogen and Vector Attraction Reduction Record Keeping Requirements for Land Application of **Biosolids**[1]

	Description of How Class A Pathogen Requirement Was Met	Description of How Class B Pathogen Requirement Was Met	Description of How the Class B Site Restrictions Were Met at Each Site Where Sewage Sludge Was Applied	Description of How Pathogen Requirement for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site Was Met	Description of How Vector Attraction Requirement Was Met	Certification Statement that the Requirement Was Met
Biosolids - Pathogen Requirements						
Person preparing Class A bulk biosolids	√					√
Person preparing Class A biosolids for sale or give away in a bag or other container	√					√
Person preparing Class B biosolids		√				√
Person applying Class B biosolids			√			√
Biosolids - Vector-Attraction Reduction Requirements						
Person preparing biosolids that meet one of the treatment-related vector attraction reduction requirements (Options 1-8)					√	√
Person applying biosolids if a barrier-related option (Optioins **9-11)** is used to meet the vector attraction reduction requirement					√	√
Domestic Septage						
Person applying domestic septage to agricultural land, a forest, or a reclamation site				√	√	√

'Other record keeping requirements, not covered in this document; apply to pollutant limits and management practices.

Table 3-6. Summary of Pathogen and Vector Attraction Reduction Record Keeping Requirements for Surface Disposal of **Biosolids**[1]

	Required Records		
	Description of How Class A or B Pathogen Requirement was Met	Description of How Vector Attraction Requirement was Met	Certification Statement that the Requirement was Met
Biosolids - Pathogen Requirements			
Person preparing the biosolids	√		√
Sewage Slude - Vector Attraction Reduction Requirements			
Person preparing biosolids that meet one of the treatment-related vector attraction reduction requirements (Options I-8)		√	
Owner/operator of the surface disposal **site** if a barrier-related option (Option 9-11) is used to meet the vector attraction reduction requirement		√	√

continued

Table 3-6. (Continued)

	Required Records		
	Description of How Class A or B Pathogen Requirement was Met	Description of How Vector Attraction Requirement was Met	Certification Statement that the Requirement was Met
Domestic Septage			
Person who places domestic septage on the surface disposal site if the domestic septage meets Option 12 for vector attraction reduction		√	√
Owner/operator of the surface disposal site if a barrier-related option (Optioin 9-11) is used to meet the vector attraction reduction requirement		√	√

[1]Other record keeping requirements, not covered in this document, apply to pollutant limits and management practices.

works either with a design flow rate equal to or greater than 1 million gallons per day or that serve 10,000 or more people, or if specifically required by the permitting authority. Reports must be submitted to the regulatory authority (see Tables 3-5 and 3-6) and/or as the owner/operators of surface disposal sites (see Table 3-6) on February 19 of each year. There are no reporting requirements associated with the use or disposal of domestic septage, but records must be kept and made available to the regulatory authority upon request.

3.9 Permits and Direct Enforceability [503.3]

Permits

Under Part 503.3(a), the requirements in Part 503 may be implemented through (1) NPDES permits issued to treatment works treating domestic sewage by EPA permits issued by states with an EPA-approved sludge management program, and (2) by permits issued under Subtitle C of the Solid Waste Disposal Act; Part C of the Safe Drinking Water Act; the Marine Protection, Research, and Sanctuaries Act of 1972; or the Clean Air Act. Treatment works treating domestic sewage should submit a permit application[6] to the approved state program, or, if there is no such program, to the EPA Regional Sludge Coordinator (see Appendix A).

Direct Enforceability

Under Part 503.3(b), the requirements of Part 503 automatically apply and are directly enforceable even when no federal permit has been issued for the use or disposal of biosolids.

[6]See 40 CFR Parts 122.123, and 501; 54 FR 18716/May 2, 1989; and 58 FR 9404/ February 19, 1993, for regulations establishing permit requirements and procedures, as well as requirements for states wishing to implement approved sewage sludge management programs as either part of their NPDES programs or under separate authority.

Chapter 4
Class A Pathogen Requirements

4.1 Introduction

This chapter principally discusses the Class A pathogen requirements in Subpart D of the 40 CFR Part 503 regulation. Biosolids that are sold or given away in a bag or other container for application to land **must** meet these requirements (see Section 3.4). Bulk biosolids applied to a lawn or home garden also **must** meet these requirements. Bulk biosolids applied to other types of land **must** meet these requirements if site restrictions are not met (see Chapter 5 for guidance on Class B biosolids). Some discussion is, however, presented of vector attraction reduction.

There are six alternative methods for demonstrating Class A pathogen reduction. Two of these alternatives provide continuity with 40 CFR Part 257 by allowing use of Processes to Further Reduce Pathogens (PFRPs) and equivalent technologies (see Sections 4.8 and 4.9). Any one of these six alternatives may be met for the sewage sludge to be Class A with respect to pathogens. The implicit objective of all these requirements is to reduce pathogen densities to below detectable limits which are:

Salmonella sp.	less than 3 MPN per 4 grams total solids biosolids (dry weight basis)
Enteric viruses[1]	less than 1 PFU per 4 grams total solids biosolids (dry weight basis)
Viable helminth ova	less than 1 viable helminth ova/ 4 gram total solids biosolids (dry weight basis)

One of the vector attraction reduction requirements (see Chapter 8) also must be met when biosolids are applied to the land or placed on a surface disposal site. To meet the Part 503 regulatory requirements, pathogen reduction must be met before vector attraction reduction or at the same time vector attraction reduction is achieved.

For the following sections, the title of each section provides the number of the Subpart D requirement discussed in the section. The exact regulatory language can be found in Appendix B, which is a reproduction of Subpart D. Chapters 9 and 10 provide guidance on the sampling and analysis needed to meet the Class A microbiological monitoring requirements.

4.2 Vector Attraction Reduction to Occur With or After Class A Pathogen Reduction[503.32(a)(2)]

Although vector attraction reduction and pathogen reduction are separate requirements, they are often related steps of a process. Chapter 8 discusses the vector attraction reduction options in greater detail.

The order of Class A pathogen reduction in relation to the reduction of vector attraction is important when certain vector attraction reduction options are used. Part 503.32(a)(2) requires that Class A pathogen reduction be accomplished before or at the same time as vector attraction reduction, except for vector attraction reduction by alkali addition [503.33(b)(6)] or drying [503.33(b)(7) and (8)] (see Chapter 8).

This requirement is necessary to prevent the growth of bacterial pathogens after sewage sludge is treated. Contamination of biosolids with a bacterial pathogen after one of the Class A pathogen reduction alternatives has been conducted may allow extensive bacterial growth unless: a) an inhibitory chemical is present, b) the biosolids are too dry to allow bacterial growth, c) little food remains for the microorganisms to consume, or d) an abundant population of non-pathogenic bacteria is present. Vegetative cells of non-pathogenic bacteria repress the growth of pathogenic bacteria by "competitive inhibition" which is in substantial part due to competition for nutrients. It should be noted that vector attraction reduction by alkali addition [503.3(b)(6)] or drying [503.3(b)(7)] and (8) is based on the characteristic of the biosolids (pH or total solids) remaining elevated. Should the pH drop or the biosolids absorb moisture, the biosolids may be more hospitable to microorganisms, and pathogenic bacteria, if introduced, may grow. Therefore it is recommended that biosolids treated with these methods be stored appropriately.

Biological treatment processes like anaerobic digestion, aerobic digestion, and cornposting produce changes in the

[1]Enteric viruses are monitored using a method that detects several enterovirus species-a subset of enteric viruses. This method is presumed to be a good indicator of enteric viruses. Since the objective of the Part 503 regulation is to reduce al enteric viruses to less than 1 PFU per 4 grams total solids sewage sludge, this document refers to "enteric viruses" when discussing this requirement, although, in reality, the detection method enumerates only enteroviruses.

sewage sludge so that it satisfies one of the vector attraction reduction requirements [503.3(b)(l) through (5)]. They repress bacterial growth by minimizing the food supply and providing competition for the remaining food from non-pathogenic organisms. The pathogen reduction alternative must precede the vector attraction reduction process: otherwise, the large number of non-pathogenic bacterial cells would be killed and growth of pathogenic bacteria could occur. Certain pathogen reduction processes such as composting accomplish vector attraction reduction by a biological process simultaneously with thermal reduction of pathogens. A non-pathogenic bacterial community survives which adequately suppresses growth of pathogenic bacteria.

In the case of Class B biosolids, a population of non-pathogenic bacteria is retained and inhibits the growth of pathogenic bacteria through competition, and site restrictions are imposed with their land application to reduce the risk of exposure to pathogens. Therefore, bacterial growth is not a concern for Class B biosolids, and vector attraction reduction and pathogen reduction for compliance with the Part 503 Rule requirements may be met in any order.

4.3 Monitoring of Fecal Coliform or *Salmonella* sp. to Detect Growth of Bacterial Pathogens [503.32(a)(3)-(8)]

The goal of Class A processes is to reduce the level of pathogens to below detectable levels and below the level at which they are infectious. The Class A processes have been shown to sufficiently reduce pathogen levels in biosolids, and studies to date have not found that the growth of pathogenic bacteria may occur in materials after processes take place or during storage. Favorable conditions for the growth of pathogenic bacteria would be: adequate moisture, absence of an inhibitory chemical, and inadequate reduction of nutritive value of the sewage sludges.

Because Class A biosolids may **be** used without site restrictions, all Class A material must be tested to show that the microbiological requirements are met at the *time* when *it is ready to be used or disposed.* In addition to meeting process requirements, Class A biosolids must meet one of the following requirements:

- Either the density of fecal coliforms in the sewage sludge be less than 1,000 MPN[2] per gram total solids (dry weight basis),
- *Or* the density of *Salmonella* sp. bacteria in the sewage be less than 3 MPN per 4 grams of total solids (dry weight basis).

Although the Part 503 regulation does not specify the number of samples that should be taken to show compliance with Class A density requirements, sampling programs should provide adequate representation of the biosolids generated. Chapter 9 provides guidance for calculating the number of samples that should be taken per sampling event. Unlike Class B biosolids, compliance with Class A requirements is not based on an average value. **Each sample analyzed must comply with the numerical requirements.**

The microbiological requirement must be met either:

- At the time of use or disposal[3], or
- At the time the biosolids are prepared for sale or give away in a bag or other container for land application, or
- At the time the biosolids or material derived from the biosolids is prepared to meet the requirements in 503.1 O(b), 503. 1 O(c), 503. 1 O(e), or 503. 1 0(f)[4].

If a facility stores material before it is distributed for use or disposal, microbiological testing should take place after storage.

In each case, the timing represents the last practical monitoring point before the biosolids are applied to the land or placed on a surface disposal site. Biosolids that are sold or given away cannot be monitored just prior to actual use or disposal; instead monitoring is required as **it** is prepared for sale or give away. Biosolids that meet the 503.1 O(b, c, d, ore) requirements are considered "Exceptional Quality" and are therefore not subject to further control (see Section 1.4). For this reason, the microbiological requirements must be met at the time the biosolids are prepared to meet the 503.10 requirements, which in most cases is the last time the biosolids are under the control of a biosolids preparer.

As discussed in Chapter 9, the timing of pathogen sampling is also a function of laboratory turnaround time. Obtaining results for fecal coliform and *Salmonella* sp. analysis may take several days if tests are performed in-house, but commercial labs may require more time to process and report results. It is not unusual for laboratories to have a turnaround time of 2 weeks, even for simple tests such as fecal coliform. If this is the case, this time should be factored into the sampling program so that results can be obtained before biosolids are distributed for use or disposal.

Monitoring Fecal Coliforms or Salmonella sp.

Fecal coliforms are used in the Part 503 as an indicator organism, meaning that they were selected to be monitored because reduction in fecal coliforms correlates to reduction in *Salmonella* sp. and other organisms. The re-

[2]The membrane filter method is not allowed for Class A because, at the low fecal coliform densities expected, the filter would have too high a loading of sewage sludge solids to permit a reliable count of the number of fecal coliform colonies.

[3]Minus the time needed to test the biosolids and obtain the test results prior to use or disposal (see Chapter 10).

[4]The 503.10(b)(c)(e) and (f) requirements are not discussed in this document.

quirements were based on experimental work by Yanko (1987) and correlations developed from Yanko's data by Farrell (1993) which show that this level of fecal coliforms correlate with a very low level of *Salmonella* sp. detection in composted sewage sludge (EPA, 1992).

Anecdotal reports suggest that some composting facilities may have diff iculty meeting this requirement even when *Salmonella* sp. are not detected. This might be expected under several circumstances. For example, very severe thermal treatment of sewage sludge during composting can totally eliminate *Salmonella* sp. yet leave residual fecal coliforms. If the sewage sludge has been poorly composted and thus is a good food source, fecal coliforms may grow after the compost cools down from thermophilic temperatures. Because the *Salmonella* sp. are absent, they cannot grow. An even more probable circumstance could occur if the sewage sludge is treated with lime before composting. Lime effectively destroys Salmonella sp. in sewage sludge and leaves surviving fecal coliforms (Farrell et al., 1974). Under conditions favorable for growth, the fecal coliforms can regrow to levels higher than 1,000 MPN per gram. Research has shown that detection of *Salmonella* sp. is much rarer in composted sewage sludge that has been lime treated and composted than detection of fecal coliforms. *Fecal coliform densities maybe high therefore compared to pathogen densities in such cases and maybe overly conservative.* For this reason, all of the Part 503 Class A alternatives allow the direct measurement of *Salmonella* sp. **or** fecal coliform analysis, but do not require both.

4.4 Alternative 1: Thermally Treated Sewage Sludge [503.32(a)(3)]

This alternative may be used when the pathogen reduction process uses specific time-temperature regimes to reduce pathogens. Under these circumstances, time-consuming and expensive tests for the presence of specific pathogens can be avoided. It is only necessary to demonstrate that:

- Either fecal coliform densities are below 1,000 MPN per gram of total solids (dry weight basis), or *Salmonella* sp. bacteria are below detection limits (3 MPN per 4 grams total solids [dry weight basis]) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or given away in a bag or other container for land application, **or** at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).
- **And** the required time-temperature regimes are met.

Time- Temperature Requirement

Four different time-temperature regimes are given in Alternative 1. Each regime is based on the percent solids of the sewage sludge and on operating parameters of the treatment process. Experimental evidence (EPA, 1992) demonstrates that these four time-temperature regimes reduce the pathogenic organisms to below detectable levels.

The four time-temperature regimes are summarized in Table 4-l. They involve two different time-temperature equations. The equation used in Regimes A through C results in requirements that are more stringent than the requirement obtained using the equation in Regime D. For any given time, the temperature calculated for the Regime D equation will be 3 Celsius degrees (5.4 Fahrenheit degrees) lower than the temperature calculated for the Regimes A through C equation.

The time-temperature relationships described for Alternative 1 are based on extensive research conducted to correlate the reduction of various pathogens in sewage sludge to varying degrees of thermal treatment. The resulting time-temperature relationship which is the basis for Alternative 1 is shown in Figure 4-l. These requirements are similar to the FDA requirements for treatment of eggnog, a food product with flow characteristics similar to those of liquid sewage sludge. The Regimes A through D differ depending on the characteristics of sewage sludge treated and the type of process used because of the varying efficiency of heat transfer under different conditions.

It is important to note that **it is mandatory for all sewage sludge particles to meet the time-temperature regime.** Therefore, testing of temperatures throughout the sewage sludge mass and agitating the material to ensure uniformity would be appropriate. For processes such as thermophilic digestion, it is important that the digester design not allow for short circuiting of untreated **sewage** sludge. One approach that has been used to overcome this problem has been to draw off treated sewage sludge and charge feed intermittently with a sufficient time period between draw-down and feeding to meet the time-temperature requirement of Alternative 1. Another option would be to carry out the process in two or more vessels in series so as to prevent bypassing.

These time-temperature regimes are not intended to be used for composting (the time-temperature regime for composting is covered in Alternative 5: Processes to Further Reduce Pathogens).

A more conservative equation is required for sewage sludges with 7% or more solids (i.e., those covered by Regimes A and B) because these sewage sludges form an internal structure that inhibits the mixing that contrib utes to uniform distribution of temperature. The more stringent equation is also used in Regime C (even though this regime applies to sewage sludges with less than 7% solids) because insufficient information is available to apply the less stringent equation for times less than 30 minutes.

The time-temperature requirements apply to every particle of sewage sludge processed. Time at the desired temperature is readily determined for batch or plug flow operations, or even laminar flow in pipes. Time of contact also can be calculated for a number of completely mixed

Table 4-l. The Four Time-Temperature Regimes for Alternative 1 (Thermally Treated Sewage Sludge) [503.32(a)(3)]

Regime	Part 503 Section	Applies to	Required Time-Temperature'
A	503.32(a)(3)(ii)(A)	Sewage sludge with at least 7% solids (except those covered by Regime B)	$D = 131{,}700{,}000/10^{0.1400t}$ $t \geq 50°C$ (122°F)[2] $D \geq 0.0139$ (i.e., 20 minutes)[3]
B	503.32(a)(3)(ii)(B)	Sewage sludge with at least 7% solids that are small particles heated by contact with either warmed gases or an immiscible liquid[4]	$D = 131{,}700{,}000/10^{0.1400t}$ $t \geq 50°C$ (122°F)[2] $D \geq 1.74 \times 10^{-4}$ (i.e., 15 seconds)[5]
C	503.32(a)(3)(ii)(C)	Sewage sludge with less than 7% solids treated in processes with less than 30 minutes contact time	$D = 131{,}700{,}000/10^{0.1400t}$ 1.74×10^{-4} (i.e., 15 seconds) $\leq D \leq 0.021$ (i.e. 30 minutes)[6]
D	503.32(a)(3)(ii)(D)	Sewage sludge with less than 7% solids treated in processes with at least 30 minutes contact time	$D = 50{,}070{,}000/10^{0.1400t}$ $t \geq 50°C$ (122° F)[2] $D \geq 0.021$ (i.e. 30 minutes)'

[1]D = time in days; t = temperature ('C).
[2]The restriction to temperatures of at least 50°C (122°F) is imposed because information on the time-temperature relationship at lower temperatures is uncertain.
[3]A minimum time at 20 minutes is required to ensure that the sewage sludge has been uniformly heated.
[4]Two examples of sewage sludge to which this requirement applies are:
- Sewage sludge cake that is mixed with previously dried solids to make the entire mass a mixture of separate particles, and is then dried by contact with a hot gas stream in a rotary drier.
- Sewage sludge dried in a multiple-effect evaporator system in which the system sludge particles are suspended in a hot oil that is heated by indirect heat transfer with condensing steam.

[5]Time-at-temperature of as little as 15 seconds is allowed because, for this type of sewage sludge, heat transfer between particles and the healing fluid is excellent. Note that the temperature is the temperature achieved by the sewage sludge particles, not the temperature of the carrier medium.
[6]Time-at-temperature of as little as 15 seconds is allowed because heat transfer and uniformity of temperature is excellent in this type of sewage sludge. The maximum time of 30 minutes is specified because a less stringent regime (D) applies when time-at-temperature is 30 minutes or more.
'Time-at-temperature of at least 30 minutes is required because information on the effectiveness of this time-temperature regime for reducing

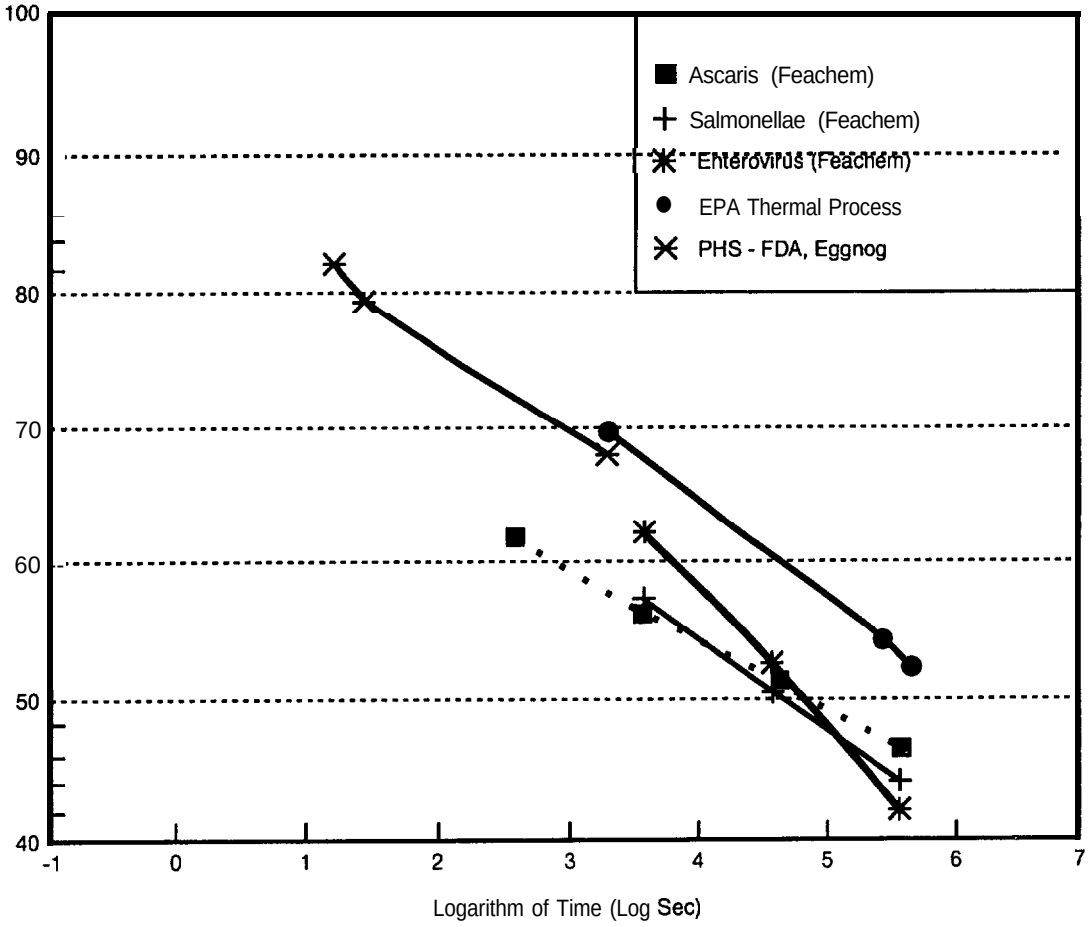


Figure 4-l. EPA's time-temperature relationship for thermal disinfection compared with other time-temperature relationships.

reactors in series (Schafer, et al, 1994). However, there are concerns that flow-through systems may permit some sludge to pass through without adequate treatment. It is recommended that facilities wishing to use this alternative for a flow-through system conduct tracer studies to demonstrate that sewage sludge is treated at the required temperature for sufficient time.

Vector Attraction Reduction

Thermally treated sewage sludge must be treated by an additional vector attraction reduction process since thermal treatment does not necessarily break down the volatile solids in sewage sludge. Vector attraction reduction can be met by further processing the sewage sludge with pH adjustment or heat drying (Options 6 and 7), or by meeting one of the other options (Options 8 – 11). Options 1 through 5 would not be applicable to thermally treated sludge unless the sludge were subject to biological digestion after or during thermal treatment.

Example of Meeting Class A Pathogen and Vector Attraction Reduction Requirements

Type of Facility	Thermophilic Anaerobic Digester
Class	A
	Digested sewage sludge is retained for at least 5 days at 50° C (Regime D). Sewage sludge is agitated regularly to ensure thorough mixing, and temperatures are monitored continually in a batch mode of operation.
Testing	Sewage sludge is sampled 6 times each year for pollutants and fecal coliforms: Compliance wiih vector attraction reduction is also monitored.
Vector Attraction Reduction	VAR is met by reducing volatile solids by over 38 percent. Five samples of input and output sewage sludge from each batch are analyzed for volatile solids content over a period of two weeks.
Use or Disposal	The Class A biosolids are land applied.

Microbiological Microbiological Requirement

Microbiological monitoring for either fecal coliforms or *Salmonella* sp. is required to ensure that growth of bacterial pathogens has not occurred.

4.5 Alternative 2: Sewage Sludge Treated in a High pH-High Temperature Process (Alkaline Treatment) [503.32(a)(4)]

This alternative describes conditions of a high temperature-high pH process that has proven effective in reducing pathogens to below detectable levels. The process conditions required by the Part 503 regulation are:

- Elevating pH to greater than 12 and maintaining the pH for more than 72 hours.
- Maintaining the temperature above 52°C (126°F) throughout the sewage sludge for at least 12 hours during the period that the pH is greater than 12.
- Air drying to over 50% solids after the 72-hour period of elevated pH.

The hostile conditions of high pH, high temperature, and reduced moisture for prolonged time periods allow a variance to a less stringent time-temperature regime than for the thermal requirements under Alternative 1. The pH of the sewage sludge is measured at 25°C (77°F) or an appropriate correction is applied (see Section 10.7).

Example of Meeting Class A Pathogen and Vector Attraction Reduction

Type of Process	Alkaline Treatment
Class	
Pathogen Reduction	Alkaline material is used to bring sewage sludge pH to 12 for 72 hours during which time temperatures are above 52°C for 72 hours. Sewage sludge is agitated during the heat pulse phase to maintain even distribution, and temperature and pH are measured at multiple points within the sewage sludge. The sewage sludge is then moved to piles and maintained until moisture is reduced to 50 percent.
Testing	Piles are tested quarterly for pollutants and *Salmonella* sp. Samples are taken from stockpiled material, and material is not distributed for use or disposal until test results are received.
Vector Attraction Reductionis	VAR Option 6, pH adjustment; pH to remain elevated until use/disposal
Use or Disposal	During winter months (Nov - March), biosolids remain on site. In the spring, biosolids are retested for pathogens before being distributed.

Operational Issues

Because the elevated pH and temperature regimes must be met by the entire sewage sludge mass, operational protocols which include monitoring pH and temperature at various points in a batch and agitating the sewage sludge during operations to ensure consistent temperature and pH are appropriate.

Vector A ttraction Reduction

The pH requirement of vector attraction reduction Option 6 is met when Alternative 2 is met. Compliance with Alternative 2 exceeds the pH requirements of Option 6.

Microbiological Requirements

As with all the Class Aalternatives, microbiological monitoring for fecal coliforms or *Salmonella* sp. is required (see Section 4.3) to ensure that pathogens have been reduced and growth of pathogenic bacteria has not occurred.

4.6 Alternative 3: Sewage Sludge Treated in Other Processes [503.32(a)(5)]

This alternative applies to sewage sludge treated by processes that do not meet the process conditions required by Alternatives 1 and 2. This requirement relies on comprehensive monitoring of bacteria, enteric viruses and viable helminth ova to demonstrate adequate reduction of pathogens:

- Either the density of fecal coliforms in the sewage sludge must be less than 1000 MPN per gram of total solids (dry weight basis), or the *Salmonella* sp. bacteria in sewage sludge must be less than 3 MPN per 4 grams of total solids (dry weight basis) at the time the sewage is used or disposed, at the time the sewage sludge is prepared for sale or given away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).
- The density of enteric viruses in the sewage sludge after pathogen treatment must be less than 1 PFU per 4 grams of total solids (dry weight basis).
- The density of viable helminth ova in the sewage sludge after pathogen treatment must be less than 1 per 4 grams of total solids (dry weight basis).

Testing for enteric viruses and viable helminth ova can be complicated by the fact that they are sometimes not present in the untreated sewage sludge. In this case, an absence of the organisms in the treated sewage sludge does not demonstrate that the process can reduce them to below detectable limits. For this reason, Alternative 3 requires that the feed sewage sludge be analyzed for enteric viruses and viable helminth ova. If these organisms are not detected in the feed sewage sludge, the sewage sludge is presumed to be acceptable as a Class A material until the next monitoring episode. Monitoring is continued until enteric viruses and/or viable helminth ova are detected in the feed sewage sludge (i.e., the density of enteric viruses is greater than or equal to 1 PFU per 4 grams total solids (dry weight basis) and/or the density of viable helminth ova is greater than or equal to 1 per 4grams total solids (dry weight basis). At this point, the treated sewage sludge is analyzed to see if these organisms survived treatment. If enteric viruses densities are below detection limits, the sewage sludge meets Class A requirements for enteric viruses, and will continue to do so as long as the treatment process is operated under the same conditions that successfully reduced the enteric virus densities. If the viable helminth ova densities are below detection limits, the process meets the Class A requirements for enteric viruses and will continue to do so as long as the treatment process is operated under the same conditions that successfully reduced the viable helminth ova densities. Thus, it is essential to monitor and document operating conditions until adequate enteric virus and helminth ova reduction have been successfully demonstrated. Samples of untreated and treated sewage sludge must correspond (see Section 7.4).

Enteric Virus and Viable Helminth Ova Testing

Tests for enteric viruses and viable helminth ova take substantial time: 4 weeks to determine whether helminth ova are viable, and 2 weeks or longer for enteric viruses. The treatment works operator does not know whether the feed sewage sludge has enteric viruses or helminth ova until at least 2 to 4 weeks after the first samples for testing feed densities are taken. This works with rapid processes but long-term process systems need to have temporally related samples. In such cases, it may be feasible to obtain results within the processing time constraints. For enteric viruses, the sewage sludge should be stored frozen, unless the sample can be processed within 24 hours, in which case the samples may be stored at 4°C (39°F). For viable helminth ova, the sewage sludge should be stored at 4°C (39°F) (see Section 9.6).

Finding a laboratory that performs viable helminth ova and virus testing has been difficult for some sewage sludge preparers. Chapter 9 has more information on how to select a laboratory. State and Regional EPA sludge coordinators should also be contacted for information on qualified labs in the region.

Since this option relies on testing, rather than process and testing, to protect public health additional testings should be completed. At a minimum, a detailed sampling plan should be submitted to the permitting authority for review.

Vector A ttractiun Reduction

For both Alternatives 3 and 4, meeting vector attraction reduction depends on the process by which pathogen reduction is met. For example, sewage sludge subject to long term storage may meet vector attraction reduction through volatile solids reduction (Options 1 – 3). Sewage sludges may also undergo additional processing or be applied following the requirements in Options 8 – 11.

Microbiological Requirements

As with all the Class A alternatives, microbiological monitoring for fecal coliforms or *Salmonella* sp. is required (see Section 4.3) to ensure that pathogens have been reduced and growth of pathogenic bacteria has not occurred.

4.7 Alternative 4: Sewage Sludge Treated in Unknown Processes [503.32(a)(6)]

The sewage sludge must meet the following limits at the time the biosolids (or material derived from sludge) are used or disposed , at the time the sewage sludge is prepared for sale or given away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.1 O(f):

- The density of enteric viruses in the sewage sludge must be less than 1 PFU per 4 grams of total solids (dry weight basis).
- The density of viable helminth ova in the sewage sludge must be less than 1 per 4 grams of total solids (dry weight basis).

In addition, as for all Class A biosolids, the sewage sludge must meet fecal coliform or *Salmonella* sp. limits. As with Alternative 3, Alternative 4 depends on a successful sampling program that provides accurate representation of the sewage sludge's microbial quality (see Chapter 9).

Example Of Meeting Class A Pathogen And Vector Attraction Reduction

Type of Facility	Unknown Process
Class	A
Pathogen Reduction	Sewage sludge is digested 'arid retained in a lagoon for up to 2 years. Sewage sludge is then moved to a stockpiling area where it may stay for up to 2 years.
Testing	Before sewage sludge is distributed, each pile, representing approximately 1 1 year of sewage sludge production, is tested 'for *Salmonella* sp., viable helminth ova, and enteric viruses. Since quarterly testing. is mandated, based on the amount of sewage sludge which is used or disposed, four samples per pile are submitted.
Vector Attraction Reduction	VAR is demonstrated by showing a 38 percent reduction -in vqlatile Solids. Records of 'incoming material and volume, bulk density, and percent volatile solids of outgoing material are used to calculate the reduction.
Distribution	Biosolids are distributed for land application on agricultural land.

Examples of situations where Alternative 4 may be used:

- Sewage sludge treatment process is unknown.
- The sewage sludge was produced with the process operating at conditions less stringent than the operating conditions at which the sewage sludge could qualify as Class A under other alternatives.

Enteric Virus and Viable Helminth Ova Testing

Tests for enteric viruses and viable helminth ova take substantial time: 4 weeks to determine whether helminth ova are viable, and 2 weeks or longer for enteric viruses. The treatment works operator does not know whether the feed sewage sludge has enteric viruses or helminth ova until at least 2 to 4 weeks after the first samples for testing feed densities are taken. This option works with rapid processes but long-term process systems need to have temporally related samples. In such cases, it may be feasible to obtain results within the processing time constraints. For enteric viruses, the sewage sludge should be stored frozen, unless the sample can be processed within 24 hours, in which case the samples may be stored at 4°C (39°F). For viable helminth ova, the sewage sludge should be stored at 4°C (39°F) (see Section 9.6).

Finding a laboratory that performs viable helminth ova and virus testing has been difficult for some sewage sludge preparers. Chapter 9 has more information on how to select a laboratory. State and Regional EPA sludge coordinators should also be contacted for information on qualified labs in the region.

Since this option relies on testing, rather than process and testing, to protect public health additional testings should be completed. At a minimum, a detailed sampling plan should be submitted to the permitting authority for review.

Vector A ttraction Reduction

For both Alternatives 3 and 4, meeting vector attraction reduction depends on the process by which pathogen reduction is met. For example, sewage sludge subject to long-term storage may meet vector attraction reduction through volatile solids reduction (Options l-3). Sewage sludges may also undergo additional processing or be applied following the requirement in Options 8-11.

4.8 Alternative 5: Use of PFRP [503.32(a)(7)]

Alternative 5 provides continuity with the 40 CFR Part 257 regulation. This alternative states that sewage sludge is considered to be Class A if:

- It has been treated in one of the Processes to Further Reduce Pathogens (PFRPs) listed in Appendix B of the regulation, and
- Either the density of fecal coliforms in the sewage sludge is less than 1,000 MPN per gram total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in the sewage sludge is less than 3 MPN per 4 grams total solids (dry weight basis) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the

time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).

To meet this requirement, the sewage sludge treatment processes must be operated according to the conditions listed in Appendix B of the regulation.

The Appendix B list of PFRPs is reproduced in Table 4-2. This list is very similar to the PFRP technologies listed in 40 CFR Part 257, with two major differences:

- All requirements related to vector attraction reduction have been removed.
- All the "add-on" processes listed in Part 257 are now full-fledged PFRPs.

Under this Alternative, treatment processes classified as PFRP under 40 CFR Part 257 can continue to be operated; however, microbiological monitoring must now be performed to ensure that the pathogen density levels are below detection limits and to ensure that growth of *Salmonella* sp. bacteria does not occur between treatment and use or disposal.

For all PFRP processes, the goal of temperature monitoring should be to represent all areas of a batch or pile and to ensure that temperature profiles from multiple points in the process all meet mandated temperatures. In some instances it may be possible to monitor representative areas of a batch or pile or a reasonable worst case area to insure compliance. Chapter 7 contains more guidelines about the operation of PFRP processes.

4.9 Alternative 6: Use of a Process Equivalent to PFRP [503.32(a)(8)]

The 40 CFR Part 257 regulation allowed any treatment process to be determined *equivalent* to a PFRP. Under Alternative 6, sewage sludge is considered to be a Class A sewage sludge if:

- It is treated by any process equivalent to a PFRP, **and**
- Either the density of fecal coliforms in the sewage sludge is less than 1,000 MPN per gram total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in the sewage sludge is less than 3 MPN per 4 grams total solids (dry weight basis) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.1 O(b), 503.1 O(c), 503.10(e), or 503.1 O(f).

Facilities that meet Alternative 6 for pathogen reduction must still meet vector attraction reduction requirements.

Processes Already Recommended as Equivalent

Processes recommended to be equivalent to PFRP are shown in Table 11.2. Products of all equivalent processes must still meet the Class A fecal coliform or *Salmonella* sp. requirements.

Who Determines Equivalency?

Part 503 gives the permitting authority responsibility for determining equivalency under Alternative 6. The EPA's Pathogen Equivalency Committee (PEC) is available as a resource to provide guidance and recommendations on equivalency determinations to both the permitting authority and the regulated community (see Chapter 11).

4.10 Frequency of Testing

The Part 503 regulation sets forth minimum sampling and monitoring requirements. Table 3-4 in Chapter 3 de-

Table 4-2. Processes to Further Reduce Pathogens (PFRPs) Listed in Appendix B of 40 CFR Part 503[1]

Composting	Using either the within-vessel composting method or the static aerated pile composting method, the temperature of sewage sludge is maintained at 55°C (131°F) or higher for 3 consecutive days. Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 consecutive days or longer. During the period when the compost is maintained at 55°C (131°F) or higher, there shall be a minimum of five turnings of the windrow.
Heat Drying	Sewage sludge is dried by direct or indirect contact with hot gases to reduce the moisture content of the sewage sludge to 10% or lower. Either the temperature of the sewage sludge particles exceeds 80°C (176°F) or the wet bulb temperature of the gas in contact with the sewage sludge as the sewage sludge leaves the dryer exceeds 80°C (176°F).
Heat Treatment	Liquid sewage sludge is heated to a temperature of 180°C (356°F) or higher for 30 minutes.
Thermophilic Aerobic Digestion	Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean cell residence time (i.e., the solids retention time) of the sewage sludge is 10 days at 55°C (131°F) to 60°C (140°F).
Beta Ray Irradiation	Sewage sludge is irradiated with beta rays from an electron accelerator at dosages of at least 1.0 megarad at room temperature (ca. 20°C [68°F]).
Gamma Ray Irradiation	Sewage sludge is irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137, at dosages of at least 1 .0 megarad at room temperature (ca. 20°C [68°F]).
Pasteurization	The temperature of the sewage sludge is maintained at 70°C (158°F) or higher for 30 minutes or longer.

[1] Chapter 7 provides a detailed description of these technologies.

scribes the minimum frequency frequency at which the sewage sludge must be sampled and analyzed for pathogens or vector attraction reduction in order to meet regulatory requirements. In addition to meeting these minimal requirements, the EPA recommends that sewage sludge generators and preparers also consider the potential public health impact pathways and possible liability issues when designing a sampling program. In some cases, it may be appropriate to sample more frequently than the required minimum.

Classification of biosolids as Class A or Class B is based on the most recent test results available. For example, if a facility produces a Class A compost, and sampling is performed once each quarter, the compost produced after each test result verifying Class A is returned is also assumed to be Class A, assuming that the same process continues to be followed. If a test result indicates that compost is not achieving Class A, all compost subsequently generated would be classified as Class B (assuming it meets Class B requirements). The Class B classification would remain until a test result confirming Class A quality is returned.

This raises several issues. Land application of Class B biosolids without site restrictions is a violation of the 503 regulation. In addition, if material is mistakenly classified as EQ biosolids and land applied without restriction to the public, the biosolids preparer may be inadvertently creating a public health risk as well as opening the facility to liability. The key issues to consider are:

At what point between the two sampling events does the ***material change*** from ***C/ass A*** to ***C/ass B?*** This depends on the particular situation. The Class B test result may be an exception – the result of cross contamination or faulty sampling or monitoring for one pile. On the other hand, the test result could be indicative of an operation which is not adequately reducing pathogens. The piles which were actually sampled may have been used or distributed under the classification of the previous lab results while lab results were pending (it generally takes 2 weeks to get lab results back). Because distribution of this material as Class A would constitute a violation of the Part 503 regulation, it is recommended that material generated during and subsequent to a sampling event remain on site until lab results are available.

What can you do if you suspect Class B biosolids has been distributed as Class A biosolids? The first question to answer is: has this material created a public health risk. The material should be resampled to determine if it is indeed Class B and not Class A. The Part 503 requires that Class A biosolids meet either the fecal coliform or the Salmonella sp. requirements (except for Alternatives 3 and 4). If the material is out of compliance for fecal coliforms, it should immediately be tested for Salmonella sp. (and vice versa). In addition, the validity of the test results should be checked by contacting the lab and reviewing the data.

Material distribution should then be tracked to determine where material has been used. Businesses and individuals to whom material has been distributed should be notified and informed of the potential quality issue. If material is stockpiled at distribution points such as at a soil blender or landscaper, the material should be retested for pathogen levels, and distribution be curtailed until the process is reviewed and acceptable results are achieved. The facility may even consider recalling the biosolids from the users.

If material has already been distributed to public access areas, including homes, gardens, parks, or other public areas, the biosolids preparer may consider testing the soil. If the testing indicates problems, corrective actions may be necessary.

How can a situation like this be avoided? There are several sampling practices that a facility should follow in order to avoid a situation like this.

First, sampling should take place close enough to the time of distribution so that results accurately reflect material quality.

If possible, material sampled and subsequently produced material should not be distributed until the results are available; there is usually a 2-week waiting period for lab results for fecal coliform or Salmonella sp. analysis.

More frequent sampling can help pinpoint when operational conditions change. This may allow more rapid correction of operations.

Stockpile biosolids in discrete batches and take multiple samples per sampling event. This will allow better identification of which piles may be out of compliance and will allow for the distribution of material that is identified as Class A.

References and Additional Resources

Barker, T.A. 1970. Pasteurization and sterilization of sludges. Proc. Biochem, August. p 44-45.

Farrell, J.B., J.E. Smith, Jr., S.W. Hathaway, and R.B. Dean. 1974. Lime stabilization of primary sludges. J. WPCF 46(1):113-122.

Farrell, J.B. 1993. Fecal pathogen control during cornposting. p 282-300 in "Science & Engineering of Composting: Design, Environmental, Microbiological, and Utilization Aspects." edit. H.A. J. Hoitink and H.M. Keenerg.

Feachem, R-G., DJ. Bradley, H. Garelick, and D.D. Mara. 1983. "Sanitation and Disease: Health Aspects of Excreta and Wastewater Management." Pub. for World Bank by J. Wiley and Sons, NY.

Foess, Gerald W., and Ronald B. Sieger. 1993. Pathogen/vector attraction reduction requirements of the sludge rules. Water/Engineering & Management, June. p 25

IRGRD (International Research Group on Refuse Disposal) 1968. Information Bulletin No. 2I-31. August 1964 - December 1967. p 3230 - 3340. Reprinted by US Dept. HEW, Bureau of Solid Waste Management (1969).

Lee, K.M., C.A. Brunner, J.B.Farrell, and A.E. Eralp. 1989. Destruction of enteric bacteria and viruses during two-phase digestion. J. WPCF 61(8): 1422-l 429.

Martin, J.H., Jr., H.E. Bostian, and G. Stern. 1990. Reduction of enteric microorganisms during aerobic sludge digestion. Wat. Res. 24(11):1377-l 385.

Schafer, P.L., J.B. Farrell, W.R. Uhte, and B. Rabinowitz. 1994. Pre-pasteurization, European and North American assessment and experience. p IO-39 to IO-50 in "The Management of Water and Wastewater Solids for the 21st Century: A Global Perspective." Conference Proceedings, June 19-22, 1992. Water Environment Federation.

U.S. Dept. of Health & Human Services. 1989. Grade A Pasteurized Milk Ordinance, 1989 Revision, Public Health Service/Food and Drug Administration Publication No. 229.

U.S. EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No: PB93-11069. Springfield, VA: National Technical Information Service.

Yanko, W.A. 1987. Occurrence of pathogens in distribution and marketing municipal sludges. Report No.: EPA/600/1-87/014. (NTIS: PB88-154273/AS.) Springfield, VA: National Technical Information Service.

Chapter 5
Class B Pathogen Requirements and Requirements for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site

5.1 Introduction

Class B pathogen requirements can be met in three different ways. The implicit objective of all three alternatives is to ensure that pathogenic bacteria and enteric viruses are reduced in density, as demonstrated by a fecal coliform density in the treated sewage sludge (biosolids) of 2 million MPN or CFU per gram total solids biosolids (dry weight basis)'. Viable helminth ova are not necessarily reduced in a Class B biosolids.

Unlike Class A biosolids, which are essentially pathogen free, Class B biosolids may contain some pathogens. Site restrictions that restrict crop harvesting, animal grazing, and public access for a certain period of time are required. This allows environmental factors to further reduce pathogens. Where appropriate, these restrictions are designed to ensure sufficient reduction in viable helminth ova, one of the hardiest of pathogens, since these pathogens may not have been reduced during sewage sludge treatment.

The Class B requirements apply to bulk biosolids that are land applied to such areas as agricultural land, forests, public contact sites, or reclamation sites. Biosolids that are placed on a surface disposal site also must meet the Class B pathogen requirements, unless the active biosolids unit on which the biosolids are placed is covered at the end of each operating day (see Table 3-l). Because the use of Class B biosolids must be closely monitored, Class B biosolids cannot be given away or sold in bags or other containers.

Domestic septage applied to agricultural land, forest, or a reclamation site must meet all of the Class B site restrictions under 503.32(b)(5) unless the domestic septage has met specific pH requirements (see Section 5.6).

Class B biosolids and domestic septage also must mee? one of the vector attraction reduction requirements (see Chapter 8). Note that the choice of vector attraction options may affect the duration of site restrictions in some cases. Specifically, if Option 9 or 10 (injection or incorporation) is used to reduce vector attraction, the restriction on harvesting for food crops grown below the soil surface (potatoes, carrots, etc.) is increased from 20 months to 38 months.

Sections 5.2 to 5.4 discuss the three alternative Class B pathogen requirements for sewage sludge. Section 5.5 discusses the site restrictions for land applied Class B biosolids, and Section 5.6 presents the requirements for domestic septage applied to agricultural land, forests, or reclamation sites. The title of each section provides the number of the Subpart D requirement discussed in the section. A copy of Subpart D can be found in Appendix B. Chapters 9 and 10 provide guidance on the sampling and analysis necessary to meet the Class B microbiological requirements.

5.2 Sewage Sludge Alternative 1: Monitoring of Fecal Coliform [503.32(b)(2)]

Alternative 1 requires that seven samples of treated sewage sludge (biosolids) be collected and that the geometric mean fecal coliform density of these samples be less than 2 million CFU or MPN per gram of biosolids (dry weight basis). This approach uses fecal coliform density as an indicator of the average density of bacterial and viral pathogens. Over the long term, fecal coliform density is expected to correlate with bacterial and viral pathogen density in biosolids treated by biological treatment processes (EPA, 1992).

Use of at least seven samples is expected to reduce the standard error to a reasonable value. The standard deviation can be a useful predictive tool. A relatively high standard deviation for the fecal coliform density indicates a wide range in the densities of the individual samples. This may be due to sampling variability or variability in the laboratory analysis, or it may indicate that the treatment process is not consistent in its reduction of pathogens. A high standard deviation can therefore alert the preparer that the sampling, analysis, and treatment processes should be reviewed.

'Farrell et al. (1985) have shown that if a processed sewage sludge is processed by aerobic or anaerobic digestion it has a fecal coliform density of 2 million MPN or CFU per gram, enteric viruses and bacteria are significantly reduced. A comparison of suspended solids densities in entering wastewater to suspended solids densities in treated sewage sludge shows that this density of fecal coliform in treated sewage sludge represents a 1 OO-fold (Z-log) reduction in fecal coliform density, and is expected to correlatewith an approximately 1.5 log (approximately 32-fold) reduction in Salmonella sp. density and an approximately 1.3 log (20-fold) reduction in the density of enteric viruses.

Each of the multiple samples taken for fecal coliform analysis should be taken at the same point in the process so that treatment of each sample has been equal. Samples must be handled correctly and analyzed within 24 hours in order to minimize the effect of the holding time of the sample on the microbial population.

Laboratory sampling should follow Standard Methods as outlined in the Appendix of this document. Standard QA/QC practices, including duplicates to verify laboratory protocols should be followed. Generally a log standard deviation between duplicate samples under 0.3 is acceptable for lab analyses.

Calculating the Geometric Mean for Class B Alternative 1

- Take seven samples over a 2-week period.
- Analyze samples for fecal coliform using the membrane filter or MPN dilution method.
- Take the log (Base 10) of each result.
- Take the average (arithmetic) of the logs.
- Take the anti-log of the arithmetic average. This is ttie geometric mean of the results.

Example: The results of analysis of seven samples of sewage sludge are shown below. The second column of the table shows the log of each result.

	Fecal Coliform (MPN/dry gram sewage sludge)	Log
Sample 1	6.4×10^6	6.81
Sample 2	4.8×10^4	4.68
Sample 3	6.0×10^5	5.78
Sample 4	5.7×10^5	5.76
Sample 5	5.8×10^5	5.76
Sample 6	4.4×10^6	6.64
Sample 7	6.2 x 10'	7.80
Average (Arithmetic)		6.18
Antilog (geometric mean)		1.5×10^6
Log standard deviation		1.00*

Note that this sewage sludge would meet Class B fecal coliform requirements even though several of the analysis results exceed the 2.0×10^6/dry gram limit.

* Duplicate analyses on the same sample would give a much lower standard deviation. Variability is inflated by differences in feed and product over a 2-week sampling period.

Process parameters including retention time and temperature should be examined in order to verify that the process is running as specified. Monitoring equipment should be calibrated regularly.

The seven samples should be taken over a 2-week period in order to represent the performance of the facility under a range of conditions. For small facilities that are required to sample infrequently, sampling should be performed under worst case conditions, for example, during the winter when the climatic conditions are the most adverse.

It has been found that* for *Class B compliance, the MPN dilution method for fecal coliform analysis is more appropriate than the membrane filtration test. This is because colloidal and suspended solids may interfere with media transport through the membrane filter. Furthermore, concentration of toxic or inhibitory substances at the filter surface may affect results. It is therefore recommended that the membrane filter procedure be used *only after demonstrating comparability between the membrane filter test and the MPN method for a given sewage sludge.*

Example of Meeting Class B Pathogen and Vector Attraction Reduction Requirements

Type of Facility	Extended Aeration
Class	B
Pathogen Reduction Testing	Quarterly testing for pollutants and for fecal coliform to determine if Class B, Alternative 1 requirements are met.
Vector Attraction Reduction	The SOUR test is used to demonstrate compliance with VAR Option 4.
Use' or Disposal	The Class B biosolids are delivered to farmers along with information regarding analysis and site restrictions.

5.3 Sewage Sludge Alternative 2: Use of a Process to Significantly Reduce Pathogens (PSRP) [503.32(b)(3)]

The PSRP Class B alternative provides continuity with the 40 CFR Part 257 regulation. Under this Alternative, treated sewage sludge (biosolids) is considered to be Class B if it is treated in one of the "Processes to Significantly Reduce Pathogens" (PSRPs) listed in Appendix B of Part 503. The biological PSRP processes are sewage sludge treatment processes that have been demonstrated to result in a 2-log reduction in fecal coliform density. See chapter 7.

The PSRPs in the Part 503 are reproduced in Table 5-l and described in detail in Chapter 6. They are similar to the PSRPs listed in the Part 257 regulation, except that all conditions related to reduction of vector attraction have been removed. Under this alternative, sewage sludge treated by processes that are PSRPs under 40 CFR Part 257 are Class B with respect to pathogens. Unlike the comparable Class A requirement (see Section 4.8), this Class B alternative does not require microbiological monitoring.

However, monitoring of process requirements such as time, temperature, and pH is required.

Table 5-1. Processes to Significantly Reduce Pathogens (PSRPs) Listed in Appendix B of 40 CFR Part 503

1. Aerobic Digestion	Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C (68°F) and 60 days at 15°C (59°F).
2. Air Drying	Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C (32°F).
3. Anaerobic Digestion	Sewage sludge is treated in the absence of air for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35°C to 55°C (131 °F) and 60 days at 20°C (68°F).
4. Composting	Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 days. For 4 hours during the 5day period, the temperature in the compost pile exceeds 55°C (131°F).
5. Lime Stabilization	Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 for $2 hours of contact.

5.4 Sewage Sludge Alternative 3: Use of Processes Equivalent to PSRP [503.32(b)(4)]

The Part 257 regulation allowed the sewage sludge to be treated by a process determined to be equivalent to a PSRP. Under Class B Alternative 3, sewage sludge treated by any process determined to be equivalent to a PSRP is considered to be Class B biosolids. A list of processes that have been recommended as equivalent to PSRP are shown in Table 11.1.

Part 503 gives the regulatory authority responsibility for determining equivalency. The Pathogen Equivalency Committee is available as a resource to provide guidance and recommendations on equivalency determinations to the regulatory authorities (see Chapter 11).

5.5 Site Restrictions for Land Application of Biosolids [503.32(b)(5)]

Potential exposure to pathogens in Class B biosolids via food crops is a function of three factors: first there must be pathogens in the biosolids; second, the application of Class B biosolids to food crops must transfer the pathogens to the harvested crop, and third, the crop must be ingested before it is processed to reduce the pathogens. Elimination of one of these steps eliminates the pathway by which public's health may be affected. The use of Class A biosolids protects public health by reducing pathogens in sewage sludge to below detectable levels. Biosolids that meet the Class B requirements may contain reduced but still significant densities of pathogenic bacteria, viruses, protozoans, and viable helminth ova. Thus, site restrictions are to allow time for further reduction in the pathogen population. Harvest restrictions are imposed in order to reduce the possibility that food will be harvested and ingested before pathogens which may be present on the food have died off. Harvest restrictions vary, depending on the type of crop, because the amount of contact a crop will have with biosolids or pathogens in biosolids varies.

The site restrictions are primarily based in the survival rates of viable helminth ova, one of the hardiest of pathogens that may be present on sewage sludge. The survival of pathogens, including the helminth ova, depends on exposure to the environment. Some of the factors that affect pathogen survival include pH, temperature, moisture, cations, sunlight, presence of soil microflora, and organic material content. On the soil surface, helminth ova has been found to die off within 4 months, but survival is longer if pathogens are within the soil. Helminth ova have been found to survive in soil for several years (Smith, 1997; Kowal 1985). Site restrictions take this into account by making a distinction between biosolids that are applied to the land surface, biosolids that are incorporated into the soil after at least 4 months on the soil surface, and biosolids that are incorporated into the soil within 4 months of being applied.

Site restrictions also take the potential pathways of exposure into account. For example, crops that do not contact the soil, such as oat or wheat, may be exposed to biosolids, but pathogens on crop surfaces have been found to be reduced very quickly (30 days) due to exposure to sunlight, desiccation, and other environmental factors. Crops that touch the soil, such as melons or cucumbers, may also come into contact with biosolids particles, but pathogens in this scenario are also subject to the harsh effects of sunlight and rain and will die off quickly. Crops grown in soil such as potatoes are surrounded by biosolids amended soil, and pathogen die-off is much slower below the soil surface.

These pathways should be considered when determining which site restriction is appropriate for a given situation. The actual farming and harvesting practices as well as the intended use of the food crop should also be considered. For example, oranges are generally considered a food crop that does not touch the ground. However, some oranges grow very low to the ground and may come into contact with soil. If the oranges that have fallen to the ground or grew touching the ground are harvested for direct consumption without processing, the 14-month harvest restriction for crops that touch the soil should be followed. Orange crops which do not touch the ground at all would not fall under the 14-month harvest restriction; harvest would be restricted for 30 days under 503.32(b)(5)(iv)

which covers food crops that do not have harvested parts in contact with the soil. For similar situations, the potential for public health impacts must be considered. Harvest practices such as the use of fallen fruit or washing or processing crops should be written into permits so that restrictions and limits are completely clear. Figure 5-1 illustrates the steps of exposure that should be considered when making a decision about harvest and site restrictions. In addition, several examples of permit conditions are included. The site restrictions for land applied Class B biosolids are summarized below. The regulatory language is given in italics. Note that the restrictions apply only to the harvesting of food crops, but not to the planting or cultivation of crops.

Food Crops with Harvested Parts That Touch the Sewage Sludge/Soil Mixture

503.32(b)(5)(I): Food crops with harvested parts that touch the sewage sludge/soil mixture and are total/y above the land surface shall not be harvested for 14 months after application of sewage sludge.

This time frame is sufficient to enable environmental conditions such as sunlight, temperature, and desiccation to further reduce pathogens on the land surface. Note that the restriction applies only to harvesting. Food crops can be planted at any time before or after biosolids application, as long as they are not harvested within 14 months

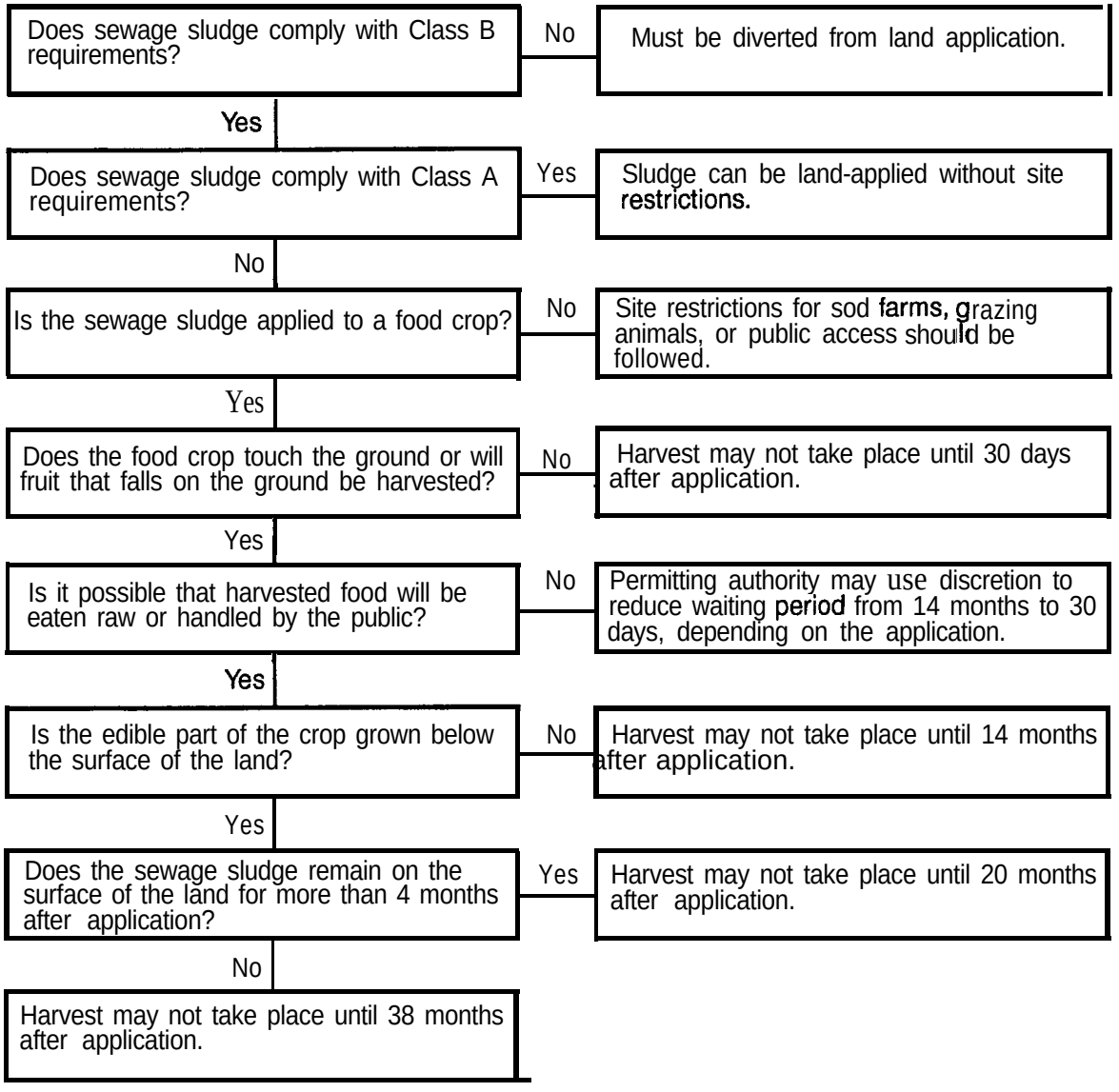


Figure 5-1. Decision tree for harvesting and site restrictions.

after sludge application. Examples of food crops grown on or above the soil surface with harvested parts that typically touch the sewage sludge/soil mixture include lettuce, cabbage, melons, strawberries, and herbs. Land application should be scheduled so that crop harvests are not lost due to harvest restrictions.

Food Crops with *Harvested Parts Below the Land Surface*

503.32(b)(5)(ii): Food crops with harvested parts below the surface of the land shall not be harvested for20 months after application of sewage sludge when the sewage sludge remains on the land surface for 4 months or longer prior to incorporation into the soil.

Pathogens on the soil surface will be exposed to environmental stresses which greatly reduce their populations. Helminth ova have been found to die off after 4 months on the soil surface (Kowal, 1994). Therefore, a distinction is made between biosolids left on the soil surface for 4 months and biosolids which are disced or plowed into soil more quickly.

For a September 1999 harvest, biosolids could be applied to the soil surface up to the end of December 1997, plowed or disced into the soil in April 1998, and the crop planted in order to allow it to be harvested in September 1999. Examples of crops with harvested parts below the land surface are potatoes, radishes, beets, onions and carrots.

Examples Of Site Restrictions for Questionable Food Crop Situations

Tree Nut Crops - Nuts which are washed, hulled, and dehydrated before being distributed for'public consumption must followthe 30-day restriction. Nuts which are harvested from the ground and sold in their shell without processing are subject to the 14-month restriction.

Sugar Beets - Sugar beets aren't expected to be. eaten raw. If the beets are transported off site and considerable biosolids amended soil is carried off with. them, the restrictions apply.: If biosolids are left on the 'soil surface for 4 months or longer before being incorporated, the 20-month restriction 'applies.' If biosolids are incorporated within 4 months of application, the 38-month restriction applies.

Tomatoes (and peppers) - Fruit often comes in contact with the ground. Tomatoes are sold both to processors and to farm stands. Tomatoes may be eaten raw by the public without further 'processing. The 14-month restriction applies.

503.32(b)(5)(iii): Food crops with harvested parts below the surface of the land shall not be harvested for38 months after application of sewage sludge when the sewage sludge remains on the land surface for less than 4 months prior to incorporation info the soil.

Exposure of the surface of root crops such as potatoes and carrots to viable helminth ova is a principal concern under these circumstances. Four months is considered the minimum time for environmental conditions to reduce viable helminth ova in biosolids on the land surface. Class B biosolids incorporated into the soil surface less than 4 months after application may contain significant numbers of viable helminth ova. Once incorporated into the soil, die-off of these organisms proceeds much more slowly; therefore, a substantially longer waiting period is required to protect public health. Thirty-eight months after biosolids application is usually sufficient to reduce helminth ova to below detectable levels.

Food Crops, Feed Crops, and Fiber Crops

503.32(b)(5)(iv): Food crops, feed crops, and fiber crops shall not be harvested for 30 days after application of sewage sludge.

This restriction covers food crops that are not covered by 503.32(b)(I-iii) This would include crops with harvested parts that do not typically touch the biosolids/soil mixture and which are not collected from the ground after they have fallen from trees or plants. The restriction also applies to all feed and fiber crops. These crops may be exposed to pathogens when biosolids are applied to the land. Harvesting of these crops could result in the transport of biosolids pathogens from the growing site to the outside environment. After 30 days, however, any pathogens in biosolids that may have adhered to the crop during application will likely have been reduced to non-detectable levels. Hay, corn, soybeans, or cotton are examples of a crop covered by this restriction.

Animal Grazing

503.32(b)(5)(v): Animals shall not be allowed to graze on the land for 30 days after application of sewage sludge.

Biosolids can adhere to animals that walk on biosolids amended land and thereby be brought into potential contact with humans who come in contact with the animals (for example, horses and milking cows allowed to graze on a biosolids amended pasture). Thirty days is sufficient to substantially reduce the pathogens in surface applied biosolids, thereby significantly reducing the risk of human and animal contamination.

Turf Harvesting

503.32(b)(5)(vi): Turf grown on land where sewage sludge is applied shall not be harvested for 1 year after application of the sewage sludge when the harvested turf is placed on either land with a high potential for public exposure or a lawn, unless otherwise specified by fhe permitting authority.

The l-year waiting period is designed to significantly reduce pathogens in the soil so that subsequent contact

of the turf layer will not pose a risk to public health and animals. A permitting authority may reduce this time period in cases in which the turf is not used on areas with high potential for public access.

Public Access

503.32(b)(5)(vii): Public access to land with a high potential for public exposure shall be restricted for 1 year after application of the sewage sludge.

As with the turf requirement above, a l-year waiting period is necessary to protect public health and the environment in a potential high-exposure situation. A baseball diamond, playground, public park, or a soccer field are examples of land with a high potential for public exposure. The land gets heavy use and contact with the soil is substantial (children or ball players fall on it and dust is raised which is inhaled and ingested).

503.32(b)(5)(viii): Public access to land with a low potential for public exposure shall be restricted for 30 days after application of the sewage sludge.

A farm field used to grow corn or soybeans is an example of land with low potential for public exposure. Even farm workers and family members walk about very little on such fields. Public access restrictions do not apply to farm workers, but workers should be aware of the public health implications of land application and the land application schedule, and should follow good hygiene practice during the 30-day period. For example, workers should be instructed to wash their hands after handling soil or crops that come into contact with soil. Protective clothing and footwear are recommended for workers who work on fields that have recently been applied with Class B biosolids. More safety recommendations for workers handling biosolids are included in Section 2.2.

5.6 Domestic Septage [503.32(c)]

Under Part 503.32(c), pathogen reduction in domestic septage applied to agricultural land, forest, or reclamation sites* may be reduced in one of two ways:

- Either all the Class B site restrictions under 503.32(b)(5) -see Section 5.5- must be met,
- Or the pH of the domestic septage must be raised to 12 or higher by alkali addition and maintained at pH 12 or higher for 30 minutes without adding more alkali, and the site restrictions on crop harvesting in 503.32(b)(5)(I-iv) must be met (see Section 5.5). The Part 503 regulation uses the term alkali in the broad sense to mean any substance that causes an increase in pH.

Vector attraction reduction can be met with Options 9, 10, or 13. Domestic septage can be incorporated or injected into the soil to prevent vector attraction, or the pH of the domestic septage can be adjusted as outlined in Option 12 (see Section 8). pH adjustment can fulfill both pathogen and vector attraction reduction.

The pH requirement applies to every container of domestic septage applied to the land, which means that the pH of each container must be monitored. The first alternative reduces exposure to pathogens in land applied domestic septage while environmental factors attenuate pathogens. The second alternative relies on alkali treatment to reduce pathogens and contains the added safeyuard of restricting crop harvesting, which prevents exposure to crops grown on domestic septage amended soils.

References and Additional Resources

Farrell, J.B., G. Stern, and A.D. Venosa. 1985. Microbial destructions achieved by full-scale anaerobic digestion. Workshop on Control of Sludge Pathogens, Series IV. Alexandria, VA: Water Pollution Control Federation.

Farrell, J.B., B.V. Salotto, and A.D. Venosa. 1990. Reduction in bacterial densities of wastewater solids by three secondary treatment processes. Res. Jour. WPCF 62(2): 177-l 84.

Gerba, C.P., C. Wallis, and J.L. Melmick. 1975. Fate of wastewater bacteria and viruses in soil. J. Irrig. Drain Div. Am. Soc. Civ. Engineers. 101 :157-1 74.

Kowal, N.E. 1985. Health effects of land application of municipal sludge. Pub. No.: EPA/600/1-85/01 5. Research Triangle Park, NC: U.S. EPA Health Effects Research Laboratory.

Kowal, N.E. 1994. Pathogen risk assessment: Status and potential application in the development of Round II regulations. Proceedings of the June 19-20, 1994 Speciality Conference. The Management of Water and Wastewater Solids for the 21 st Century: A Global Perspective. Water Environment Federation. Alexandria, VA.

Moore, B.E., D.E. Camann, G.A. Turk, and C.A. Sorber. 1988. Microbial characterization of municipal wastewater at a spray irrigation site: The Lubbock infection surveillance study. J. Water Pollut. Control Fed. 60(7): 1222-l 230.

Smith, J.E., Jr. 1988. Fate of pathogens during the sewage sludge treatment process and after land application. In Proceedings of the January 21-22, 1998 California Plant and Soil Conference: Agricultural challenges in an urbanizing state, Sacramento, CA.

Sobsey, M.D., and P.A. Shields. 1987. Survival and transport of viruses in soils: Model studies. Pp. 155-l 77 in V.C. Rao and J.L. Melnick, eds. Human viruses in sediments, sludges, and soils. Boca Raton, FL: CRC Press.

Sorber, C.A., and B.E. Moore. 1986. Survival and transport of pathogens in sludge-amended soil, a critical literature review. Report No.: EPA/600/2-87/028. Cincinnati, OH: Office of Research and Development.

Storey, G.W. and R.A. Phillips. 1985. The survival of parasite eggs throughout the soil profile. Parasitology. 91:585-590.

U.S. EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No.: PB93-11069. Springfield, VA: National Technical Information Service.

Chapter 6
Processes to Significantly Reduce Pathogens (PSRPs)

6.1 Introduction

Processes to Significantly Reduce Pathogens (PSRPs) are listed in Appendix B of Part 503. There are five PSRPs: aerobic and anaerobic digestion, air drying, composting, and lime stabilization. Under Part 503.32(b)(3), sewage sludge meeting the requirements of these processes is considered to be Class B with respect to pathogens (see Section 5.3). When operated under the conditions specified in Appendix B, PSRPs reduce fecal coliform densities to less than 2 million CFU or MPN per gram of total solids (dry weight basis) and reduce Salmonella sp. and enteric virus densities in sewage sludge by approximately a factor of 10 (Farrell, et al., 1985).

This level of pathogen reduction is required, as a minimum, by the Part 503 regulation if the sewage sludge is applied to agricultural land, a public contact site, a forest, or a reclamation site or placed on a surface disposal site[1]. Because Class B biosolids may contain some pathogens, land application of Class B biosolids is allowed only if crop harvesting, animal grazing, and public access are limited for specific periods of time following application of Class B biosolids so that pathogens can be further reduced by environmental factors (see Section 5.5).

The PSRPs listed in Part 503 are essentially identical to the PSRPs that were listed under the 40 CFR Part 257 regulation, except that all requirements related solely to reduction of vector attraction have been removed. Vector attraction reduction is now covered under separate requirements (see Chapter 8) that include some of the requirements that were part of the PSRP requirements under Part 257, as well as some new options for demonstrating vector attraction reduction. These new options provide greater flexibility to the regulated community in meeting the vector attraction reduction requirements.

Although theoretically two or more PSRP processes, each of which fails to meet its specified requirements, could be combined and effectively reduce pathogens (i.e. partial treatment in digestion followed by partial treatment by air drying) it cannot be assumed that the pathogen reduction contribution of each of the operations will result in the 2-log reduction in fecal coliform necessary to define the combination as a PSRP. Therefore, to comply with Class B pathogen requirements, one of the PSRP processes must be conducted as outlined in this chapter, or fecal coliform testing must be conducted in compliance with Class B Alternative 1. The biosolids preparer also has the option of applying for PSRP equivalency for the combination of processes. Achieving PSRP equivalency enables the preparer to stop monitoring for fecal coliform density.

This chapter provides detailed descriptions of the PSRPs listed in Appendix B. Since the conditions for the PSRPs, particularly aerobic and anaerobic digestion, are designed to meet pathogen reduction requirements, they are not necessarily the same conditions as those traditionally recommended by environmental engineering texts and manuals.

6.2 Aerobic Digestion

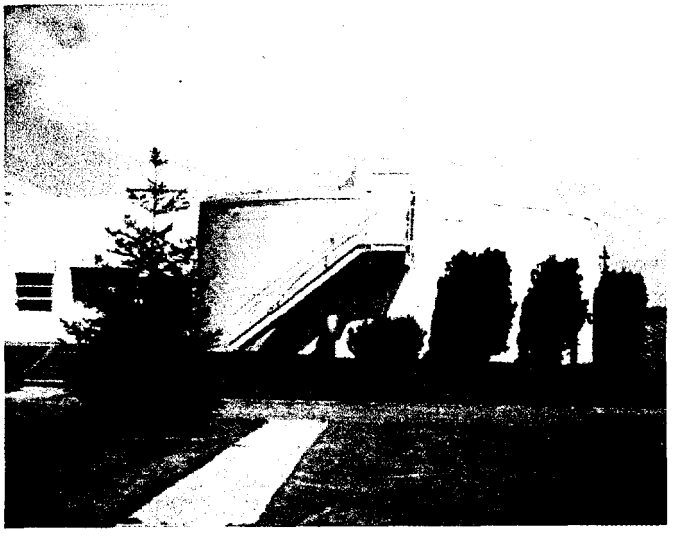
In aerobic digestion, sewage sludge is biochemically oxidized by bacteria in an open or enclosed vessel (see photo). To supply these aerobic microorganisms with enough oxygen, either the sewage sludge must be agitated by a mixer, or air must be forcibly injected (Figure 6-1). Under proper operating conditions, the volatile solids in sewage sludge are converted to carbon dioxide, water, and nitrate nitrogen.

Aerobic systems operate in either batch or continuous mode. In batch mode, the tank is filled with untreated sewage sludge and aerated for 2 to 3 weeks or longer, depending on the type of sewage sludge, ambient temperature, and average oxygen levels. Following aeration, the stabilized solids are allowed to settle and are then separated from the clarified supernatant. The process is begun again by inoculating a new batch of untreated sewage sludge with some of the solids from the previous batch to supply the necessary biological decomposers. In continuous mode, untreated sewage sludge is fed into the digester once a day or more frequently; thickened, clarified solids are removed at the same rate.

The PSRP description in Part 503 for aerobic digestion is:

- Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell resi-

[1]Unless the active biosolids surface disposal unit is covered al the end of each operating day, in which case no pathogen requirement applies.



Digester in Vancouver, Washington.

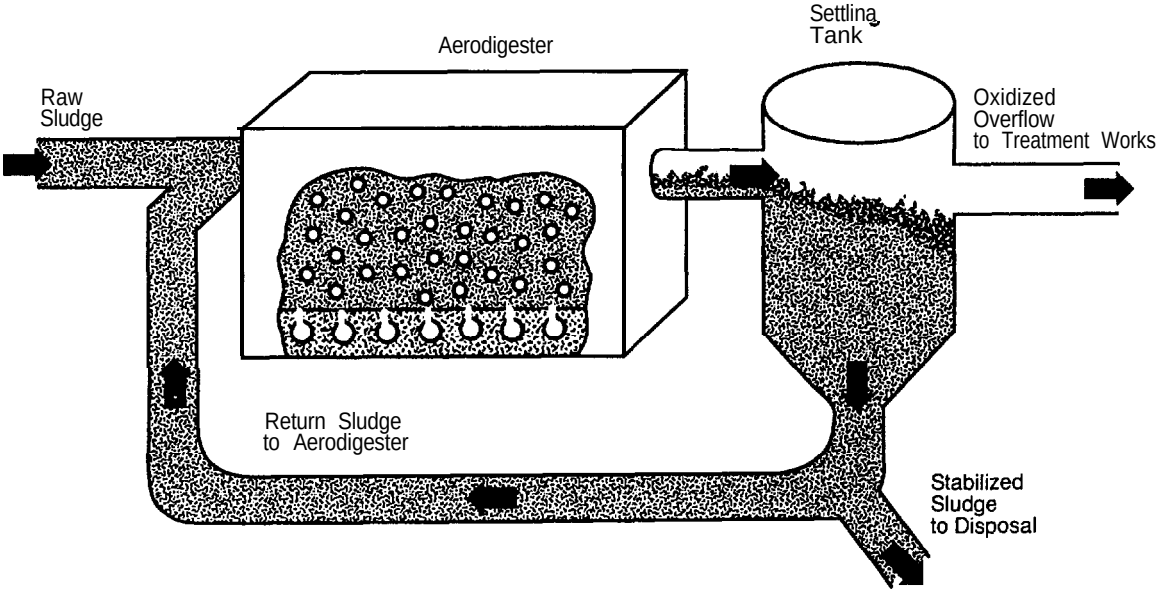


Figure 6-l. Aerobic digestion.

dence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C (68°F) and 60 days at 15°C (59°F).

For temperatures between 15°C (59°F) and 20°C (68°F) use the relationship between time and temperature provided below to determine the required mean cell residence time.

$$\frac{\text{Time @T°C}}{40\text{ d}} = 1.08\ (20\text{-T})$$

The regulation does not differentiate between batch, intermittently fed, and continuous operation, so any method is acceptable. The mean cell residence time is considered the residence time of the sewage sludge solids. The appropriate method for calculating residence time depends on the type of digester operation used (see Appendix E).

Continuous-Mode, No Supernatant Removal For continuous-mode digesters where no supernatant is removed, nominal residence times may be calculated by dividing liquid volume in the digester by the average daily flow rate in or out of the digester.

Continuous-Mode, Supernatant Removal In systems where the supernatant is removed from the digester and recycled, the output volume of sewage sludge can be much less than the input volume of sewage sludge. For these systems, the flow rate of the sewage sludge out of the digester is used to calculate residence times.

Continuous-Mode Feeding, Batch Removal of Sewage Sludge For some aerobic systems, the digester is initially filled above the diffusers with treated effluent, and sewage sludge is wasted daily into the digester. Periodically, aeration is stopped to allow solids to settle and supernatant to be removed. As the supernatant is drawn off, the solids content in the digester gradually increases. The process is complete when either settling or supernatant removal is inadequate to provide space for the daily sewage sludge wasting requirement, or sufficient time for digestion has been provided. The batch of digested sewage sludge is then removed and the process begun again. If the daily mass of sewage sludge solids introduced has been constant, nominal residence time is one-half the total time from initial charge to final withdrawal of the digested sewage sludge.

Batch or Staged Reactor Mode A batch reactor or two or more completely-mixed reactors in series are more effective in reducing pathogens than is a single well-mixed reactor at the same overall residence time. The residence time required for this type of system to meet pathogen reduction goals may be 30% lower than the residence time required in the PSRP definition for aerobic digestion (see Appendix E). However, since lower residence times would not comply with PSRP conditions required for aerobic digestion in the regulation, approval of the process as a PSRP by the permitting authority would be required.

Other Digesters are frequently operated in unique ways that do not fall into the categories above. Appendix E provides information that should be helpful in developing a calculation procedure for these cases. Aerobic digestion carried out according to the Part 503 requirements typically reduces bacterial organisms by 2-log and viral pathogens by l-log. Helminth ova are reduced to varying degrees, depending on the hardiness of the individual species. Aerobic digestion typically reduces the volatile solids content (the microbes' food source) of the sewage sludge by 40% to 50%, depending on the conditions maintained in the system.

Vector Attraction Reduction

Vector attraction reduction for aerobically digested sewage sludges is demonstrated either when the percent volatile solids reduction during sewage sludge treatment equals or exceeds 38%, or when the specific oxygen uptake rate (SOUR) at 20°C (68°F) is less than or equal to 1.5 mg of oxygen per hour per gram of total solids, or when additional volatile solids reduction during bench-scale aerobic batch digestion for 30 additional days at 20°C (68°F) is less than 15% (see Chapter 8).

Thermophilic aerobic systems (operating at higher temperatures) capable of producing Class A biosolids are described in Section 7.5.

6.3 Anaerobic Digestion

Anaerobic digestion is a biological process that uses bacteria that function in an oxygen-free environment to convert volatile solids into carbon dioxide, methane, and ammonia. These reactions take place in an enclosed tank (see Figure 6-2) that may or may not be heated. Because the biological activity consumes most of the volatile solids needed for further bacterial growth, microbial activity in the treated sewage sludge is limited. Currently, anaerobic digestion is one of the most widely used treatments for sewage sludge treatment, especially in treatment works with average wastewater flow rates greater than 19,000 cubic meters/day (5 million gallons per day).

Most anaerobic digestion systems are classified as either standard-rate or high-rate systems. Standard-rate systems take place in a simple storage tank with sewage sludge added intermittently. The only agitation that occurs comes from the natural mixing caused by sewage sludge gases rising to the surface. Standard-rate operation can be carried out at ambient temperature, though heat is sometimes added to speed the biological activity.

High-rate systems use a combination of active mixing and carefully controlled, elevated temperature to increase the rate of volatile solids destruction. These systems sometimes use pre-thickened sewage sludge introduced at a uniform rate to maintain constant conditions in the reactor. Operating conditions in high-rate systems foster more efficient sewage sludge digestion.

The PSRP description in Part 503 for anaerobic digestion is:

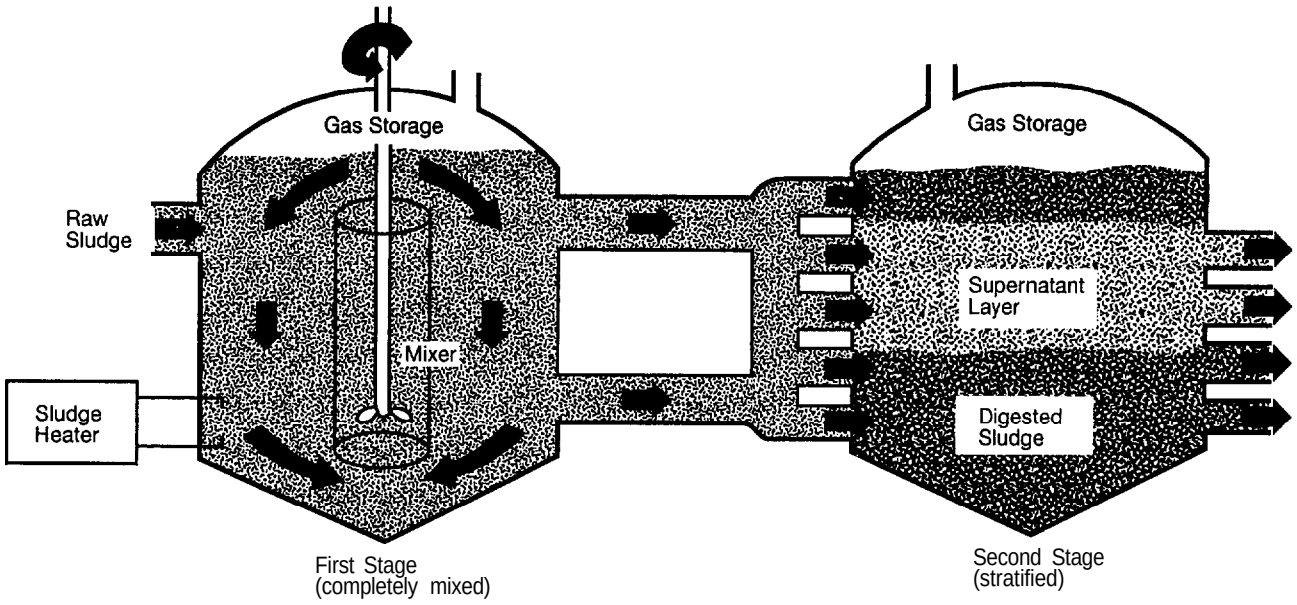


Figure 6-2. Two-stage anaerobic digestion (high rate).

- Sewage sludge is treated in the absence of air for a specific mean cell residence time at a specified temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35°C to 55°C (95°F to 131 °F) and 60 days at 20°C(68°F).

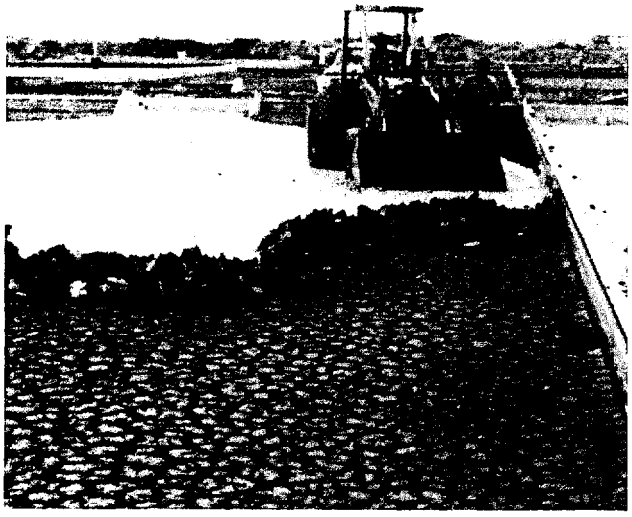
Straight-line interoolation to calculate mean cell residence time is allowable when the temperature falls between 35°C and 20°C.

Section 6.2 provides information on calculating residence times. Anaerobic digestion that meets the required residence times and temperatures typically reduces bacterial and viral pathogens by 90% or more. Viable helminth ova are not substantially reduced under mesophilic conditions (32°C to 38°C [90°F to 1 00°F]) and may not be completely reduced at temperatures between 38°C (100°F) and 50°C (122°F).

Anaerobic systems reduce volatile solids by 35% to 60%, depending on the nature of the sewage sludge and the system's operating conditions. Sewage sludges produced by systems that meet the operating conditions specified under Part 503 will typically have volatile solids reduced by at least 38%, which satisfies vector attraction reduction requirements. Alternatively, vector attraction reduction can be demonstrated by Option 2 of the vector attraction reduction requirements, which requires that additional volatile solids loss during bench-scale anaerobic batch digestion of the sewage sludge for 40 additional days at 30°C to 37°C (86°F to 99°F) be less than 17% (see Section 8.3). The SOUR test is an aerobic test and cannot be used for anaerobically digested sewage sludge.

6.4 Air Drying

Air drying allows partially digested sewage sludge to dry naturally in the open air (see photo). Wet sewage sludge is usually applied to a depth of approximately 23 cm (9 inches) onto sand drying beds, or even deeper on paved or unpaved basins. The sewage sludge is left to drain and dry by evaporation. Sand beds have an underlying drainage system; some type of mechanical mixing or turning is frequently added to paved or unpaved basins. The effectiveness of the air drying process depends very much on the local climate: drying occurs faster and more completely in warm, dry weather, and slower and less completely in cold, wet weather. During the drying/storage period in the bed, the sewage sludge is undergoing physical, chemical, and biological changes. These include biological decomposition of organic material, ammonia production, and desiccation.



Sludge drying operation. (Photo credit: East Bay Municipal Utility District)

The PSRP description in Part 503 for air drying is:

- Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C (32°F).

Although not required by the Part 503, it is advisable to ensure that the sewage sludge drying beds are exposed to the atmosphere (i.e., not covered with snow) during the 2 months that the daily temperature is above 0°C (32°F). Also, the sewage sludge should be at least partially digested before air drying. Under these conditions, air drying will reduce the density of pathogenic viruses by l-log and bacteria by approximately 2-log. Viable helminth ova also are reduced, except for some hardy species that remain substantially unaffected.

Vector Attraction Reduction

Frequently sand-bed drying follows an aerobic or anaerobic digestion process that does not meet the specified process requirements and does not produce 38% volatile solids destruction. However, it may be that the volatile solids reduction produced by the sequential steps of digestion and drying will meet the vector attraction reduction requirement of 38% volatile solids reduction. If this is the case, vector attraction reduction requirements are satisfied.

Example of Meeting PSRP and Vector Attraction Reduction Requirements

Type of Facility	Air Drying
Class	B
Pathogen Reduction	Partially digested sewage sludge is thickened and spread in drying beds. Filling of beds starts in June, and the beds accommodate sewage sludge generated over 1 full year. Beds are then emptied the following September so that all sewage sludge is retained over an entire summer (> 0°C ambient temperatures).
Testing	Sewage sludge is tested for pollutants 2 weeks before material is removed and distributed.
Vector Attraction Reduction	Biosolids are land applied and plowed immediately into the soil.
Use or Disposal	Biosolids are delivered to local farmers. Farmers are given information on site restrictions, and must follow harvest, grazing, and public access restrictions.

Vector Attraction Reduction

Air-dried sewage sludge typically is treated by aerobic or anaerobic digestion before it is placed on drying beds. Usually, the easiest vector attraction reduction requirement to meet is a demonstration of 38% reduction in volatile solids (Option 1, See Section 8.2), including the reduction that occurs during its residence on the drying beds.

In dry climates, vector attraction reduction can be achieved by moisture reduction (see Option 7 in Section 8.8, and Option 8 in Section 8.9).

6.5 Cornposting

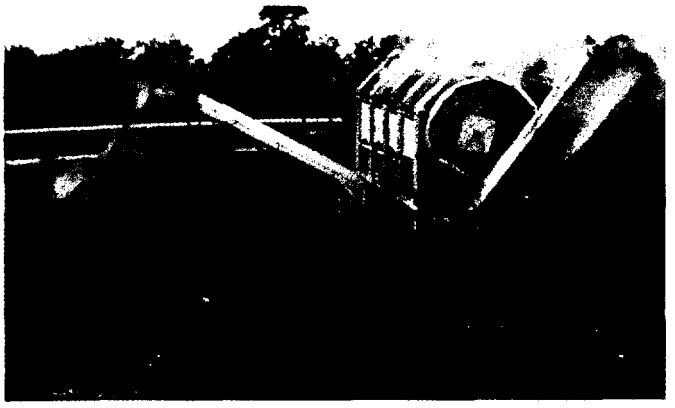
Composting involves the aerobic decomposition of organic material using controlled temperature, moisture, and oxygen levels. Several different composting methods are currently in use in the United States. The three most common are **windrow,** aerated static pile, and within-vessel composing, are described below.

Composting can yield either Class A or Class B biosolids, depending on the time and temperature variables involved in the operation.

All composting methods rely on the same basic processes. Bulking agents such as wood chips, bark, sawdust, straw, rice hulls, or even-finished compost are added to the sewage sludge to absorb moisture, increase porosity, and add a source of carbon. This mixture is stored (in windrows, static piles, or enclosed tanks) for a period of intensive decomposition, during which temperatures can rise well above 55°C (131 °F). Depending on ambient temperatures and the process chosen, the time required to reduce pathogens and produce Class B biosolids can range from 3 to 4 weeks. Aeration and/or frequent mixing or turning are needed to supply oxygen and remove excess heat. Following this active stage, bulking agents may or may not be screened from the completed compost for recycling (see photo), and the composted biosolids are "cured" for an additional period.

Windrow composting involves stacking the sewage sludge/bulking agent mixture into long piles, or windrows, generally 1.5 to 2.7 meters high (5 to 9 feet) and 2.7 to 6.1 meters wide (9 to 20 feet). These rows are regularly turned or mixed with a turning machine or front-end loader to fluff up the material and increase porosity which allows better convective oxygen flow into the material. Turning also breaks up compacted material and reduces the moisture content of the composting media (see photo, next page). Active **windrows** are typically placed in the open air, except in areas with heavy rainfall. In colder climates, winter weather can significantly increase the amount of time needed to attain temperatures needed for pathogen reduction.

Aerated static pile composting uses forced-air rather than mechanical mixing (see Figure 6-3) to both supply sufficient oxygen for decomposition and carry off moisture. The sewage sludge/bulking agent mixture is placed on top of



Composted sludge is screened to remove the bulking agent prior to land application.

Compost mixing equipment turns over a windrow of compost for solar drying prior to screening. [Photo credit: East Bay Municipal Utility District)

either (1) a fixed underlying forced aeration system, or (2) a system of perforated piping laid on the composting pad surface and topped with a bed of bulking agent. The entire pile is covered with a layer of cured compost for insulation and odor control. Pumps are used to blow air into the compost pile or suck air through it. The latter provides greater odor control because the compost air can be easily collected and then filtered or scrubbed.

Within-vessel composting systems vary greatly in design, but they share two basic techniques: the process takes place in a reactor vessel where the operating conditions can be carefully controlled (see photo page 49), and active aeration meets the system's high oxygen demand. Agitated bed systems (one type of within-vessel composting) depend on continuous or periodic mixing within the vessel, followed by a curing period

Pathogen reduction during composting depends on time and temperature variables (see photo page 49). Part 503 provides the following definition of PSRP requirement for pathogen reduction during composting:

- Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 days. For 4 hours during the 5-day period, the temperature in the compost pile exceeds 55°C (131 °F).

These conditions, achieved using either within-vessel, aerated static pile, or windrow methods, reduce bacterial pathogens by 2-log and viral pathogens by l-log.

A process time of only 5 days is not long enough to fully break down the volatile solids in sewage sludge, so the composted sewage sludge produced under these conditions will not be able to meet any of the requirements for reduced vector attraction. In addition, sewage sludge that has been composted for only 5 days may still be odorous. Breakdown of volatile solids may require 14 to 21 days for within-vessel; 21 or more days for aerated static pile; and 30 or more days for windrow composting. Many treatment works allow the finished sewage sludge compost to further mature or cure for at least several weeks following active composting during which time pile turning or active aeration may continue.

Composting is most often used to meet Class A requirements. More guidance for composting operations and how to meet Class A time and temperature requirements is provided in Chapter 7.

Vector Attraction Reduction

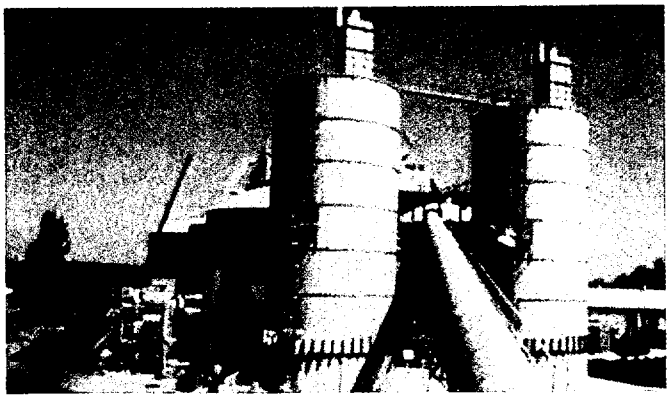
Vector attraction reduction must be conducted in accordance with Option 5, or compost must be incorporated into soil when land applied. This option requires aerobic treatment (i.e., cornposting) of the sewage sludge for at least 14 days at over 40°C (104°F) with an average temperature of over 45°C (113°F).

6.6 Lime Stabilization

The lime stabilization process is relatively straightforward: lime — either hydrated lime, $Ca(OH)_2$; quicklime, CaO; or lime containing kiln dust or fly ash — is added to sewage sludge in sufficient quantities to raise the pH above 12 for 2 hours or more after contact, as specified in the Part 503 PSRP description for lime stabilization:

- Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 after 2 hours of contact.

For the Class B lime stabilization process, the alkaline material must be a form of lime. Use of other alkaline ma-



Taulman Weiss in-vessel compostlng facility in Portland, Oregon.

Compost operator measures compost pile temperature as part of process monltorlng. (Photo credit: East **Bay** Municipal Utioity District, Oakland, California)

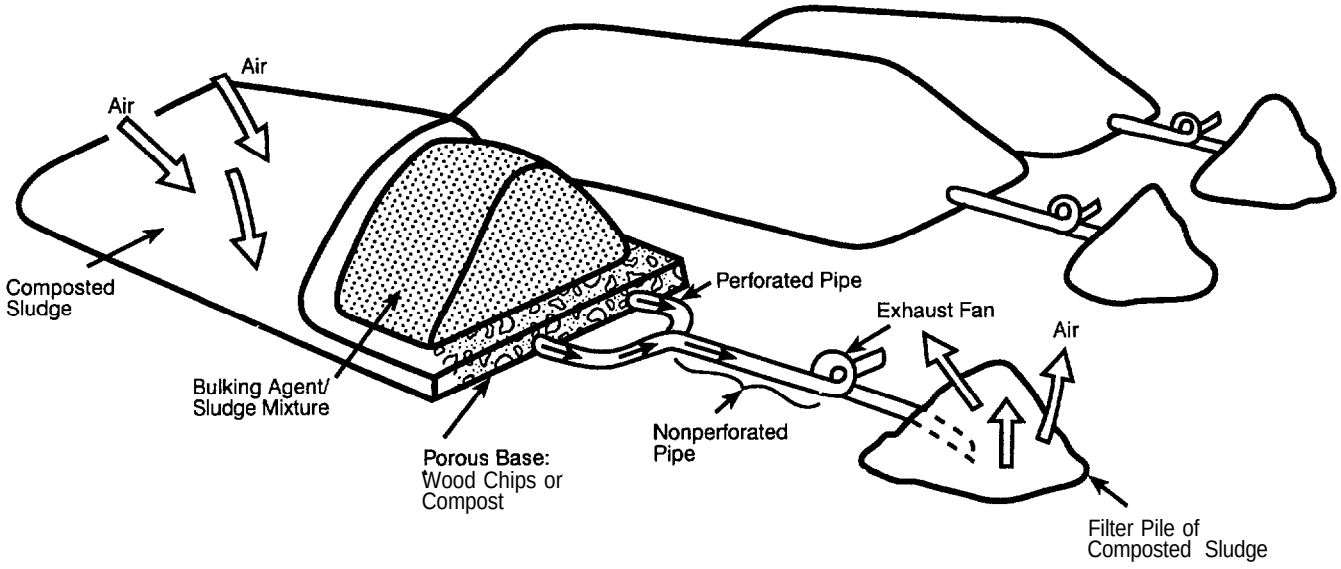


Figure 6-3. Static aerated pile composting.

terials must first be demonstrated to be equivalent to a PSRP. Elevation of pH to 12 for 2 hours is expected to reduce bacterial and viral density effectively.

Lime may be introduced to liquid sewage sludge in a mixing tank or combined with dewatered sewage sludge, providing the mixing is complete and the sewage sludge cake is moist enough to allow aqueous contact between the sewage sludge and lime.

Mixing must be sufficient to ensure that the entire mass of sewage sludge comes into contact with the lime and undergoes the increase in pH and to ensure that samples are representative of the overall mixture (see Chapter 9). pH should be measured at several locations to ensure that the pH is raised throughout the sewage sludge.

A variety of lime stabilization processes are currently in use. The effectiveness of any lime stabilization process for controlling pathogens depends on maintaining the pH at levels that reduce microorganisms in the sewage sludge. Field experience has shown that the application of lime stablized material after the pH has dropped below 10.5 may, in some cases, create odor problems. Therefore it is

recommended that biosolids application take place while the pH remains elevated. If this is not possible, and odor problems develop, alternate management practices in the field include injection or incorporation or top dressing the applied biosolids with additional lime. Alternate management practices if the biosolids have not yet left the wastewater treatment plant may include adding additional lime to maintain the elevated pH or additional treatment through drying or cornposting. Lime stabilization can reduce bacterial and viral pathogens by 99% or more. Such alkaline conditions have little effect on hardy species of helminth ova, however.

Vector Attraction Reduction

For lime-treated sewage sludge, vector attraction reduction is best demonstrated by Option 6 of the vector attraction reduction requirements. This option requires that the sewage sludge pH remain at 12 or higher for at least 2 hours, and then at 11.5 or more for an additional 22 hours (see Section 8.7).

Lime stabilization does not reduce volatile solids. Field experience has shown that the application of lime stabilized material after the pH has dropped below 10.5 may create odor problems. Therefore it is recommended that land application of biosolids take place as soon as possible after vector attraction reduction is completed and while pH remains elevated.

6.7 Equivalent Processes

Table 11 .1 in Chapter 11 lists some of the processes that the EPA's Pathogen Equivalency Committee has recommended as being equivalent to PSRP under Part 257. Information on the PEC and how to apply for equivalency are discussed in Chapter 11.

References and Other Resources

Berg G. and D. Berman. 1980. Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. Appl. Envir. Microbiol. 39 (2):361-368.

Farrell, J.B., G. Stern, and A.D. Venosa. 1990. Microbial destructions achieved by full-scale anaerobic digestion. Paper presented at Municipal Wastewater Sludge Disinfection Workshop. Kansas City, MO. Water Pollution Control Federation, October 1995.

U.S. EPA. 1992. Technical support document for reduction of pathogens and vector attraction in sewage sludge. EPA 822/R-93-004.

Chapter 7
Processes to Further Reduce Pathogens (PFRPs)

7.1 Introduction

Processes to Further Reduce Pathogens (PFRPs) are listed in Appendix B of the Part 503. There are seven PFRPs: composting, heat drying, heat treatment, thermophilic aerobic digestion, beta ray irradiation, gamma ray irradiation, and pasteurization. When these processes are operated under the conditions specified in Appendix B, pathogenic bacteria, enteric viruses, and viable helminth ova are reduced to below detectable levels. The PFRPs listed in Part 503 are essentially identical to the PFRPs listed under the 40 CFR Part 257 regulation, except that all requirements related solely to reduction of vector attraction have been removed.

This chapter provides detailed descriptions of the seven PFRPs listed in Part 503. Because the purpose of these processes is to produce Class A biosolids, the pathogen reduction process must be conducted concurrent to or prior to the vector attraction reduction process (see Section 4.2).

Under Part 503.32(a)(7), sewage sludge treated in these processes is considered to be Class A with respect to helminth ova, enteric viruses, and pathogenic bacteria. In addition, Class A biosolids must be monitored for fecal coliform or *Salmonella* sp. bacteria at the time of use or disposal, at the time the biosolids are prepared for sale or give away in a bag or other container for land application, or at the time the biosolids are prepared to meet the requirements for "exceptional quality" sludge (see Chapter 2) in 503.10(b), 503.10(c), 503.10(e), or 503.10(f) to ensure that growth of bacteria has not occurred (see Section 4.3). Guidelines regarding the frequency of pathogen sampling and sampling protocols are included in Chapter 9.

7.2 Cornposting

Composting is the controlled, aerobic decomposition of organic matter which produces a humus-like material. Sewage sludge which is to be composted is generally mixed with a bulking agent such as wood chips which increases porosity in the sewage sludge, allowing air to more easily pass through the composting material and maintain aerobic conditions. There are three commonly used methods of cornposting: windrow, static aerated pile, and within-vessel.

To be considered a PFRP under Part 503, the composting operation must meet certain operating conditions:

- Using either the within-vessel composting method or the static aerated pile composting method, the temperature of the sewage sludge is maintained at 55°C (131 °F) or higher for 3 consecutive days.
- Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 consecutive days or longer. During the period when the compost is maintained at 55°C (131°F) or higher, there shall be a minimum of five turnings of the windrow.

For aerated static pile and in-vessel composting processes, temperatures should be taken at multiple points at a range of depths throughout the composting medium. Points which are likely to be slightly cooler than the center of the pile, such as the toes of piles, also should be monitored. Because the entire mass of sewage sludge must attain the required temperatures for the required duration, the temperature profiles from every monitoring point, not just the average of the points, should reflect PFRP conditions.

It has been found that points within 0.3 m (1 foot) of the surface of aerated static piles may be unable to reach PFRP temperatures, and for this reason, it is recommended that a 0.3 m (1 foot) or greater layer of insulating material be placed over all surfaces of the pile. Finished compost is often used for insulation. It must be noted that because the insulation will most likely be mixed into the composted material during post-processing or curing, compost used as an insulation material must be a Class A material so as not to reintroduce pathogens into the composting sewage sludge.

For windrow composting, the operational requirements are based on the same time-temperature relationship as aerated static pile and in-vessel composting. The material in the core of the windrow attains at least 55°C and must remain at that temperature for 3 consecutive days. Windrow turning moves new material from the surface of the windrow into the core so that this material may also undergo pathogen reduction. After five turnings, all material in the windrow must have spent 3 days at the core of the pile. The time-temperature regime takes place over a period of at least 15 consecutive days during which time the temperature in the core of the windrow is at least 55*o* C. See Appendix J for additional guidance.

Pathogen reduction is a function of three parameters:

- Ensuring that all sewage sludge is mixed into the core of the pile at some point during active cornposting
- Ensuring that all sewage sludge particles spend 3 consecutive days in the core during which time the temperatures are at 55°C
- Preventing growth of pathogenic bacteria in composted material

The first issue, ensuring that all material is mixed into the core of the pile, depends on the configuration of the windrows and the turning methodology. Pile size and shape as well as material characteristics determine how much of the pile is in the "hot zone" at any given time. Additional turning and maintenance of temperatures after the mandated 15 days are recommended, depending on the windrow configuration. For example, the Los Angeles County Sanitation District found that as many as 12-15 turnings were necessary to reduce pathogens in windrow composted sewage sludge (Personal Communication, Ross Caballero, Los Angeles County Sanitation District, 1998).

Second, it is important that once that material is in the pile core it be subject to the full time-temperature regime necessary to reduce pathogens. Therefore, the turning schedule and the recovery of the core zone to 55°C are important factors. If pile turning is not evenly distributed throughout the 15-day period, some material may not spend adequate time in the core of the pile. Additionally, pile temperatures generally drop off immediately after turning; if temperatures in the pile core do not quickly recover to 55°C (within 24 hours), the necessary pathogen reduction period of 3 days will not be achieved.

Because of the operational variability, pathogen reduction in windrow cornposting has been found to be less predictable than pathogen reduction in aerated static pile or in-vessel cornposting. In order to improve pathogen reduction, the following operational guidelines are recommended.

- Windrow turning should take place after the pile core has met pathogen reduction temperatures for 3 consecutive days. Windrow turnings should be evenly spaced within the 15 days so that all material remains in the core zone for 3 consecutive days; allowing additional time as needed for the core temperature to come up to 55°C.
- Pathogen reduction temperatures (55°C) must be met for 15 consecutive days at the pile core;
- Temperatures should be taken at approximately the same time each day in order to demonstrate that 55°C has been reached in the pile core within 24 hours after pile turning;
- Testing frequency should be increased; a large sewage sludge windrow cornposting operation recommends testing each windrow for Salmonella sp. before piles are distributed (Personal Communication, Ross Caballero, Los Angeles County Sanitation District, 1998). Samples are taken after turning is completed, and piles which do not comply with Class A requirements are retained on site for further cornposting.

Vector Affracfion Reduction *(VAR)*

The options for demonstrating vector attraction reduction for both PFRP and PSRP cornposting are the same. Option 5 is the most appropriate for cornposting operations. This option requires aerobic treatment (e.g. cornposting) of the sewage sludge for at least 14 consecutive days at over 40°C (104°F) with an average temperature of over 45°C (113°F). This is usually easily attained by sewage sludge cornposting.

The PFRP and VAR requirements can be met concurrently in cornposting. For within-vessel or aerated static pile cornposting, the temperature profile should show PFRP temperatures at each of the temperature monitoring points for 3 consecutive days, followed by a minimum of 11 more days during which time the average temperature of the pile complies with VAR requirements. For windrow piles, the compliance with PFRP temperatures will also fulfill VAR requirements.

PFRP temperatures should be met before or at the same time that VAR requirements are fulfilled in order to reduce the potential for pathogen regrowth. However, continued curing of the cornposting material will most likely further prevent the growth of pathogenic bacteria from taking place.

Like all microbiological processes, cornposting can only take place with sufficient moisture (45-60%). Excessive aeration of cornposting piles or arid ambient condition may dry cornposting piles to the point at which microbial activity slows or stops. The cessation of microbial activity results in lowered pile temperatures which can easily be mistaken for the end-point of cornposting. Although cornposting may appear to have ended, and compost may even meet vector attraction reduction via Option 7, overly dried compost can cause both odor problems and vector attraction if moisture is reintroduced into the material and microbial activity resumes. It is therefore recommended that the cornposting process be maintained at moisture levels between 45-60% (40-55% total solids) (Epstein, 1997).

Microbiological Requiremen fs

If the conditions specified by the Part 503 regulation are met, all pathogenic viruses, bacteria, and parasites will be reduced to below detectable levels However, it may be difficult to meet the Class A microbiological requirement for fecal coliforms even when *Salmonella* sp. bacteria are not present. Biological sewage sludge treatment processes involving high temperatures, such as cornposting, can reduce *Salmonella* sp. to below detectable levels while leaving some surviving fecal coliforms. If sufficient nutrients remain in the sewage sludge, bacteria can later grow to

significant numbers. It may be preferable, therefore, to test composted sewage sludge directly for Salmonella sp., rather than using fecal coliforms as an indicator of pathogen control.

Although not mandated by the Part 503 regulation, compost is usually maintained on site for longer than the required PFRP and VAR duration. In order to produce a high-quality, marketable product, it has been found that a curing period, or the period during which the volatile solids in the sewage sludge continue to decompose, odor potential decreases, and temperatures decrease into the mesophilic (40-45°C) range, is necessary. Depending on the feedstock and the particular process, the curing period may last an additional 30 - 50 days after regulatory requirements are met.

In general, compost is not considered marketable until the piles are no longer self-heating. It is important to note that compost piles that are cooled by excessive aeration or that do not self-heat because the material is too dry to support microbial activity may not actually be fully decomposed.

It has been found that further reduction of organic material takes place during the curing phase of composting (Epstein, 1997). Therefore microbiological testing should take place at the end of the curing process when compost is prepared for sale or distribution. Compost which is stored on site for extended periods of time until it can be sold or distributed must be tested for compliance with microbiological limits when it is to be used or disposed.

7.3 Heat Drying

Heat drying is used to reduce both pathogens and the water content of sewage sludge. The Part 503 PFRP description of heat drying is:

- Sewage sludge is dried by direct or indirect contact with hot gases to reduce the moisture content to 10% or lower. Either the temperature of the sewage sludge particles exceeds 80°C (176°F) or the wet bulb temperature of the gas in contact with the sewage sludge as it leaves the dryer exceeds 80°C (176°F).

Properly conducted heat drying will reduce pathogenic viruses, bacteria, and helminth ova to below detectable levels. Four processes are commonly used for heat drying sewage sludge: flash dryers, spray dryers, rotary dryers, and steam dryers. Flash dryers used to be the most common heat drying process installed at treatment works, but current practice favors rotary dryers. These processes are briefly described below. More detailed descriptions are provided in EPA's Process Design Manual (EPA, 1979).

Flash Dryers

Flash dryers pulverize sewage sludge in the presence of hot gases. The process is based on exposing fine sewage sludge particles to turbulent hot gases long enough to attain at least 90% solids content.

Spray Dryers

A spray dryer typically uses centrifugal force to atomize liquid sewage sludge into a spray that is directed into a drying chamber. The drying chamber contains hot gases that rapidly dry the sewage sludge mist. Some spray drying systems use a nozzle to atomize sewage sludge.

Rotary Dryers

Rotary dryers function as horizontal cylindrical kilns. The drum rotates and may have plows or louvers that mechanically mix the sewage sludge as the drum turns. There are many different rotary kiln designs, utilizing either direct heating or indirect heating systems. Direct heating designs maintain contact between the sewage sludge and the hot gases. Indirect heating separates the two with steel shells.

Steam Dryers

Indirect steam dryers utilize steam to heat the surface of the dryers which will come into contact with the sewage sludge. The heat transfer surface may consist of discs or paddles, which rotate to increase their contact with the sewage sludge.

Vector Attraction Reduction

No further processing is required because the PFRP requirements for heat drying also meet the requirements of Option 8 for vector attraction reduction (the percent solids must be at least 90% before mixing the sewage sludge with other materials). This fulfills the requirement of Option 7 if the sewage sludge being dried contains no unstabilized solids.

Drying of sewage sludge to 90% solids deters the attraction of vectors, however, unstabilized dried biosolids which are rewet may become odorous and attract vectors. Therefore, it is recommended that materials be used or disposed while the level of solids remains high and that dried material be stored and maintained under dry conditions.

Some operators have found that maintaining stored material at solids levels above 95% helps to deter reheating because microbiological activity is halted. However, storage of materials approaching 90% total solids can lead to spontaneous combustion with subsequent fires and risk of explosion. While there is little likelihood of an explosion occurring with storage of materials like pellets, precautionary measures such as maintaining proper oxygen levels and minimizing dust levels in storage silos and monitoring temperatures in material can reduce the risk of fires.

Microbiological Requirements

Heat dried biosolids must be tested for fecal coliform or Salmonella sp. at the last point before being used or disposed. For example, biosolids should be tested immediately before they are bagged or before they leave the site for bulk distribution. If material is stored for a long period of time, it should be re-tested, even if previous testing has

shown the biosolids to have met the Part 503 regulation. This is particularly important if material has been rewetted.

7.4 Heat Treatment

Heat treatment processes are used to disinfect sewage sludge and reduce pathogens to below detectable levels. The processes involve heating sewage sludge under pressure for a short period of time. The sewage sludge becomes sterilized and bacterial slime layers are solubilized, making it easier to dewater the remaining sewage sludge solids. The Part 503 PFRP description for heat treatment is:

- Liquid sewage sludge is heated to a temperature of 180°C (356°F) or higher for 30 minutes.

Two processes have principally been used for heat treating sludge in preparation for dewatering: the Porteous and the Zimpro process. In the Porteous process the sewage sludge is preheated and then injected into a reactor vessel. Steam is also injected into the vessel under pressure. The sewage sludge is retained in the vessel for approximately 30 minutes after which it is discharged to a decant tank. The resulting sewage sludge can generally be concentrated and dewatered to high solids concentrations. Further dewatering may be desirable to facilitate sewage sludge handling.

The Zimpro process is similar to the Porteous process. However, air is injected into the sewage sludge before it enters the reactor and the vessel is then heated by steam to reach the required temperature. Temperatures and pressures are approximately the same for the two processes.

Vector Attraction Reduction

Heat treatment in most cases must be followed by vector attraction reduction. Vector attraction reduction Options 6 to 11 (pH adjustment, heat drying, or injection, incorporation, or daily cover) may be used (see Chapter 8). Options 1 through 5 would not typically be applicable to heat treated sludge unless the sludge was digested or otherwise stabilized during or after heat treatment (e.g. through the use of wet air oxidation during heat treatment).

Microbiological Requirements

When operated according to the Pan 503 requirements, the process effectively reduces pathogenic viruses, bacteria, and viable helminth ova to below detectable levels. Sewage sludge must be properly stored after processing because organic matter has not been reduced, and therefore, growth of bacteria can occur.

Heat treated sewage sludge must be tested for fecal coliform or Salmonella sp. at the time of use or disposal or as it is prepared for sale or distribution. If heat treated biosolids are subsequently composted or otherwise treated, pathogen testing should take place after that processing is complete.

7.5 Thermophilic Aerobic Digestion

Thermophilic aerobic digestion is a refinement of the conventional aerobic digestion processes discussed in Section 6.2. In this process, feed sewage sludge is generally pre-thickened and an efficient aerator is used. In some modifications, oxygen is used instead of air. Because there is less sewage sludge volume and less air to carry away heat, the heat released from biological oxidation warms the sewage sludge in the digester to as high as 60°C (140°F).

Because of the increased temperatures, this process achieves higher rates of organic solids reduction than are achieved by conventional aerobic digestion which operates at ambient air temperature. The biodegradable volatile solids content of the sewage sludge can be reduced by up to 70% in a relatively short time. The digested sewage sludge is effectively pasteurized due to the high temperatures. Pathogenic viruses, bacteria, viable helminth ova and other parasites are reduced to below detectable limits if the process is carried out at temperatures exceeding 55°C (131°F).

This process can either be accomplished using auxiliary heating of the digestion tanks or through special designs that allow the energy naturally released by the microbial digestion process to heat the sewage sludge. The Part 503 PFRP description of thermophilic aerobic digestion is:

- Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean cell residence time of the sewage sludge is 10 consecutive days at 55°C to 60°C (131 °F to 140°F).

The thermophilic process requires significantly lower residence times (i.e., solids retention time) than conventional aerobic processes designed to qualify as a PSRP, which must operate 40 to 60 days at 20°C to 15°C (68°F to 59°F), respectively. Residence time is normally determined by dividing the volume of sewage sludge in the vessel by the volumetric flow rate. Facility operation should minimize the potential for bypassing by withdrawing treated sewage sludge before feeding, and feeding no more than once a day.

In the years following the publication of the Part 503 regulation, advances in thermophilic digestion have been made. It should be noted, however, that complete-mix reactors with continuous feeding may not be adequate to meet Class A pathogen reduction because of the potential for bypassing or short-circuiting of untreated sewage sludge.

Vector Attraction Reduction

Vector attraction reduction must be demonstrated. Although all options, except Options 2, 4, and 12 are possible, Options 1 and 3 which involve the demonstration of volatile solids loss are the most suitable. (Option 2 is appropriate only for anaerobically digested sludge, and Option 4 is not possible because it is not yet known how to translate SOUR measurements obtained at high temperatures to 20°C [68°F].)

Thermophilically aerobically digested biosolids must be tested for fecal coliform or *Salmonella* sp. at the time of

use or disposal or as it is prepared for sale or distribution. If digested biosolids are subsequently composted or otherwise treated, pathogen testing for either fecal coliform or *Salmonella* sp. should take place after processing is complete.

7.6 Beta Ray and Gamma Ray Radiation

Radiation can be used to disinfect sewage sludge. Radiation destroys certain organisms by altering the colloidal nature of the cell contents (protoplasm). Gamma rays and beta rays are the two potential energy sources for use in sewage sludge disinfection. Gamma rays are high-energy photons produced by certain radioactive elements. Beta rays are electrons accelerated in velocity by electrical potentials in the vicinity of 1 millions volts. Both types of radiation destroy pathogens that they penetrate if the doses are adequate. The Part 503 PFRP descriptions for irradiation systems are:

Beta Ray Irradiation

- Sewage sludge is irradiated with beta rays from an accelerator at dosages of at least 1 .O megarad at room temperature (ca. 20°C [68°F]).

Gamma Ray Irradiation

- Sewage sludge is irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137 [at dosages of at least 1 .O megarad] at room temperature (ca. 20°C [68°F]).

The effectiveness of beta radiation in reducing pathogens depends on the radiation dose, which is measured in rads. A dose of 1 megarad or more will reduce pathogenic viruses, bacteria, and helminths to below detectable levels. Lower doses may successfully reduce bacteria and helminth ova but not viruses. Since organic matter has not been destroyed with process, sewage sludge must be properly stored after processing to prevent contamination.

Although the two types of radiation function similarly to inactivate pathogens, there are important differences. Gamma rays can penetrate substantial thicknesses of sewage sludge and can therefore be introduced to sewage sludge by either piping liquid sewage sludge into a vessel that surrounds the radiation source (Figure 7-l) or by carrying composted or dried sewage sludge by hopper conveyor to the radiation source. Beta rays have limited penetration ability and therefore are introduced by passing a thin layer of sewage sludge under the radiation source (Figure 7-2).

Vector Attraction Reduction

Radiation treatment must be followed by vector attraction reduction. The appropriate options for demonstrating vector attraction reduction are the same as for heat treatment (see Section 7.4), namely Options 6 to 11. Options l-5 are not applicable unless the sewage sludge is subsequently digested.

Microbiological Requirements

Irradiated sewage sludge must be tested for fecal coliform or *Salmonella* sp. at the time of use or disposal or as it is prepared for sale or distribution.

7.7 Pasteurization

Pasteurization involves heating sewage sludge to above a predetermined temperature for a minimum time period. For pasteurization, the Part 503 PFRP description is:

- The temperature of the sewage sludge is maintained at 70°C (158°F) or higher for 30 minutes or longer.

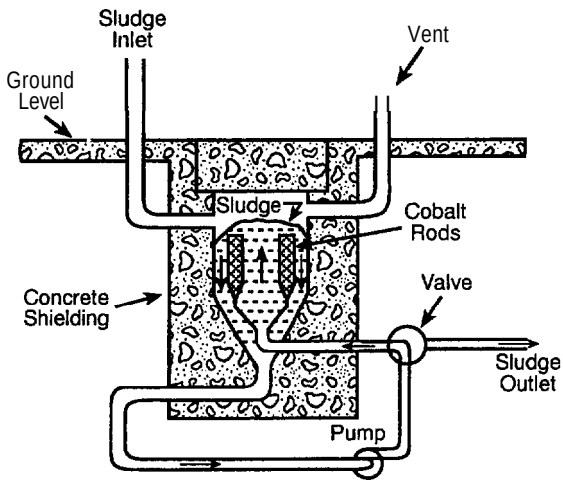


Figure 7-1. Schematic representation of cobalt-60 (gamma ray) irradiation facility at Geiselbullach, Germany. Source: EPA. 1979.

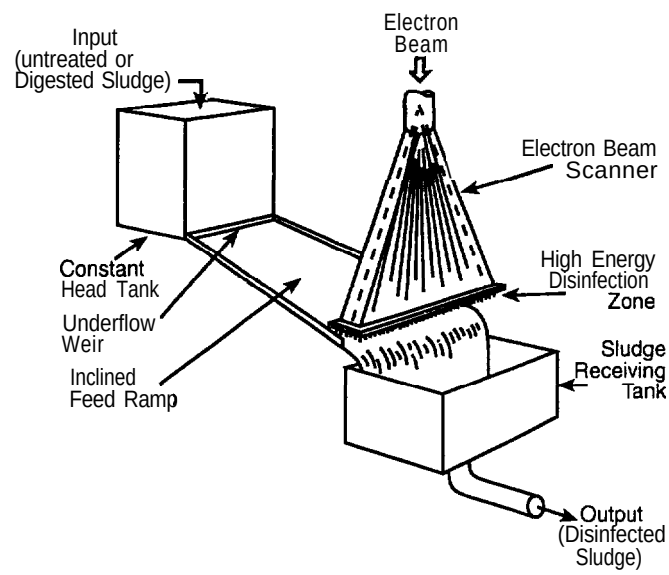


Figure 7-2. Beta ray scanner and sludge spreader. Source: EPA, 1979.

Pasteurization reduces bacteria, enteric viruses, and viable helminth ova to below detectable values. Sewage sludge can be heated by heat exchangers or by steam injection. Although sewage sludge pasteurization is uncommon in the United States, it is widely used in Europe. The steam injection method is preferred because it is more effective at maintaining even temperatures throughout the sewage sludge batch being processed. Sewage sludge is pasteurized in batches to prevent recontamination that might occur in a continuous process. Sewage sludge must be properly stored after processing because the organic matter has not been stabilized and therefore odors and growth of pathogenic bacteria can occur if sewage sludge is re-inoculated.

In theory, quicklime can be used to meet the requirements for pasteurization of sewage sludge. The water in the sludge slakes the lime, forming calcium hydroxide, and generates heat. However, it is difficult to ensure that the entire mass of sewage sludge comes into contact with the lime and achieves the required 70°C for 30 minutes. This is particularly true for dewatered sewage sludges. Processes must be designed to 1) maximize contact between the lime and the sewage sludge, 2) ensure that adequate moisture is present, 3) ensure that heat loss is minimal, and 4) if necessary, provide an auxiliary heat source. Pasteurization cannot be accomplished in open piles.

In addition, in order for pasteurization to be conducted properly, facility operators must be trained with regard to 1) the proper steps to be taken to ensure complete hydration of the alkaline reagent used, 2) the evaluation of the slaking rate of the lime based alkaline material required for their particular process, specifying the reactivity rate required, 3) the proper measurement of pH, 4) an awareness of the effect of ammonia gassing off and how this affects the lime dose, and 5) the necessity for maintaining sufficient moisture in the sewage sludge/alkaline mixture during the mixing process to ensure the complete hydration of the quicklime and migration of hydroxyl ions throughout the sewage sludge mass. This is to ensure that the entire sewage sludge mass is disinfected.

EPA -sponsored studies showed that pasteurization of liquid sewage sludge at 70°C (158°F) for 30 minutes inactivates parasite ova and cysts and reduces the population of measurable viruses and pathogenic bacteria to below detectable levels (U.S. EPA, 1979). This process is based on the pasteurization of milk which must be heated to at least 63°C (145°F) for at least 30 minutes.

Vector Attraction Reduction

Pasteurization must be followed by a vector attraction reduction process unless the vector attraction reduction conditions of Option 6 (pH adjustment) have been met. The options appropriate for demonstrating vector attraction reduction are the same as those for heat treatment (see Section 10.4), namely Options 6 to 11. Options 1 to 5 are not applicable unless the sludge is subsequently digested.

Microbiological Requirements

Pasteurized sludge must be tested for fecal coliform or *Salmonella* sp. at the time of use or disposal or as it is prepared for sale or distribution. In Europe, serious problems with regrowth of *Salmonella* sp. have occurred, so pasteurization is rarely used now as a terminal treatment process. Pre-pasteurization followed by mesophilic digestion has replaced the use of pasteurization after digestion in many European communities.

7.8 Equivalent Processes

Under Class A Alternative 6, sewage sludge treated in processes that are determined to be equivalent to PFRP are considered to be Class A with respect to pathogens (assuming the treated sewage sludges also meet the Class A microbiological requirement). Table 11-2 in Chapter 11 lists some of the processes that were found, based on the recommendation of EPA's Pathogen Equivalency Committee, to be equivalent to PFRP under Part 257. Chapter 11 discusses how the PEC makes a recommendation of equivalency.

References and Additional Resources

Caballero, Ross. 1984. Experience at a windrow composting facility: LA County site technology transfer. US EPA, Municipal Environmental Research. Cincinnati, Ohio.

Composting Council. 1994. Compost facility operating guide: A reference guide for composting facility and process management. Alexandria, Virginia.

Epstein, Eliot. 1997. The science of cornposting. Technomic Publishing Company.

Farrell, J.B. 1992. Fecal pathogen control during cornposting. presented at International Composting Research Symposium, Columbus, Ohio.

Haug, Roger T. 1993. The practical handbook of compost engineering. Lewis Publishers.

lacaboni, M.D., J.R. Livingston, and T.J. LeBrun. 1984. Windrow and static pile composting of municipal sewage sludges. Report No.: EPA/600/2-84-122 (NTIS PB84-215748).

U.S. EPA. 1979. Process design manual for sludge treatment and disposal. Repot-f No.: EPA/625/1-79/001. Cincinnati, OH: Water Engineering Research Laboratory and Center for Environmental Research Information.

USDA/U.S. EPA. 1980. Manual for composting sewage sludge by the Beltsville aerated-pile method. Report No.: EPA/600/8-80-022.

WEF/ASCE. 1998. WEF Manual of Practice No. 8, Design of Municipal Wastewater Treatment Plants. Pub. WEF (Alexandria, VA) and ASCE (New York, NY).

Yanko, W.A. 1987. Occurrence of pathogens in distribution and marketing municipal sludges. Report No.: EPA/600/1-87/014. (NTIS PB88-154273/AS.) Springfield, VA: National Technical Information Service.

Chapter 8
Requirements for Reducing Vector Attraction

8.1 Introduction

The pathogens in sewage sludge pose a disease risk only if there are routes by which the pathogens are brought into contact with humans or animals. A principal route for transport of pathogens is vector transmission. Vectors are any living organisms capable of transmitting a pathogen from one organism to another either mechanically (by simply transporting the pathogen) or biologically by playing a specific role in the life cycle of the pathogen. Vectors for sewage sludge pathogens would most likely include insects, rodents, and birds.

Suitable methods for measuring vector attraction directly are not available. Vector attraction reduction is accomplished by employing one of the following:

- Biological processes which breakdown volatile solids, reducing the available food nutrients for microbial activities and odor producing potential
- Chemical or physical conditions which stop microbial activity
- Physical barriers between vectors and volatile solids in the sewage sludge

At the present time there is no vector attraction equivalency committee that evaluates other options for vector attraction reduction. The creation of one is being considered. The specific options outlined in the Part 503 regulation are currently the only available means for demonstrating vector attraction reduction.

The term "stability" is often used to describe sewage sludge. Although it is associated with vector attraction reduction, stability is not regulated by the Part 503 Rule. With regard to sewage sludge, stability is generally defined as the point at which food for rapid microbial activity is no longer available. Sewage sludge which is stable will generally meet vector attraction reduction (VAR) requirements. The converse is not necessarily true; meeting VAR requirements does not ensure sewage sludge stability. Because stability is also related to odor generation and the continued degradation of sewage sludge, it is often considered an important parameter when producing biosolids for sale or distribution. Table 8-l lists some of the common methods for measuring stability.

Table 8-l. Stability Assessment

Process	Monitoring Methods
Composting	CO, respiration, 0, uptake
Heat Drying	Moisture content
Alkaline Stabilization	pH; pH change with storage; moisture; ammonia evolution; temperature
Aerobic Digestion	SOUR; volatile solids reduction, additional volatile solids reduction
Anaerobic Digestion	Gas production; volatile solids reduction, additional volatile solids reduction

More information on stability can be found in the WERF publication, "Defining Biosolids Stability: A Basis for Public and Regulatory Acceptance" (1997).

The Part 503 regulation contains 12 options, described below and summarized in Table 8-2, for demonstrating a reduction in vector attraction of sewage sludge. These requirements are designed to either reduce the attractiveness of sewage sludge to vectors (Options 1 through 8 and Option 12) or prevent the vectors from coming in contact with the sewage sludge (Options 9 through 11).

Guidance on when and where to sample sewage sludge to meet these requirements is provided in Chapter 10.

As mentioned in Chapter 3, meeting the vector attraction reduction requirements must be demonstrated separately from meeting the pathogen reduction requirements. Therefore, demonstration of vector attraction reduction (e.g., through reduction of volatile solids by 38% as described below) does not demonstrate achievement of pathogen reduction. It should be noted that for Class A biosolids, vector attraction reduction must be met after or concurrent with pathogen reduction to prevent growth of pathogenic bacteria.

8.2 Option 1: Reduction in Volatile Solids Content [503.33(b)(l)]

This option is intended for use with biological treatment systems only. Under Option 1, reduction of vector attraction is achieved if the mass of volatile solids in the sewage sludge is reduced by at least 38%. This is the percentage

Table 8-2. Vector Attraction Reduction Options

Requirement	What is Required?	Most Appropriate For:
Option 1 503.33(b)(1)	At least 38% reduction in volatile solids during sewage sludge treatment	Sewage sludge processed by: Anaerobic biological treatment Aerobic biological treatment
Option 2 503.33(b)(2)	Less than 17% additional volatile solids loss during bench-scale anaerobic batch digestion of the sewage sludge for 40 additional days at 30°C to 37°C (86°F to 99°F)	Only for anaerobically digested sewage sludge that cannot meet the requirements of Option 1
Option 3 503.33(b)(3)	Less than 15% additional volatile solids reduction during bench-scale aerobic batch digestion for 30 additional days at 20°C (68°F)	Only for aerobically digested liquid sewage sludge with 2% or less solids that cannot meet the requirements of Option 1 — e.g., sewage sludges treated in extended aeration plants. Sludges with , 2% solids must be diluted.
Option 4 503.33(b)(4)	SOUR at 20°C (68°F) is #1.5 mg oxygen/hr/g total sewage sludge solids	Liquid sewage sludges from aerobic processes run at temperatures between 10 to 30° C. (should not be used for composted sewage sludges).
Option 5 503.33(b)(5)	Aerobic treatment of the sewage sludge for at least 14 days at over 40°C (104°F) with an average temperature of over 45°C (113°F)	Compcsted sewage sludge (Options 3 and 4 are likely to be easier to meet for sewage sludges from other aerobic processes)
Option 6 503.33(b)(6)	Addition of sufficient alkali to raise the pH to at least 12 at 25°C (77°F) and maintain a pH $12 for 2 hours and a pH $11.5 for 22 more hours	Alkali-treated sewage sludge (alkaline materials include lime, fly ash, kiln dust, and wood ash)
Option 7 503.33(b)(7)	Percent solids $75% prior to mixing with other materials	Sewage sludges treated by an aerobic or anaerobic process (i.e., sewage sludges that do not contain unstabilized solids generated in primary wastewater treatment)
Option 8 503.33(b)(8)	Percent solids $90% prior to mixing with other materials	Sewage sludges that contain unstabilized solids generated in primary wastewater treatment (e.g., heat-dried sewage sludges)
Option 9 503.33(b)(9)	Sewage sludge is injected into soil so that no significant amount of sewage sludge is present on the land surface 1 hour after injection, except Class A sewage sludge which must be injected within 8 hours after the pathogen reduction process.	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, a forest, or a reclamation site, or placed on a surface disposal site
Option 10 503.33(b)(10)	Sewage sludge is incorporated into the soil within 6 hours after application to land or placement on a surface disposal site, except Class A sewage sludge which must be applied to or placed on the land surface within 8 hours after the pathogen reduction process.	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, forest, or a reclamation site, or placed on a surface disposal site
Option 11 503.33(b)(11)	Sewage sludge placed on a surface disposal site must be covered with soil or other material at the end of each operating day.	Sewage sludge or domestic septage placed on a surface disposal site
Option 12 503.33(b)(12)	pH of domestic septage must be raised to $12 at 25°C (77°F) by alkali addition and maintained at $12 for 30 minutes without adding more alkali.	Domestic septage applied to agricultural land, a forest, or a reclamation site or placed on a surface disposal site

of volatile solids reduction that can generally be attained by the "good practice" recommended conditions for anaerobic digestion of 15 days residence time at 35°C [95°F] in a completely mixed high-rate digester. The percent volatile solids reduction can include any additional volatile solids reduction that occurs before the biosolids leave the treatment works, such as might occur when the sewage sludge is processed on drying beds or in lagoons.

The starting point for measuring volatile solids in sewage sludge is at the point at which sewage sludge enters a sewage sludge treatment process. This can be problem-

atic for facilities in which wastewater is treated in systems like oxidation ditches or by extended aeration. Sewage sludges generated in these processes are already substantially reduced in volatile solids content by their long exposure to oxidizing conditions in the process. If sewage sludge removed from these processes is further treated by anaerobic or aerobic digestion to meet VAR requirements, it is unlikely that the 38% reduction required to meet Option 1 can be met. In these cases, use of Options 2,3, or 4 is more appropriate.

The end point where volatile solids are measured to calculate volatile solids losses can be at any point in the process. Because volatile solids continue to degrade throughout sewage sludge treatment, it is recommended that samples be taken at the end point of treatment.

Volatile solids reduction is calculated by a volatile solids balance around the digester or by the Van Kleeck formula (Fisher, 1984). Guidance on methods of calculation is provided in Appendix C.

Volatile solids reduction is typically achieved by anaerobic or aerobic digestion. These processes degrade most of the biodegradable material to lower activity forms. Any biodegradable material that remains characteristically degrades so slowly that vectors are not drawn to it.

8.3 Option 2: Additional Digestion of Anaerobically Digested Sewage Sludge [503.33(b)(2)]

Under this option, an *anaerobically* digested sewage sludge is considered to have achieved satisfactory vector attraction reduction if it loses less than 17% additional volatile solids when it is anaerobically batch-digested in the laboratory in a bench-scale unit at 30°C to 37°C (86°F to 99°F) for an additional 40 days. Procedures for this test are presented in Appendix D. As noted in Appendix D, the material balance method for calculating additional volatile solids reduction will likely show greater reductions than the Van Kleeck method.

Frequently, return activated sludges have been recycled through the biological wastewater treatment section of a treatment works or have resided for long periods of time in the wastewater collection system. During this time they undergo substantial biological degradation. If they are subsequently treated by anaerobic digestion for a period of time, they are adequately reduced in vector attraction, but because they entered the digester with volatile solids already partially reduced, the volatile solids reduction after treatment is frequently less than 38%. The additional digestion test is used to demonstrate that these sewage sludges are indeed satisfactorily reduced in vector attraction.

It is not necessary to demonstrate that Option 1 cannot be met before using Option 2 or 3 to comply with VAR requirements.

This additional anaerobic digestion test may have utility beyond use for sewage sludge from the classical anaerobic digestion process. The regulation states that the test can be used for a previously anaerobically digested sewage sludge. One possible application is for sewage sludge that is to be removed from a wastewater lagoon. Such sewage sludge may have been stored in such a lagoon for many years, during which time it has undergone anaerobic digestion and lost most of its volatile solids. It is only recognized by the regulations as a sewage sludge when it is removed from the lagoon. If it were to be further processed by anaerobic digestion, the likelihood of achieving 38% volatile solids reduction is very low. The additional anaerobic digestion test which requires a long period of batch digestion at temperatures between 30 and 37°C would seem to be an appropriate test to determine whether such a sewage sludge has the potential to attract vectors.

8.4 Option 3: Additional Digestion of Aerobically Digested Sewage Sludge [503.33(b)(3)]

Under this option, an aerobically digested sewage sludge with 2% or less solids is considered to have achieved satisfactory vector attraction reduction if it loses less than 15% additional volatile solids when it is aerobically batch-digested in the laboratory in a bench-scale unit at 20°C (68°F) for an additional 30 days. Procedures for this test and the method for calculating additional volatile solids destruction are presented in Appendix D. The test can be run on sewage sludges up to 2% solids and does not require a temperature correction for sewage sludges not initially digested at 20°C (68°F). Liquid sludges with greater than 2% solids can be diluted to 2% solids with unchlorinated effluent, and the test can then be run on the diluted sludge. This option should not be used for non-liquid sewage sludge such as dewatered cake or compost.

This option is appropriate for aerobically digested sewage sludges that cannot meet the 38% volatile solids reduction required by Option 1. These include sewage sludges from extended aeration and oxidation ditch processes, where the nominal residence time of sewage sludge leaving the wastewater treatment processes section generally exceeds 20 days. In these cases, the sewage sludge may already have been substantially reduced in biological degradability prior to aerobic digestion.

As was suggested for the additional anaerobic digestion test, the additional aerobic digestion test may have application to sewage sludges that have been aerobically treated by other means than classical aerobic digestion.

8.5 Option 4: Specific Oxygen Uptake Rate (SOUR) for Aerobically Digested Sewage Sludge [503.33(b)(4)]

For an aerobically digested sewage sludge with a total solids content equal to or less than 2% which has been processed at a temperature between 10 – 30° C, reduction in vector attraction can also be demonstrated using the SOUR test. The SOUR of the sewage sludge to be used or disposed must be less than or equal to 1.5 mg of

oxygen per hour per gram of total sewage sludge solids (dry weight basis) at 20°C (68°F).[1] This test is based on the fact that if the aerobically treated sewage sludge consumes very little oxygen, its value as a food source for vectors is very low and therefore vectors are unlikely to be attracted to it. Frequently aerobically digested sewage sludges are circulated through the aerobic biological wastewater treatment process for as long as 30 days. In these cases, the sewage sludge entering the aerobic digester is already partially digested, which makes it difficult to demonstrate the 38% reduction required by Option 1.

The oxygen uptake rate depends on the conditions of the test and, to some degree, on the nature of the original sewage sludge before aerobic treatment. The SOUR test should not be used on sewage sludge products such as heat or air dried sludge or compost. Because of the reduction of microbial populations that occur in these processes, the SOUR results are not accurate and should not be used. SOUR testing on sewage sludges with a total solids content below 0.5% may give inaccurately high results. Farrell, et al. (1996) cite the work of several investigators indicating such an effect. Farrell, et al. (1996) also note that storage for up to two hours did not cause a significant change in the SOUR measurement. It is therefore suggested that a dilute sewage sludge could be thickened to a solids content less than 2% solids and then tested, provided that the thickening period is not in excess of two hours.

The SOUR test requires a poorly defined temperature correction at temperatures differing substantially from 20°C (68°F). SOUR cannot be applied to sewage sludges digested outside the 10-30° C range (50-86°F). The actual temperature of the sewage sludge tested cannot be adjusted because temperature changes can cause short-term instability in the oxygen uptake rate (Benedict, et al. (1973); Farrell, et al. [1996]), and this would invalidate the results of the test. Guidance on performing the SOUR test and on sewage sludgedependent factors are provided in Appendix D.

It should be noted that the limit on the use of the SOUR test for sewage sludges at different solids and temperature levels is due to the lack of research and data on different sewage sludges. EPA encourages the collection of SOUR data for different sewage sludges so that at some point, Option 4 may be expanded to include more sewage sludge materials.

[1]SOUR is defined in Part 503 as the mass of oxygen consumed per unit time per unit mass of total solids (dry weight basis) in the sewage sludge. SOUR is usually based on total suspended volatile solids rather than total solids because it is assumed that it is the volatile matter in the sewage sludge that is being oxidized. The SOUR definition in Part 503 is based on the total solids primarily to reduce the number of different determinations needed and for consistency with application rates, which are measured in total solids per unit area. Generally, the range in the ratio of volatile solids to total solids in aerobically digested sewage sludges is not large. The SOUR based on total solids will merely be slightly lower than if it had been based on volatile suspended solids to indicate the same endpoint.

8.6 Option 5: Aerobic Processes at Greater Than 40°C [503.33(b)(5)]

The sewage sludge must be aerobically treated for 14 days or longer during which time the temperature must be over 40°C (104°F) and the average temperature higher than 45°C (113°F). This option applies primarily, but not exclusively, to composted sewage sludge. These processed sewage sludges generally contain substantial amounts of partially decomposed organic bulking agents, in addition to sewage sludge. This option must be used for composted sewage sludge; other options are either not appropriate or have not adequately been investigated for use with compost.

The Part 503 regulation does not specifically mention or limit this option to cornposting. This option can be applied to sewage sludge from other aerobic processes such as aerobic digestion as long as temperature requirements can be met and the sewage sludge is maintained in an aerobic state for the treatment period, but other methods such as Options 3 and 4 are likely to be easier to meet for these sewage sludges.

If cornposting is used to comply with Class A pathogen requirements, the VAR time-temperature regime must be met along with or after compliance with the pathogen reduction time-temperature regime.

8.7 Option 6: Addition of Alkali [503.33(b)(6)]

Sewage sludge is considered to have undergone adequate vector attraction reduction if sufficient alkali is added to:

- Raise the pH to at least 12
- Maintain a pH of at least 12 without addition of more alkali for 2 hours
- Maintain a pH of at least 11.5 without addition of more alkali for an additional 22 hours

pH should be measured in a slurry at 25°C. For more information on making a slurry, see Section 10.7. Either sewage sludge samples may be taken and heated or cooled to 25°C, or results can be adjusted based on the ambient temperature where pH is measured and the following calculation:

$$\text{Correction Factor} = \frac{0.03 \text{ pH units} \times (T - 25\,^{\circ}C)}{1.0\,^{\circ}C}$$

Actual pH = Measured pH +/- the Correction Factor

T= Temperature measured

Example of Using the pH Correction Factor

If the measured pH is 12.304 at 30° C, the actual pH can be calculated as follows:

Correction Factor = 0.03 X (30-25) = +0.15
Actual pH = 12.304+ 0.15 = 12.454

It should be noted that temperature compensation devices on pH meters correct only for variations in the conductance of pH probes, and not for the variability in solution concentration. Therefore, the temperature correction noted above should be applied to pH measurements, even if a pH meter with temperature settings is used.

As noted in Section 5.6, the term "alkali" means a substance that causes an increase in pH. Raising sewage sludge pH through alkali addition reduces vector attraction by reducing or stopping biological activity. However, this reduction in biological activity is not permanent. If the pH drops, surviving bacteria become biologically active and the sewage sludge will again putrefy and potentially attract vectors. The more soluble the alkali, the less likely it is that there will be an excess present when a pH of 12 is reached. Consequently, the subsequent drop in pH with time will be more rapid than if a less soluble alkali is used.

The conditions required under this option are designed to ensure that the sewage sludge can be stored for at least several days at the treatment works, transported, and applied to soil without the pH falling to the point where biological activity results in vector attraction. The requirement of raising the pH to 12 increases the probability that the material will be used before pH drops to a level at which putrefaction can occur. The requirements for pH adjustment of domestic septage are less stringent because it is unlikely that septage haulers will hold domestic septage for long periods of time.

Raising the pH to 12 and maintaining this pH for two hours and a pH of 11.5 for an additional 22 hours ensures that the pH will stay at adequately high levels to prevent putrefaction before disposal in all but unusual cases. In any event, it is prudent in a timely manner to apply sludge in a thin layer or incorporate it into the soil for the prevention of odors and vector attraction.

More information on alkali addition and measurement of pH are included in Chapter 10.

8.8 Option 7: Moisture Reduction of Sewage Sludge Containing No Unstabilized Solids [503.33(b)(7)]

Under this option, vector attraction is considered to be reduced if the sewage sludge does not contain unstabilized solids generated during primary wastewater treatment and if the solids content of the sewage sludge is at least 75% before the sewage sludge is mixed with other materials. Thus, the reduction must be achieved by removing water, not by adding inert materials.

It is important that the sewage sludge not contain unstabilized solids because the partially degraded food scraps likely to be present in such a sewage sludge could attract birds, some mammals, and possibly insects, even if the solids content of the sewage sludge exceeds 75%.

The way dried sewage sludge is handled or stored before use or disposal can create or prevent vector attraction. If dried sewage sludge is exposed to high humidity, the outer surface of the sewage sludge could equilibrate to a lower solids content and attract vectors. Proper management should be conducted to prevent this from happening.

8.9 Option 8: Moisture Reduction of Sewage Sludge Containing Unstabilized Solids [503.33(b)(8)]

Vector attraction of any sewage sludge is considered to be reduced if the solids content of the sewage sludge is increased to 90% or greater. This extreme desiccation deters vectors in all but the most unusual situations. As noted for Option 7, the solids increase should be achieved by removal of water and not by dilution with inert solids. Drying to this extent severely limits biological activity and strips off or decomposes the volatile compounds that attract vectors.

Because sewage sludge meeting vector attraction reduction with this option may contain unstabilized solids, material that absorbs moisture or is rewet may putrefy and attract vectors. Proper storage and use of this material should be considered in order to prevent potential pathogen growth and vector attraction.

8.10 Option 9: Injection [503.33(b)(9)]

Vector attraction reduction can be achieved by injecting the sewage sludge below the ground. Under this option, no significant amount of the sewage sludge can be present on the land surface within 1 hour after injection, and, if the sewage sludge is Class A with respect to pathogens, it must be injected within 8 hours after discharge from the pathogen-reduction process.

Injection of sewage sludge beneath the soil places a barrier of earth between the sewage sludge and vectors. The soil quickly removes water from the sewage sludge, which reduces the mobility and odor of the sewage sludge. Odor is usually present at the site during the injection process, but it quickly dissipates when injection is complete.

The special restriction requiring injection within 8 hours for Class A sewage sludge is needed because these sewage sludges are likely devoid of actively growing bacteria and are thus an ideal medium for growth of pathogenic bacteria (see Section 4.3). If pathogenic bacteria are present (survivors or introduced by contamination), their numbers increase slowly for the first 8 hours after treatment, but after this period, their numbers can rapidly increase. This kind of explosive growth is not likely to happen with Class B sludge because high densities of non-pathogenic bacteria are present which suppresses the growth of pathogenic bacteria. In addition, the use of Class B biosolids requires site restrictions which reduce the risk of public exposure to pathogens. Consequently, this special requirement is not needed for Class B biosolids

8.11 Option 10: Incorporation of Sewage Sludge into the Soil [503.33(b)(10)]

Under this option, sewage sludge applied to the land surface or placed on a surface disposal site must be incorporated into the soil within 6 hours after application to or placement on the land. If the sewage sludge is Class A with respect to pathogens, the time between processing and incorporation after application or placement must not exceed 8 hours -the same as for injection under Option 9.

When applied at agronomic rates, the loading of sewage sludge solids typically is about l/l 00th or less of the mass of soil in the plow layer (approximately the top six inches of soil). If mixing is reasonably good, the dilution of sewage sludge in the soil surface is equivalent to that achieved with soil injection. Odor will be present and vectors will be attracted temporarily, as the sewage sludge dewaters on the soil surface. This attraction diminishes and is virtually eliminated when the sewage sludge is mixed with the soil. The mixing method applies to liquid sewage sludges, dewatered sewage sludge cake, and even to dry sewage sludges that have not already met the vector attraction reduction requirements of the regulation by one of the other options.

The 6 hours allowed to complete the mixing of sewage sludge into the soil should be adequate to allow for proper incorporation. As a practical matter, it may be wise to complete the incorporation in a much shorter time. Clay soils tend to become unmanageably slippery and muddy if the liquid sewage sludge is allowed to soak into the first inch or two of topsoil.

8.12 Option 11: Covering Sewage Sludge [503.33(b)(11)]

Under this option, sewage sludge placed on a surface disposal site must be covered with soil or other material at the end of each operating day. Daily covering reduces vector attraction by creating a physical barrier between the sewage sludge and vectors, while environmental factors work to reduce pathogens.

8.13 Option 12: Raising the pH of Domestic Septage [503.33(b)(12)]

This option applies only to domestic septage applied to agricultural land, forest, a reclamation site, or surface disposal site. Vector attraction is reduced if the pH is raised to at least 12 through alkali addition and maintained at 12 or higher for 30 minutes without adding more alkali. (These conditions also accomplish pathogen reduction for domestic septage-see Section 5.6.) When this option is used, every container (truckload) must be monitored to demonstrate that it meets the requirement. As noted in Section 5.6, "alkali" refers to a substance that causes an increase in pH.

This vector attraction reduction requirement is slightly less stringent than the alkali addition requirement for sewage sludge. The method is geared to the practicalities of the use or disposal of domestic septage, which is typically treated by lime addition in the domestic septage hauling truck. The treated septage is typically applied to the land shortly after lime addition. During the very short time interval, the pH is unlikely to fall to a level at which vector attraction could occur.

If domestic septage is not applied soon after pH adjustment, it is recommended that pH be retested, and additional alkali be added to the domestic septage to raise the pH to 12 if necessary. Alternatively, if pH has dropped and the domestic septage begins to putrefy, it is advisable to cover or incorporate the domestic septage in order to prevent vector attraction.

8.14 Number of Samples and Timing

Unlike pathogenic bacteria, volatile solids cannot regenerate once they are destroyed, so samples can be taken at any point along the process. However, since volatile solids are destroyed throughout treatment, it is recommended that samples be taken at the end of processing.

Facilities which use Option 2 or 3 to demonstrate vector attraction reduction must schedule sampling to leave ample time to complete the laboratory tests before sewage sludge is used or disposed. A suggested procedure would be to take several samples at evenly spaced time intervals during the period between the required monitoring dates and calculate running averages comprised of at least four volatile solids results. This has the advantage of not basing the judgement that the process is performing adequately (or inadequately) on one or two measurements that could be erroneous because of experimental error or a poorly chosen sample inadvertently taken during a brief process upset. It also provides an important quality control measure for process operations. Since the Part 503 regulation do not specify a sampling program, it is recommended that sewage sludge preparers consult with the regulatory authority with regard to sampling schedules.

8.15 Vector Attraction Reduction Equivalency

Many of the vector attraction reduction tests are time consuming and inconvenient, particularly for small treatment plants that do not have a laboratory. Efforts to define new, simpler methods for measuring vector attraction are on-going.

Since it is infeasible to measure the actual attraction of vectors, given the large number of variables, methodology development must continue to focus on the cause of vector attraction, namely the availability of a food source (volatile solids) or odor. The tests to measure the attractants may vary depending on the technology by which the sewage sludge is processed.

Some of the parameters which might be used to measure vector attraction may include gas production or measures of microbial activity. For example, several methods

which measure carbon dioxide evolution or reheating potential are currently in use to measure compost stability, but these methods must be examined more closely to determine if they can be applied to other forms of sewage sludge and if results can be adequately correlated to vector attraction.

The responsibility to eventually develop additional vector attraction reduction test protocols lies with the scientific community and the sewage sludge industry. Since there is currently no standard procedure for considering VAR equivalency, new methods must be submitted to the EPA for consideration and potential inclusion in the next rulemaking effort.

References and Additional Resources

Benedict, A.H., and D.A. Carlson. 1973. Temperature acclimation in aerobic bio-oxidation systems. Jour. WPCF: 45(1), 10-24. January.

Farrell, Joseph B., Vinayak Bhide, James E. Smith, Jr. 1996. Development of EPA's new methods to quantify vector attraction of wastewater sludges. Water Environment Research, Volume 68, Number 3.

Fisher, W.J. 1984. Calculation of volatile solids destruction during sludge digestion. Pp 514-528 in Bruce, A., ed Sewage sludge stabilization and disinfection. Published for Water Research Center. Chichester, England: E.Harwood, Ltd.

Smith, J. E.,Jr.,and J. B. Farrell, Vector attraction reduction issues associated with the Part 503 Regulations and Supplemental Guidance, Proceedings of the Water Environment Federation's Conference, "International Management of Water and Wastewater Solids for the 21 st Century': A Global perspective, June 19-22, 1994, Washington, DC, pp 131 l-1330.

Switzenbaurm, Michael S., L.H. Moss, E.Epstein, A. B. Pincince, J.F. Donovan. 1997. Defining biosolids stability: a basis for public and regulatory acceptance. Water Environment Research Foundation.

WEF/ASCE. 1998. WEF Manual of Practice No. 8, Design of Municipal Wastewater Treatment Plants. Pub. WEF (Alexandria, VA) and ASCE (New York, NY).

WERF. 1997. Defining biosolids stability: A basis for public and regulatory acceptance. Pub. WERF. Alexandria, VA.

U.S. EPA 1992. Technical support document for reduction of pathogens and vector attraction in sewage sludge. EPA 822/R-93-004. EPA, Washington, D.C.

Chapter 9
Sampling Procedures and Analytical Methods

9.1 Introduction

Many of the Part 503 Subpart D pathogen and vector attraction reduction requirements call for monitoring and analysis of the sewage sludge to ensure that microbiological quality and vector attraction reduction meet specified requirements (see Chapters 4 to 6 for a description of the requirements).The purpose of this chapter is to describe procedures for obtaining representative samples and insuring their quality and integrity. It also summarizes the analytical methods required under Part 503, and directs the reader to other sections of this document that describe some of those methods.

Sampling personnel will also benefit from reading expanded presentations on the subject. "Standard Methods" (APHA, 1992), "Principles of Environmental Sampling" (Keith, 1988), "Samplers and Sampling Procedures for Hazardous Waste Streams" (EPA, 1980), 'Sludge Sampling and Analysis Guidance Document" (EPA, 1993), and ASTM Standard E 300-86, "Standard Practice for Sampling Industrial Chemicals" (ASTM, 1992a) are highly recommended. The latter publication provides an in-depth description of available sampling devices and procedures.

When referring to other publications, it is important to note that most guidance on specific sampling techniques is directed toward chemical analysis. Procedures described may be inappropriate for microbiological sampling because they expose the samples to possible contamination, or may be appropriate only after some modification to reduce the risk of microbial contamination during sampling.

9.2 Laboratory Selection

A very important, but often overlooked component of pathogen and vector attraction monitoring is selecting an appropriate analytical laboratory. The analysis of sewage sludge or biosolids for indicator and pathogenic organisms is more complex than water analysis. Solid samples such as biosolids are prepared differently than water samples and also typically contain a much higher background microbial population than water contains. Biosolids products such as compost can be very heterogeneous, requiring special sample preparation procedures. It is therefore important to use a laboratory that has developed an expertise through the routine analysis of biosolids products. Regional and state sludge coordinators should be contacted for assistance in selecting a qualified laboratory.

To ensure that a laboratory has adequate experience with biosolids analyses, the following information should be obtained and reviewed.

- For how long has the laboratory been analyzing biosolids for the specified parameters?
- Approximately how many biosolids samples does the laboratory analyze per week or month?
- For how many wastewater treatment facilities is the laboratory conducting the specified analyses?
- A list of references.
- Does the laboratory have a separate and distinct microbiology lab?
- Does the laboratory have microbiologists on staff? Request and review their resumes
- Who will actually perform the analyses?
- Is the laboratory familiar with the analytical procedures including sample preparation, holding times, and QA/QC protocols?

A laboratory tour and reference check is also recommended. A good laboratory should be responsive, providing requested technical information in a timely manner. It is the biosolids generator's responsibility to provide accurate analytical results. Consequently, the selection of an appropriate laboratory is an important component of developing a biosolids monitoring plan.

9.3 Safety Precautions

Sewage sludges that are being sampled should be presumed to contain pathogenic organisms, and should be handled appropriately. Both the sampler and the person carrying out the microbiological analysis must take appropriate precautions. Safety precautions that should be taken when sampling and when analyzing the samples are discussed in Standard Methods (APHA, 1992) in Sections 1060A and 1090C.

Individuals performing sampling (usually employees of wastewater treatment works) should receive training in the microbiological hazards of sewage sludge and in safety

precautions to take when sampling. Laboratory personnel should be aware that the outside of every sample container is probably contaminated with microorganisms, some of which may be pathogens. Personal hygiene and laboratory cleanliness are also extremely important. Several safety practices that should be standard procedures during sample collection and analysis are:

- Gloves should be worn when handling or sampling untreated sewage sludge or treated biosolids.
- Personnel taking the samples should clean sample containers, gloves, and hands before delivering the samples to others.
- Hands should be washed frequently and always before eating, smoking, and other activities that involve hand-to-mouth contact.
- Photocell-activated or foot-activated hand washing stations are desirable to reduced spreading of contamination to others.
- !Employees should train themselves to avoid touching their lips or eyes.
- Mouth pipetting should be forbidden.
- Smoking should not be allowed inside the lab.

Employees involved in sample collection (or any other activity where they are exposed to wastewater or sewage sludges) should review their immunization history. At a minimum, employees should be immunized against tetanus. However, employees should consider immunization for other diseases, particularly hepatitis A and B. Employees should also consider having a blood sample analyzed to determine if they still have active antibodies for the various immunizations they received as children.

Personnel analyzing sewage sludge or biosolids samples should receive training in awareness and safety concerning biohazards. Because microbiological laboratories have safety programs, this subject is not covered in depth here. A facility's sampling plan should include a section on microbiological hazards and appropriate safety practices or, alternatively, refer the reader to another document where this information is presented.

9.4 Requirements for Sampling Equipment and Containers

Containers

Sampling containers may be of glass or plastic that does not contain a plasticizer (Teflon, polypropylene, and polyethylene are acceptable). Plastic bags are especially useful for thick sewage sludges and free-flowing solids. Presterilized bags are available. Stainless steel containers are acceptable, but steel or zinc coated steel vessels are not appropriate. In addition to providing guidance on appropriate containers for specific analyses, private analytical laboratories will typically provide sample containers at no cost. Sampling containers used for microbiological analyses should be sterile. Sampling tools that come in contact with the actual sample should be constructed of stainless steel, which is easily cleaned, and sterilized. Tools made of wood, which is difficult to sterilize because of porosity, should not be used.

Equipment

The sampling equipment used is primarily dependent on the type of material being sampled. For relatively high solids content biosolids, a hand trowel or scoop may be adequate, whereas, collecting stratified samples from a lagoon requires more sophisticated and specialized equipment. Automated sampling equipment, as commonly used for wastewater, should not be used. Such equipment can cause solids separation during sampling and is difficult to clean, resulting in cross contamination. Sampling equipment should be constructed of a non-corrosive materials, such as stainless steel, Teflon, or glass, that can be thoroughly cleaned. Sampling equipment should be dedicated for this task and should not be used for other applications. Equipment should be cleaned well with detergent and a nylon scrub brush after each use and stored inside in a dedicated location. The types of sampling equipment and their applications are presented in Table 9-l. The use of this equipment is discussed in greater detail in Sections 9.6 and 9.7.

Sterilization

The containers and tools used for sampling must be sterilized if the biosolids product is to be analyzed for Class A microbiological parameters. Alternatively, pre-sterilized, disposable scoops, and other sampling devices can be purchased, alleviating the need to sterilize sampling equipment. Conservative microbiological practice also requires sterilization of containers and sampling tools used for collecting samples to be tested for meeting the Class B requirements. Sample containers and equipment should be scrupulously cleaned prior to actual sample collection. After the samples are collected, the sampling equipment should be cleaned well with soap and water and put away until the next sampling event. Equipment should be dedicated to sampling and not used for other activities. Only equipment that touches the actual sample must be sterilized. Equipment such as shovels or heavy equipment used to access a sludge pile interior does not need to be sterilized, but should be clean, as long as another sterile sample collection device (such as a hand trowel) is used to access and collect the actual sample. Sterilization is not required when collecting samples of sewage sludge to be used in vector attraction reduction tests, but all equipment must be clean.

Either steam or a sterilizing solution such as sodium hypochlorite (household bleach) should be used for sterilizing equipment. If bleach is used, equipment must be rinsed thoroughly in order to prevent residual bleach from having an effect on the microbial population in the sample. Equipment should be thoroughly washed with water, soap, and a brush prior to sterilization. If an autoclave or large

Table 9-i. Equipment used for Collecting Sewage Sludge Samples

Equipment	Application
Composite Liquid Waste Sampler (Coliwasa)	The Coliwasa is a device employed to sample free-flowing sewage sludges contained in drums, shallow tanks, pits, and similar containers. It is especially useful for sampling wastes that consist of several immiscible liquid phases. The Coliwasa consists of a glass, plastic, or metal tube equipped with an end closure that can be opened and closed while the tube is submerged in the material to be sampled.
Weighted Bottle	This sampling device consists of a glass or plastic bottle, sinker, stopper, and a line that is used to lower, raise, and open the bottle. The weighted bottle is used for sampling free flowing sewage sludges and is particularly useful for obtaining samples at different depths in a lagoon. A weighted bottle with line is built to the specifications in ASTM Method D270 and E300.
Dipper	The dipper consists of a glass or plastic beaker clamped to the end of a two- or three-piece telescoping aluminum or fiberglass pole that serves as the handle. A dipper is used for obtaining samples of free flowing sewage sludges that are difficult to access. Dippers are not available commercially and must be fabricated.
Sampling Thief	A thief consists of two slotted concentric tubes, usually made of stainless steel or brass. The outer tube has a conical pointed tip that permits the sampler to penetrate the material being sampled. The inner tube is rotated to open and close the sampler. A thief is used to sample high solids content materials such as composted and heat dried biosolids for which particle diameter is less than one-third the width of the slots. Thief samplers are available from laboratory supply companies.
Trier	A trier consists of a tube cut in half lengthwise with a sharpened tip that allows the sampler to cut into sticky materials such as dewatered cake and lime stabilized biosolids. A trier samples moist or sticky solids with a particle diameter less than one-half the diameter of the trier. Triers 61 to 100 cm long and 1.27 to 2.54 cm in diameter are available from laboratory supply companies
Auger	An auger consists of sharpened spiral blades attached to a hard metal central shaft. An auger can be used to obtain samples through a cross section of a biosolids stockpile. Augers are available at hardware and laboratory supply stores.
Scoops and Shovels	Scoops are used to collect samples from sewage sludge or biosolids stockpiles, shallow containers, and conveyor belts. Stainless steel or disposable plastic scoops are available at laboratory supply houses. Due to the difficulty of sterilizing shovels and other large sampling equipment, this type of equipment should only be used for accessing the center of stockpiles and should not be used for collecting the actual sample.

pressure cooker is available, enclose the sampling tool in a kraft paper bag and place the bag in the autoclave. A minimum period of 30 minutes at a temperature of 121°C is required for sterilization. The kraft paper bag keeps the sampling device from becoming contaminated in the field. A steam cleaner can also be used to sterilize sampling equipment. Place the equipment in a heat resistant plastic bucket and direct steam onto the equipment for a minimum of 10 minutes. When done, place the sterilized equipment in a kraft paper bag.

Many facilities do not have an autoclave or steam cleaning equipment and will need to use a sterilizing solution to sterilize equipment. A 10% household bleach solution (1 part bleach, 9 parts water) is readily available and works well. However, bleach is corrosive and may also affect the microbial population of a sample and does need to be adequately removed from the equipment prior to sample collection. Make up the 10% solution in a clean plastic bucket. Immerse each piece of clean equipment in the solution for a minimum contact time of a minute. Rinse the equipment in another bucket containing sterile or boiled water. Let the equipment air dry for a few minutes or dry with sterile paper or cloth towels. After drying, place the equipment in a paper bag. Sterile plastic bags obtained from a scientific equipment supplier can also be used for short-term sterile equipment storage.

9.5 Sampling Frequency and Number of Samples Collected

The primary objective of biosolids monitoring is to assure that all of the biosolids produced meets the regulatory requirements related to land application. It is obviously not feasible to sample and analyze every load of biosolids leaving a facility, nor is it necessary. However, a sampling plan does need to adequately account for the variability of the biosolids. This entails collecting samples at an adequate frequency and analyzing a sufficient number of samples. The minimum sampling frequency and number of samples to be analyzed are shown in 40 CFR Part 503. As shown in Table 3-4, the sample collection frequency is determined by the amount of biosolids used or disposed.

The number of samples which must be analyzed for compliance with Class A microbiological parameters is not specified, however, it is strongly recommended that multiple samples per sampling event be analyzed for biosolids. The number of samples taken must be sufficient to adequately represent biosolids quality. It must be noted that for Class A biosolids, analytical results are not averaged: every sample analyzed must meet the Class A requirements.: "Either the density of fecal coliform in the sewage sludge must be less than 1,000 MPN per gram of total solids (dry weight basis), or the density of *Salmonella* sp.

bacteria in sewage sludge must be less than 3 MPN per 4 grams of total solids (dry weight basis).".

To meet Class B Alternative 1 requirements, seven samples must be taken and the geometric mean of results must meet the 2.0 x 10^6 MPN fecal coliform per dry gram limit (see Chapter 5). It is recommended that the samples be taken over a two-week period in order to adequately represent variability in the sewage sludge.

The actual sampling and analysis protocol is typically developed by the facility and reported to the regulatory authority, which can require a more stringent sampling and analysis protocol than that stipulated in the Part 503 regulation. In some cases, the regulatory authority may initially require a more stringent monitoring schedule which may be relaxed once product consistency is established. The biosolids preparer should carefully consider the treatment process, analytical variability, end-use, and other factors when determining the frequency and number of samples to be analyzed. Collecting samples more frequently or analyzing more samples will help to ensure the product meets the regulatory criteria and that pathogen and vector attraction reduction goals have been met. More information on this subject is available in Chapter 10.

It is recommended that additional sampling be conducted for heterogeneous biosolids products. A single grab sample may adequately represent the sewage sludge in a digester that is being mixed, but might not adequately represent several hundred yards of compost product stored in several stockpiles. Likewise, a facility that conducts a single annual analysis should consider more frequent monitoring, particularly if the analytical results from the annual analysis are near the regulatory limit. It is a facility's responsibility and in the facility's best interest to develop a monitoring plan that assures product quality.

9.6 Sampling Free-Flowing Sewage Sludges

Sewage sludges below about 7% solids behave, at worst, like moderately viscous liquids such as an SAE 20 lubricating oil. They flow freely under small pressure gradients, and flow readily into a sample bottle. They are heterogeneous, and concentration gradients develop upon standing. Generally settling is slow and is overcome by good mixing.

Liquid sewage sludges may be flowing in pipes, undergoing processing, or stored in concrete or metal tanks, in tank cars or tank trucks, or in lagoons. This section describes procedures for sampling from these various situations, except for lagoons, which are discussed in Section 9.7.

Filling Containers

Liquid sewage sludge samples are usually transferred into wide mouth bottles or flexible plastic containers. Sewage sludges can generate gases, and pressure may build up in the container. Consequently, the bottle or container is generally not filled. If the sewage sludge is to be used for the oxygen uptake test, the sample bottle should not be more than half full, to provide some oxygen for respiration of the microorganisms in the sewage sludge. Conversely, if the sewage sludge is to be used for the additional anaerobic digestion test for vector attraction reduction, it is important that it not be exposed to oxygen more than momentarily. Consequently, sample bottles must be completely filled to the top. Bottles should have closures that can pop off, or else be made of flexible plastic that can both stretch and assume a spherical shape to relieve any internal pressure that develops.

The containers used to collect the samples can be widemouth bottles that can be capped, or pails. If a pail is used and only part of its contents will be taken as a sample, the sample should be transferred to a bottle at the sampling location. Preferably, the transfer should be made by use of a ladle rather than by pouring, since some settling can occur in the pail, particularly with primary or mixed sewage sludges of solids contents below about 3%.

Collecting Liquid Sewage Sludge Samples

If liquid sewage sludges are to be sampled, it is most desirable to sample them as they are being transferred from one vessel to another. Preferably this is done downstream of a pump that serves to mix the sewage sludge thoroughly. Ideally, the sample is taken through a probe facing upstream in the center of the discharge pipe and is withdrawn at the velocity of the liquid at the center-line of the pipe. This approach generally is not possible with sewage sludge, because fibrous deposits can build up on the probe and plug up the pipeline.

Sampling through a side tap off the main discharge pipe is adequate only if the flow is turbulent and the sample point is over ten pipe diameters downstream from the pipe inlet (e.g., for a 3-inch [7.6-cm] pipe, 30 inches [76 cm] downstream) or the tap is downstream from a pump. For any kind of a slurry, the fluid at the wall contains fewer particles than the bulk of the fluid in the pipe. The sample should be withdrawn fast enough so that it minimizes the amount of thinned-out fluid from the outside pipe wall that enters the sample.

If the sewage sludge discharges into the open as it is transferred from one vessel to another, it can be sampled by passing a sample container through the discharge. The container should be large enough to catch the whole discharge during the sampling interval, rather than, for example, just sampling the center or the edge of the discharge. The sample container could be a pail or a beaker at the end of an extension arm. Sample volume should be about three times what is needed for the analyses planned.

The collection of a representative sample often requires the use of time cornpositing procedures. For example, if a digester is being sampled during a withdrawal that takes about 15 minutes, a sample can be taken during the first, second, and third 5-minute period. The three separate

samples should be brought back to the laboratory and cornposited into a single sample. If the sample is being analyzed for bacteria, viruses, or vector attraction reduction, the composite should be prepared within an hour of collecting the first individual grab sample. A longer time period might allow microbiological changes to occur in the first sample taken. Composite sampling over 24 hours is possible for viable helminth ova analysis, provided that the ova in the sample are not exposed to chemical or thermal stress such as temperatures above 40°C (104°F) or certain chemicals such as ammonia, hydroxides, and oxidants.

Sampling Sewage Sludge in Tanks

The purpose of the sampling is to determine the properties of the entire mass of the sewage sludge, rather than, for example, to find out if there is a gradient in the property at various points in the tank. This requires that the tank be well-mixed, otherwise many subsamples must be taken throughout the tank and cornposited. Large tanks, even if they are well-mixed, have the potential for developing gradients in composition. An enclosed tank such as an anaerobic digester must be sampled through pipelines entering the digester. A minimum of three taps on a side wall of the enclosed tank is recommended. The sample tap pipe should project several feet into the tank. Precautions must be taken to minimize contamination from sample collection lines. When a sample is taken, enough material must be withdrawn to thoroughly flush the line before the sample is collected. This helps flush any contaminants in the sample line and assure that a representative sample is collected from the tank. The sample line should be back-flushed with water after the sample is withdrawn to clean out residual sewage sludge and prevent microbial growth. Sampling should be conducted when the tank is being agitated. An open tank such as an aerobic digester can be sampled by drawing a vacuum on a vacuum-filtering flask connected to a rigid tube lowered to the desired level in the tank. A weighted sampling bottle may also be used to sample the liquid at the desired depth in the tank (see ASTM E30086, Par. 21, in ASTM [1992a]).

9.7 Sampling Thick Sewage Sludges

If sewage sludges are above 7% solids, they take on "plastic" flow properties; that is, they require a finite shear stress to cause flow. This effect increases as the solids content increases. Solids may thicken in lagoons to 15% solids. At these concentrations, they will not flow easily and require a substantial hydrostatic head before they will flow into a sample bottle.

Sampling of sewage sludge stored in lagoons may be very difficult, depending on the objectives of sampling and the nature of the sewage sludge in the lagoon. The thickened sewage sludge solids are generally nonuniformly distributed in all three dimensions. It is desirable first to map the distribution of depth with length and width to determine where the sampling should be concentrated. A length-width grid should be established that takes the nonuniformity of the solids deposit into account. ASTM E300-86, Figure 19 (ASTM, 1992a), shows a grid for sampling a uniform deposit in a railroad car.

The layer of water over the sewage sludge complicates the use of many types of tube samplers because the overlying water should not be included in the sample. A thief sampler (ASTM, 1992a) that samples only the sewage sludge layer may be useful. Weighted bottle samplers (ASTM, 1992a) that can be opened at a given depth can be used to collect samples at a desired depth. Samples at three depths can be taken and cornposited. Most likely the sewage sludge will be as much as twice as high in solids content at the bottom of the sewage sludge layer as at the top. Cornpositing of equal volumes of samples from top, middle, and bottom produces an excellent mass-average sample and adjusts for this difference in solids content. Generally there is no point in determining the gradient with depth for microbiological and VAR parameters, because there is no practical way of separately removing layers of sewage sludge from a lagoon. Determining whether there are gradients with length and width makes more sense because, if desired, sewage sludge could be removed selectively from part of a lagoon, leaving behind the unacceptable material.

Sewage sludges from dewatering equipment such as belt filter presses and centrifuges can have a solids content up to 35% and even higher following some conditioning methods. High solids content sewage sludges are easy to sample if they are on moving conveyors, as described in Section 9.5. However, if the sewage sludge is stored in piles, obtaining a representative sample requires more planning and a greater overall effort. As a result of the different environment between the pile surface and interior a gradient will develop over time in the sewage sludge storage pile. The sampling methodology used needs to address this potential gradient between the pile surface and interior. Sampling devices such as augers (a deeply threaded screw) are used on high solids cakes (ASTM, 1992a) to collect a cross sectional sample. The auger is 'turned into the pile and then pulled straight out. The sample is removed from the auger with a spatula or other suitable device." Alternatively, a shovel can be used to collect subsamples for cornpositing from the pile interior. The pile should be sampled in proportion to its mass; more samples are taken where the pile is deeper.

9.8 Sampling Dry Sewage Sludges

For purposes of this discussion, "dry" sewage sludges contain as much as 60% water. They include heat dried and composted sewage sludges, and sewage sludges from dewatering processes, such as pressure filtration, that produce a cake which is usually handled by breaking it up into pieces. Some centrifuge cakes are dry enough that they are comprised of small pieces that remain discrete when piled.

Dry sewage sludges are best sampled when they are being transferred, usually on conveyors. Preferably material across the entire width of the conveyor is collected for

a short period of time. Several of these across-width samples are collected and combined into a time-composite sample. if the entire width of the conveyor cannot be sampled, the sample is collected from various points across the breadth of the conveyor, and a space and time-cornposited sample is collected.

Collecting a representative sample from a stockpile containing a dried sewage sludge product poses a greater challenge than collecting the sample from a conveyor. The settling and classification of the material and the different environments between the pile edge and interior must be considered. When a material comprised of discrete particles is formed into a pile, classification occurs. If the particles are homogeneous in size and composition, a representative sample can be easily obtained (assuming the sample is collected within 24 hours of pile construction). However if the particles are of a different size or composition, an unequal distribution of the particles may result during settling. For example, a composted sewage sludge may be heterogeneous, with respect to particle composition, even when oversized bulking agents have been removed. It is important that the edges and interior of such piles are properly weighted as part of the sample collection procedure. A sampling grid that prevents bias, such as that presented in ASTM E300-86, Item 31.4 (ASTM, 1992a), should be used.

The heterogeneous nature and presence of large particles in some composted sewage sludges causes another problem in sampling. For example, most augers and sampling thiefs will be ineffective in getting a representative sample from the interior of a pile containing large wood chips and fine composted sewage sludge. There may be no substitute for digging with a shovel to get to the desired location.

Stockpile sampling is also made more difficult by the constant evolution of the characteristics of stored material. Immediately after a sewage sludge stockpile is constructed, physical, chemical, and biological changes begin to occur within and on the surface of the stockpile. Within a period as short as 24 hours, the characteristics of the surface and outer part of the pile can differ substantially from that of the pile interior. The outer part of a pile tends to remain at or near ambient temperature, loses moisture through evaporation, and volatilizes some compounds such as ammonia. In contrast, pile interiors retain heat (achieving temperatures that can be 40°C greater than the pile surface), but lose little moisture or chemical compounds through evaporation and volatilization. As a result, the level of microbial growth and activity within the pile and on the pile surface will also differ. The potential for growth of fecal coliform bacteria in mesophilic regions of the pile is of particular concern. If a sewage sludge stockpile is more than one day old, the sample should be collected from a pile cross section. This is especially important when there is a large temperature gradient between the pile surface and interior.

9.9 Control of Temperature, pH, and Oxygenation After Sample Collection Samples for Microbial Tests

Table 9-2 summarizes the maximum holding times and temperatures for sewage sludge samples taken for microbial analyses. All samples should be cooled to appropriate temperatures immediately after they are collected to minimize changes in indicator organism and pathogen populations. For example, enteric viral and bacterial densities are noticeably reduced by even 1 hour of exposure to temperatures of 35°C (95°F) or greater. The requirement for cooling limits the practical size of the sample collection container. A gallon sample bottle takes much longer to cool than a quart bottle. Use of bottles no larger than a quart is recommended for most samples, particularly if the sewage sludge being sampled is from a process operated at above ambient temperature. Granular solids and thick sewage sludges take a long time to cool, so use of containers smaller than one quart is advised. For rapid cooling, place the sample container in a slurry of water and ice. Placing the sample container in a cooler containing bagged ice or "blue ice" is effective in maintaining low temperatures but several hours can elapse before this kind of cooling reduces sample temperature to below 10°C (50°F) (Kent and Payne, 1988). The same is true if warm samples are placed in a refrigerator. The presence or absence of oxygen is not a serious concern for the microbiological tests if the samples are promptly cooled.

Table Q-2. Analytical Methods Required Under Part 503

Analysis	Methodology	Maximum Holding Time[3]/Temperature
Enteric Viruses	American Society for Testing and Materials Method D 4994-89 (ASTM, 1992b)[1]	-18°C (0°F); up to 2 weeks
Fecal Coliform	Standard Methods Part 9221 E or Part 9222 D (APHA, 1992)[2]	4°C (39.2°F) (do not freeze); 24 hours
Salmonella sp. Bacteria	Standard Methods Part 926OD (APHA, 1992)[2] or Kenner and Clark (1974) (see Appendix G of this document)	4°C (39.2°F) (do not freeze); 24 hours
Viable Helminth Ova	Yanko (1987) (see Appendix I of this document)	4°C (39.2°F) (do not freeze); 1 month
Specific Oxygen Uptake Rate (SOUR)	Standard Methods Part 27108 (APHA, 1992)	20°C (sewage sludge must be digested in the 10-30°C range); 2 hours
Total, Fixed, and Volatile Solids	Standard Methods Part 2540G (APHA, 1992)	NA
Percent Volatile Solids Reduction	Appendix C of this document	NA

[1]Appendix H of this document presents a detailed discussion of this method.
[2]Method SM-9221 E, the MPN procedure, is required for analysis of Class A biosolids and recommended for Class B biosolids. Method SM-9221 D, the membrane filtration procedure is also allowable for Class B biosolids. See Appendix F of this document for recommended sample preparation procedures and a discussion of the reporting of results.
[3]Time between sampling and actual analysis, including shipping time.

Standard Methods (APHA, 1992) states that if analysis for bacterial species (fecal coliform and Salmonella sp.) will commence within 1 hour after sample collection, no temperature adjustment is required. If analysis will commence between 1 and to 6 hours after following collection, the sample should be immediately cooled to at least 10°C. If analysis will commence between 6 and 24 hours after collection the sample should be immediately cooled to 4°C. The sample should never be frozen and analysis must commence within 24 hours of collection.

Proper planning and coordination with the courier service and analytical laboratory are essential if bacterial analyses are to be conducted within 24 hours of sample collection. The laboratory needs to be notified several days in advance so they can be prepared to initiate the analysis within several hours of receiving the sample. If they are not notified, the laboratory may not be adequately prepared and another day may lapse before the samples are analyzed. Actual sample collection should be conducted in the afternoon, within a few hours of the sample courier's arrival. If the samples are collected in the morning, a greater than 24-hour period may pass before the laboratory actually begins the analysis.

Follow-up with the laboratory is important to determine the actual sample holding time and temperature of the sample when it was received. This information can be used to improve the overall sample collection and transfer procedure. Feedback received from the lab regarding sample condition and holding times may also provide an explanation for erroneous or unexpected test results.

The requirement for prompt chilling of samples is appropriate for viruses as well as bacteria. There are far fewer laboratories capable of carrying out virus tests than can conduct bacterial analyses, so time between sample collection and analysis can routinely exceed 24 hours. Fortunately, viruses are not harmed by freezing. Typically, virology laboratories store samples at -70°C (-94°F) before analysis. Samples can be frozen in a -18°C (0°F) freezer and stored for up to 2 weeks without harm. Samples should be frozen, packed in dry ice, and shipped overnight to the analytical laboratory.

Viable helminth ova are only slightly affected by temperatures below 35°C (95°F), provided chemicals such as lime, chlorine, or ammonia are not utilized in the treatment process. Nevertheless, chilling to 4°C (39.2°F) is advised. If the samples are held at this temperature, a period of a month can elapse between sampling and analysis. Freezing should be avoided because the effect of freezing on helminth ova is not well understood.

Vector Attraction Reduction Tests

For the vector attraction reduction tests that measure oxygen uptake, or additional anaerobic or aerobic digestion (see Appendix D), the samples must be kept at the temperature at which they were collected. This sometimes can be done just by collecting a large sample in a large container. Covering the sample with an insulating blanket or placing it in an insulated box provides adequate protection against temperature change in most cases. Desired temperature can be maintained in the box by adding a "hot water bottle" or a bag of blue ice.

Depending on the whether the sewage sludge is from an aerobic process or anaerobic digestion, the presence or lack of oxygen will determine which vector attraction reduction test is appropriate and therefore how the sample should be handled. For aerobic sewage sludges, a lack of oxygen will interfere with the metabolic rate of the aerobic microorganisms in the sample. Similarly, presence of oxygen will seriously affect or even kill the anaerobic organisms that convert organic matter to gases in anaerobic digestion. With samples taken for SOUR analysis, it has been the experience of some investigators that if the test is not run almost immediately after collection (within about 15 minutes), that erroneous results are obtained. The additional aerobic digestion test is more "forgiving" (because it is a long-term test and shocked bacteria can revive); up to 4 hours of shortage of oxygen can be tolerated. For the additional anaerobic digestion test, the sample containers should be filled to exclude air. In any subsequent operations where there is a freeboard in the sample or testing vessel, that space should be filled with an inert gas such as nitrogen.

No pH adjustment is to be made for any of the vector attraction reduction tests. For those vector attraction processes that utilize lime, the only requirement is to measure pH after the time periods indicated in the vector attraction reduction option (see Section 8.7).

9.10 Sample Cornpositing and Size Reduction

The amount of sample collected in the field generally far exceeds the amount needed for analysis. The field sample must therefore be reduced to a manageable size for the analyst to handle. As for all sample handling, sample size reduction is more difficult for microbial samples than for samples taken for vector attraction reduction tests because of the potential for microbial contamination. The laboratory may be better equipped to perform subsampling than samplers in the field.

Microbial Tests

Freely flowing liquids samples can be adequately mixed in the sample bottles by shaking the bottles. There must be room in the bottle for adequate mixing. Cornpositing of smaller samples is accomplished by pouring them into a larger bottle with adequate freeboard and mixing it by shaking or stirring it thoroughly with a sterile paddle. Pouring off a portion of the contents of a large container into a smaller bottle is not an acceptable procedure because the top layer of any slurry always contains fewer solids than do lower layers. Sampling with a pipette with a wide bore is an acceptable alternative, provided the bore of the pipette does not restrict the entry of solid particles. The

sample should be drawn into the pipette slowly and the tip moved through the sample to minimize selective collection of liquid over solid particles.

Sample size reduction for thick sewage sludges is difficult, because they cannot be mixed by shaking. Stirring with a mechanical mixer or a paddle is often inadequate (recall how long it takes to mix a can of paint). A satisfactory approach is to hand mix a composite of subsamples, and then take a large number of small grabs from the composited sample to form the smaller sample for the analyst.

Dry solids samples can generally be mixed adequately by shaking if there is sufficient head space in the sample container, but the individual particles are frequently large and must be reduced in size to get a representative sample. If the particles are large and a number of subsamples must be combined into a large composite, it may be necessary to reduce the particle size before they are cornposited. This can be done in a sterile covered chopper, blender, or grinder. The individual subsamples are then combined and mixed by shaking, rotating, and tumbling. A smaller composite is then prepared by combining a number of grabs from all parts of the combined sample. Many facilities do not have adequate equipment needed to perform this size reduction procedure. However, most analytical laboratories have this capability and will typically perform this procedure at a nominal cost. Coordination with the analytical laboratory regarding subsampling is an important part of the sampling and analysis procedure that should not be ignored. Some other sample size reduction methods, such as "coning and quartering" (ASTM, 1992a) may be use only if aseptic handling practices are observed. It should be noted that particle size reduction is not appropriate if the large pieces in the sample are not sewage sludge but are other materials which have been added to the sewage sludge for processing purposes. For the purpose of microbial or volatile solids reduction testing, additives such as wood chips should be removed from the sample before size reduction or sample preparation (see Section 10.5). It is recommended in these cases that a one-quarter inch mesh sieve be used for this purpose.

Vector Attraction Reduction Tests

The lack of a need to prevent microbial contamination makes cornpositing and size reduction easier for vector attraction reduction tests than for the microbial tests. However, there is a need to keep the aerobic samples aerobic and to prevent the anaerobic samples from coming into contact with air. Subsamples for the anaerobic tests can be collected into individual bottles at the sampling location. As noted above, these sample bottles should be filled completely and capped. A brief exposure to air will not cause a problem, but any prolonged exposure, such as might occur when several subsamples are being blended together and reduced in size for a representative composite sample, must be avoided. One acceptable sample size reduction procedure is to flush a large sterile bottle with nitrogen, then pour in the subsamples and blend them together with nitrogen still bleeding into the vessel. Alternatively, the nitrogen-filled vessel could be flushed with more nitrogen after the admission of the subsamples, capped, and then shaken thoroughly to accomplish the blending. Analytical laboratories generally can perform this size reduction procedure.

9.11 Packaging and Shipment

Proper packaging and shipment are important to ensure that the samples arrive in good condition (proper temperature, no spillage) within the specified time frame.

Sealing and Labeling Sample Containers

Sample containers should be securely taped to avoid contamination, and sealed (e.g., with gummed paper) so it is impossible to open the container without breaking the seal. Sealing ensures that sample integrity is preserved until the sample is opened in the laboratory. A permanent label should be affixed to each sample container. At a minimum the following information should be provided on each sample container:

- Type of analysis to be performed (e.g., Salmonella sp., fecal coliform bacteria, enteric virus, or viable helminth ova)
- Sample identification code (if used) or a brief description of the sample (that distinguishes it from other samples) if no sample code system is used
- Sample number (if more than one sample was collected at the same point on the same day)

Other information may include:

- Facility name, address and telephone number
- Date and time the sample was taken
- Facility contact person

This information should also be included on an enclosed chain of custody form.

Shipment Container

A soundly constructed and insulated shipment box is essential to provide the proper environment for the preserving sample at the required temperature and to ensure the sample arrives intact. Small plastic cased coolers are ideal for sample shipping. It is recommended that the outside of the shipment container be labeled with the following information:

- The complete address of the receiving laboratory (including the name of the person responsible for receiving the samples and the telephone number)
- Appropriate shipping label that conforms to the courier's standards
- Number of samples included (i.e. "This cooler contains 10 samples")

- The words “Fragile” and “This End Up”

To maintain a low temperature in the shipment box, a blue-ice type of coolant in a sealed bag should be included in the box. If the blue ice has been stored in a 0°F (-18°C) freezer (e.g., a typical household freezer), the coolant should be “tempered” to warm it up to the melting point of ice (0°C [32°F]) before it is placed around the sample. Additional packing material (bubble wrap, Styrofoam peanuts, balled-up newspaper) should be placed in the shipping container to fill in empty space to prevent sample containers from moving and potentially breaking or spilling during shipping. It is also recommended that the courier be contacted in order to determine if there are any special requirements for the shipping of this type of sample.

Adherence to Holding and Shipment Times

Adherence to sample preservation and holding time limits described in Section 9.6 is critical. Samples that are not processed within the specified time and under the proper conditions can yield erroneous results, especially with the less stable microorganisms (i.e., bacteria). Make sure the analytical laboratory reports the date and time when the samples arrived, and total holding time (period from when the sample was collected to the initiation of analysis). This information will be valuable for improving future sample events and maintaining quality control.

9.12 Documentation

Sampling Plan

It is recommended that all procedures used in sample collection, preparation, and shipment be described in a sampling plan. At a minimum, a sampling plan should provide the following information:

- Sample collection locations
- Volume of sample to be collected
- Sample cornpositing procedures
- Days and times of collection
- Required equipment
- Instructions for labeling samples and ensuring chain of custody
- A list of contact persons and telephone numbers in case unexpected difficulties arise during sampling

If a formal sampling plan is not available, a field log that includes instructions and a sample collection form may be used (EPA, 1980).

Sampling Log

All information pertinent to a sampling event should be recorded in a bound log book, preferably with consecutively numbered pages. At a minimum, the following information should be recorded in the log book.

- Purpose of sampling event
- Date and time of sample collection
- Location where samples were collected
- Grab or composite sample (for composite samples, the location, number, and volume of subsamples should be included)
- Name of the person collecting the sample(s)
- Type of sewage sludge
- Number and volume of the sample taken
- Description of sampling point
- Date and time samples were shipped

Chain of Custody

To establish the documentation necessary to trace sample possession from the time of collection, it is recommended that a chain-of-custody record be filled out and accompany every sample. This record is particularly important if the sample is to be introduced as evidence in litigation. Suggested information for the chain-of-custody record includes, at a minimum:

- Collector’s name
- Signature of collector
- Date and time of collection
- Place and address of collection
- Requested preprocessing (subsampling, compositing, particle size reduction)
- Requested analyses
- Sample code number for each sample (if used)
- Signatures of the persons involved in the chain of possession

A good rule of thumb is to record sufficient information so that the sampling situation can be reconstructed without reliance on the collector’s memory. Chain of custody forms can be obtained from the laboratory and should be used even if the laboratory is on-site and part of the treatment facility.

9.13 Analytical Methods

Part 503.8(b) of the Par-t 503 regulation specifies methods that must be used when analyzing for enteric viruses; fecal coliform; Salmonella sp.; viable helminth ova; specific oxygen uptake rate; and total, fixed, and volatile solids. Table 9-2 lists the required methods. Complete references for these methods can be found in Chapter 12, and recommended sample preparation and analytical methods can be found in the appendix as listed below.

Calculating volatile solids reduction	Appendix C
Conducting additional digestion and specific oxygen uptake rate (SOUR) tests	Appendix D
Determination of residence time in digesters	Appendix E
Sample preparation — fecal coliform and Salmonella sp. analysis	Appendix F
Analytical method — Salmonella sp.	Appendix G
Analytical method — enteroviruses in sewage sludge	Appendix H
Analytical method — viable helminth ova	Appendix I

As of the time of publication of this document, the allowable analytical methodologies are as listed above. However, in the case of fecal coliform analysis for Class B-Alternative 1, it is recommended that the MPN method be used instead of the membrane filter test (the MPN method is required for Class A fecal coliform analysis), and that the Kenner and Clark methodology be used for Salmo*nella* sp. analysis.

9.14 Quality Assurance

Quality assurance involves establishing a sampling plan and implementing quality control measures and procedures for ensuring that the results of analytical and test measurements are correct. A complete presentation of this subject is beyond the scope of this manual. A concise treatment of quality assurance is found in Standard Methods (APHA, 1992) and is strongly recommended. Parts 1000 to 1090 of Standard Methods are relevant to the entire sampling and analysis effort. Part 1020 discusses quality assurance, quality control, and quality assessment. Standard Methods (Part 10208) states that "a good quality control program consists of at least seven elements: certification of operator competence, recovery of known additions, analysis of externally supplied standards, analysis of reagent blanks, calibration with standards, analysis of duplicates, and maintenance of control charts." For most of the tests to be carried out to meet the pathogen and vector attraction reduction requirements of the Part 503 regulation, these elements cannot be met completely, but they should be kept in mind as a goal.

Microbial Tests

For the microbiological tests, quality assurance is needed to verify precision and accuracy. Quality assurance for microbiological methods is discussed in Part 9020 of Standard Methods. The quality control approach suggested is recommended for the microbiological tests required by the Part 503 regulation. In Part 9020B-4, Analytical Quality Control Procedures, it is suggested that precision be initially established by running a number of duplicates, and that thereafter duplicates (5% of total samples) be run to determine whether precision is being maintained.

Spiking and recovery tests are an important part of quality assurance. Yanko (1987) has found that spiking is useful for the viable helminth ova test, but that testing recovery effectiveness on unspiked sewage sludge is more useful for quality assurance for bacterial or viral tests. With either method, the density of the measured pathogens should be at levels that are relevant to the Part 503 regulation. For example, for viable helminth ova, samples should be spiked to density levels of approximately 100 per gram. Recovery of bacteria and viruses should be conducted on primary sewage sludges that typically contain viruses at low but consistent levels (such as primary sewage sludges from large cities).

For both commercial and in-house laboratories, quality assurance procedures should be incorporated into the analytical method and assessed routinely. Communication with the analytical personnel is an important part of developing a good sampling and analysis protocol. The sewage sludge preparer should review quality assurance data along with analysis results to ensure that laboratory performance is acceptable.

Vector Attraction Reduction Tests

It is not possible to test for accuracy for any of the vector attraction reduction tests, because standard sewage sludges with consistent qualities do not exist. Standard Methods gives guidance on precision and bias. However, for some of the vector attraction reduction options, this information was not available or was approximate. Section 10.7 provides guidance on the number of samples to take. The procedures for three of the vector attraction options developed for the Part 503 regulation (additional anaerobic and aerobic digestion and the specific oxygen uptake rate test), which are presented in Appendix D, have internal quality control procedures that include replication. Since the tests are newly proposed, the data are insufficient to judge whether agreement between replicates is adequate. This kind of information will be communicated as experience with these options accumulates.

References and Additional Resources

APHA. 1992. Standard methods for the examination of water and wastewater. 18th ed. Washington DC: American Public Health Association.

ASTM. 1992a. Annual book of ASTM standards. Philadelphia, PA: American Society for Testing and Materials.

ASTM. 1992b. Standard practice for sampling industrial chemicals. Philadelphia, PA: American Society for Testing and Materials.

Kent, R.T., and K.E. Payne. 1988. Sampling groundwater monitoring wells: Special quality assurance and quality control considerations. p 231-246 in Keith, L.H. Principles of Environmental Sampling. American Chemical Society.

Kieth, L.H., ed. 1988. Principles of environmental sampling. American Chemical Society.

U.S. EPA. 1980. Samplers and sampling procedures for hazardous waste streams. Report No.: EPA/600/2-80/

018. Cincinnati, OH: Municipal Environmental Research Laboratory.

U.S. EPA. 1993. POTW sludge sampling and analysis guidance document. EPA 833-B-89-100. Office of Water.

U.S. EPA. 1993. Sewage sludge sampling techniques (video).

U.S, EPA. 1999. Biosolids Management Handbook. U.S. EPA Region VIII, P-W-P, 999 18th Street Denver CO 80202-2466.

Yanko, W.A. 1987. Occurrence of pathogens in distribution and marketing municipal sludges. Report No.: EPA! 600/1-87/014. (NTIS PB88-154273/AS.) Springfield, VA: National Technical Information Service.

Chapter 10
Meeting the Quantitative Requirements of the Regulation

10.1 Introduction

The Part 503 regulation contains operational standards for pathogen and vector attraction reduction. It provides only minimal guidance on the amount of information that must be obtained during a monitoring event to prove that a standard has been met or to demonstrate that process conditions have been maintained. This document provides more detailed information for regulators and facilities on how to adequately satisfy the regulatory requirements. Some frequently asked questions and answers are also included at the end of this chapter.

In general, it has been found that the daily, weekly, and seasonal fluctuations that occur in wastewater treatment works and sludge quality make it difficult to adequately represent sludge quality with minimum sampling. It is therefore recommended that multiple samples be taken for any sampling event and that samples be taken over a minimum 2-week period in order to best represent the performance of a sludge treatment process. Although extensive-sampling is time consuming and facility operators are often under pressure to reduce costs, it is strongly recommended that multiple samples be included in a sampling plan so that the variable quality of sludge can fully be understood.

There are many types of wastewater treatment plants and sludge management practices. This document addresses some of the many operational variables and provides some examples of how to demonstrate compliance with the regulations, The final decision about what to monitor and how frequently to monitor it lies with the permitting authority who may impose permit conditions based on specific parameters including the type of sludge produced, its intended usage, and/or the history of the facility.

10.2 Process Conditions

Sufficient information must be collected about sludge processing conditions and made available to the permitting authority and any other interested parties to enable a qualified reviewer to determine if the Part 503 requirements have been met. How this information is collected and how much information is needed depend on the process. The following example illustrates the type of information and the level of detail that may be included in a permit application. Consider the case of a treatment works that meets the pathogen reduction requirement for a Class B sludge by using anaerobic digestion conducted at the PSRP conditions of 35°C (95°F) with a 15-day residence time. To meet the pathogen reduction requirement, the monitoring results must demonstrate that the 35°C (95°F) temperature and 15-day residence time are maintained whenever the process is being used. The example below illustrates some of the factors to be considered in assuring compliance with the regulation. In addition, a contingency plan in case the conditions are not met, and product usage should be specified.

Example

Facility	Clarksdale Wastewater Treatment Facility Anaerobic Digestion
Size:	300 dry metric tons per year
Class:	B

Sewage sludge is treated in two digesters, operated in parallel, fed by constant displacement progressive cavity pumps. The facility complies with PSRP requirements by maintaining sludge at a temperature at or above 35° C for a minimum of 15 consecutive days.

- Temperature — During the first six months of operation under this permit, the permittee shall perform temperature scans throughout the volume of the digester to establish the location of the zone at which temperature is at a minimum. Scans will be conducted under the expected range of operating conditions. Once the location of the zone is established, the permit-tee will continuously measure digester temperature in the zone of minimum temperature. Temperatures will be recorded continuously or at intervals of eight hours. The temperature measuring device will be calibrated on a monthly basis.

- Retention Time — The permittee shall calculate the working volume of the digester to determine residence time. The permittee shall provide evidence that the digester has been cleaned within the last two years, or alternatively, determine the levels of grit and scum accumulation. Residence time must be at least 15 days. Flow rate and residence time will be measured and calculated each year.

. Vector Attraction Reduction -The facility will comply with vector attraction reduction via management practices. After digestion, the sludge will be dewatered and transported to farm land where it will be land applied and disked immediately (within six hours) into the soil (see below). Sludge will not be stored at application sites.

- Reporting — The data collected throughout the year will be summarized and submitted to the permitting authority annually. Reports will include temperature and residence time records as well as records of all application sites and sludge application rates.

- Contingency Plan — If the facility fails to meet the 35° C/l 5 day requirement, it has several options. The facility can try to meet the Class B time/temperature requirement with lower temperatures and longer residence times as determined by a linear interpolation between 35°C (95°F) and 15 days and 20°C (68°F) and 60 days. If the facility does not have the flexibility to maintain sludge in the digester for longer than 15 days, it can meet Class B requirements by sampling the sludge for fecal coliform and demonstrating that the sludge contains less than 2 million CFU or MPN per gram of sludge on a dry weight basis. Alternatively, the facility can dispose of the sludge by means other than land application. In the case that the facility cannot meet the time/temperature requirements, the permitting authority must be contacted so that a sampling plan which adequately represents sludge quality and demonstrates Class B pathogen reduction can be designed. If the facility decides to divert the sludge from land application, it must notify the regulatory agency of its plans.

- Product Use — The sludge will be land applied in accordance with all Part 503 restrictions. The facility will distribute the Class B sludge to local fruit farmers. The facility will notify applicators of sludge quality and relevant site restrictions. Crop harvesting will be restricted in accordance with Part 503 site restrictions. In the case of application to fruit trees, the farmer will wait a minimum of 30 days after application to harvest the fruit. If fruit that has fallen off the trees or otherwise touched the ground will also be harvested, the farmer will wait 14 months after sludge application to harvest the* *fruit. If there is any question about the waiting period or if the facility wishes to distribute sludge to farmers of crops which touch the ground, the facility should notify the regulator. Site restrictions for crops which touch the soil or which grow below the soil surface are subject to longer waiting periods.

The number and the level of detail of a permit's conditions vary depending on the type of process. Facilities that handle sludge or septage from more than one source should be subject to more frequent testing until they can demonstrate that the product consistently meets quality standards. The permitting authority must determine at what point the facility has adequately demonstrated consistency and can reduce the level of sampling.

For example, consider a treatment facility that collects liquid sewage sludge and septage from several different sources. Although all of the sludge collected undergoes standard treatment for Class B pathogen reduction, the quality of the sludge generated may vary depending on the particular feedstock received. Initially, the permitting authority may require this facility to monitor every batch of sludge in order to demonstrate that it consistently produces sludge in compliance with regulatory and permit requirements. Eventually, if enough data is available showing that the treated sewage sludge is rarely off specification, and the sampling frequency could be reduced.

For other processes, such as static pile cornposting, a sampling plan might specify that one of several piles constructed in a day could be monitored, probably with several thermocouples at different elevations and locations in the pile, to demonstrate conformance for the whole day's production.

At times, processes do not conform to process conditions. In such cases, the operator should keep records showing that the treated sludge produced was either recycled to be processed again or diverted in some manner for use or disposal consistent with its quality (e.g., disposal in a landfill with daily cover or, if the sludge meets the Class B requirements, application as a Class B [rather than as a Class A] biosolids).

10.3 Schedule and Duration of Monitoring Events

For purposes of this discussion:

- A sampling event is defined as the period during which samples are collected. Samples may include several independently analyzed subsamples taken during the sampling event.

- A monitoring event includes the sampling period and the period to analyze the samples and provide the results needed to determine compliance.

Monitoring events are intended to reflect the typical usual performance of the treatment works. Conditions should be as stable as possible before the monitoring event. Day-to-day variations in feed rate and quality are inevitable in sewage sludge treatment, and the processes are designed to perform satisfactorily despite these variations. However, major process changes should be avoided before monitoring events, because long periods of time-as much as 3 months if anaerobic digestion is part of the process train-are required before steady state operation is reestablished.

Monitoring for Microbiological Quality

To meet the Part 503 pathogen reduction requirements, sewage sludges may have to be monitored to determine densities of fecal coliforms, *Salmonella* sp., enteric viruses,

and/ or viable helminth ova. Monitoring for these microorganisms presents special problems, primarily caused by the length of time it takes to obtain microbiological test results. This is a function of the time it takes to deliver the samples to a laboratory, have the tests conducted, and obtain the results. Microbiological analyses require a substantially longer period than conventional physical and chemical analyses. The approximate time to complete specific microbiological analyses is summarized as follows.

Fecal coliform (MPN), 4 days
Salmonella sp. (MPN) 5 to 7 days
Enteric viruses, 14 days
Viable helminth ova, 28 days

Variations in the microbiological quality of the treated sludge and intrinsic variation in the analytical methods are generally large enough that a single measurement of a microbiological parameter is inadequate to determine whether a process meets or fails to meet a requirement. The Pathogen Equivalency Committee recommends that the monitoring event include at least seven samples taken over a period of approximately 2 weeks (see Section 10.7). Based on the reliability of the treatment process and historic test results, there may be times when a reduction in this monitoring recommendation is justified.

Thus, the time required for a monitoring event could range from 3 to 7 weeks. During this time, the quality of the treated sewage sludge generated is unknown. As discussed in Section 4.10, classification of sludge as Class A or B is based on the most recent test results available. Therefore, material can continue to be distributed under its classification as Class A or B until more recent analytical results are available. However, it is recommended that material generated during the monitoring event be retained on site until results from the monitoring event are available. This will prevent misclassified sludge from being erroneously distributed.

For example, consider a facility producing a Class A sludge that is sampled for *Salmonella* sp. analysis every quarter. All historic data has shown the facility to be in compliance with Class A standards including the most recent set of lab analyses from the January monitoring event. Under these results, materials are distributed as-Class A products even throughout April when a subsequent monitoring event takes place. This is acceptable because material is still classified under the most recent available lab result. However, suppose the April results show non-compliance with Class A standards. Despite the fact that the preparer complied with regulations, it is possible that some Class B material was inadvertently distributed for Class A use.

In order to avoid this situation, it is recommended that the sludge processed during the monitoring event either be stored until it is demonstrated that the processed sludge meets the quality requirements for use as a Class A or B sludge, or - if the sludge is being monitored for Class A requirements - used or disposed as a Class B sludge (provided it meets the Class B requirements). This may take up to 3 weeks in the case of fecal coliform or *Salmonella* sp. analysis and much longer if sludge is being analyzed for helminth ova or viruses. Contingencies for this type of situation should be discussed with the regulatory authority and included in permit conditions and operational plans.(For more discussion on the timing of sampling and distribution, see Section 4.10)

Monitoring for Vector Attraction Reduction

Not all the vector attraction reduction options listed in the regulation (see Chapter 8) require lab testing. Four of the methods (treatment of sewage sludge in an aerobic process for 14 days or longer, injection below the surface of the land, incorporation of sludge into the land, and placement of sludge on a surface disposal site and covering it at the end of each day) are technology descriptions. These technologies have to be maintained throughout the year in the manner described in the regulation. Examples of the kind of information needed to demonstrate adequate performance are provided in Section 10.2.

The remaining vector attraction reduction options are based on laboratory testing for volatile solids reduction, moisture content, or oxygen uptake reduction. Some of the options can only be used with certain sludge processes. For example, the oxygen uptake rate test is only appropriate for a sludge from any aerobic digestion or wastewater treatment process. Other options, such as the 38 percent reduction in volatile solids, can be applied to a variety of biological sludge treatment processes. In any case, the technology aspect of the option, or the process by which vector attraction reduction is being attained, must be documented in the manner described in Section 10.2. Monitoring for vector attraction reduction should be performed at a minimum according to the required monitoring schedule.

Some tests for vector attraction reduction can be conducted within a few hours while others can take more than a month. For the tests that can be conducted within a few hours, the sampling event must be more than a few hours to account for the variability in the material tested and the performance of the vector attraction reduction process as affected by the changes in feedstock.

It is suggested in Section 8.14 that facilities maintain a sampling program that involves sampling at evenly spaced time intervals throughout an established monitoring period. The on-going samples can be used to calculate running averages of volatile solids reduction which are more representative than single samples or an attempt to correlate feed sludge and sludge product. As is the case for the microbiological tests, these vector attraction reduction tests should be conducted over approximately 2 weeks to minimize the expected effect of these variations. The 2-week period can be the same 2-week period during which the microbiological parameters are being determined.

The longer VAR tests present a similar problem as monitoring for microbiological quality. Some of the tests - such as the additional digestion tests -take more than a month to complete. Unless the treatment works has several sets of duplicate testing equipment, it will be impossible to run these tests on enough samples during a 2-week sampling period to assess the variability in the performance of the treatment process. Storing samples taken during this period until the equipment becomes available is not an option, because samples cannot be stored for more than a limited time period (see Section 9.6). In such circumstances, the preparer may wish to run the vector attraction reduction tests more frequently than required in order to demonstrate on-going compliance with the requirements. More frequent testing will indicate if the facility is performing consistently and will reduce the need for multiple samples during the sampling period.

The preparer may wish to conduct composite sampling which combines samples taken within a 24 hour period to better represent sludge quality. (See Section 10.6). Since some of the bench scale tests may be affected by long-term storage of samples, cornpositing should be limited to a 24 hour period. If composting is done, the composite should be held at # 5°C during compositing, and the assay must begin immediately upon completion of the composite.

Preparers should discuss specific facility parameters with the permitting authority to design a sampling program that is appropriate.

10.4 Comparison of Feed Sludge and Sludge Product Samples

The enteric virus and viable helminth ova analytical requirements to demonstrate that an existing or new sludge treatment process is equivalent to a PFRP one and some of the vector attraction reduction methods (e.g., percent volatile solids reduction) involve taking input and output samples that correspond (i.e., they are "before processing" and "after processing" samples of the same batch of sludge). The comparison of input and output samples allows for the determination of whether enteric viruses and helminth ova levels are being reduced to adequate levels and/or percent volatile solids reduction.

Obtaining samples that correspond can be difficult for sewage sludge treatment processes, such as anaerobic digestion, that characteristically treat sludge in fully mixed reactors with long residence times. For example, as mentioned in Section 10.3, it can take up to 3 months for an anaerobic digester to achieve steady state operation after some substantive change in feed sludge or process condition is made. Samples taken only after the process has reached steady state operation are considered as corresponding.

Many of the treatment processes that might be considered for demonstrating equivalency to PFRP are either batch or plug flow processes. In theory it is relatively simple to obtain corresponding samples - it is only necessary to calculate the time for the input material to pass through the system and sample the downstream sludge at that time. Achieving accurate correspondence in practice, however, is seldom easy. Consider, for example, the difficulty of obtaining good correspondence of feed and treated sludge for a composting operation in which the feed sewage sludge is to be compared to composted sludge that has been stored for 3 months.

Taking multiple samples and appropriately cornpositing the samples of feed and treated sludge averages out the composition of these sewage sludges and reduces the correspondence problem. It is the regulatory authority's task to determine how many samples should be taken and how much data is necessary to demonstrate reduction of microorganisms in corresponding samples. As indicated in Section 10.6, limitations on the periods of time over which microbiological samples can be collected limit the utility of cornpositing.

10.5 The Effect of Sludge Processing Additives on Monitoring

Many sewage sludge dewatering and stabilization processes introduce other substances into the sludge. With the exception of large bulky additives such as wood chips, there is no need to modify sampling and analytical procedures. As discussed below, additives such as wood chips can complicate sample preparation and analysis and are best removed prior to analysis.

Polymers, lime, ferric chloride, paper pulp, and recycled sludge ash are frequently used to aid in dewatering. Disinfection by alkaline treatment requires the addition of lime or other alkaline materials to increase the temperature of the sewage sludge cake to disinfecting temperature. These materials also reduce the microbial densities by dilution and increased solids content. However, the change in microbial density caused by dilution may not be substantial. For example, an increase in mass of 20% would result in a reduction in the log density of a microbiological parameter of only 0.079.

The exposure risk to human health is directly related to the mass of treated sludge. So the achievement of pathogen reduction requirements and safe end-use is dictated by the population of pathogenic organisms in the final product. This is the approach taken by the Part 503 regulation, which requires that the treated sludge, regardless of the mass of other materials added, meet the standards for Class A or Class B sludge.

For some sludges, particularly those treated by composting (these usually will be Class A biosolids), the amount of additive can be considerable. Nevertheless, the regulation requires that the biosolids meet the standard, which means that no correction need be made for dilution.

The issues of sampling and analytical procedures for employment are different when considering wood chips or other materials which are often added to sludge as a bulk-

ing agent for cornposting. Compost product may be given away or sold as a screened or unscreened product, and although regulations require that the treated sludge, as it is applied, meets 503 standards, in the case of wood chips and other large particle size bulking materials, it is appropriate to remove large pieces before analysis takes place.

Large additives are removed in order to improve the accuracy of the microbial measurements. The wood chips are so big (typically 4 cm x 4 cm x 1 cm) that a very large sample would have to be taken and blended to get a representative subsample. Sample reliability is reduced when the sample consists of a mix of sludge solids and fibrous wood-chip residue from blending. Another reason for removing the wood chips prior to microbial analysis is that the exposure of users to the compost is related to the fine particle content and not to large, physically distinct wood chips. For example, a user who handles the compost gets his or her hands covered with compost particles. Similarly, the user might breathe in a dust of compost particles. In both cases, it is the "fines" of the compost, not the wood chips that the user is exposed to.

In order to ensure that wood chips are not included in the lab's subsample, the facility should remove wood chips after sampling, being careful not to contaminate, with a sterilized sieve. The size of the sieve needed depends on the dimensions of the wood chips, but the same sieve size should be used for each sampling event. Alternatively, the laboratory should be asked to remove wood chips from samples before subsampling or analyses are conducted. Again, the sieve size should be established so that a standard size is used.

10.6 Collecting Representative Samples

Sludge quality varies depending on the inputs to the wastewater system. In addition, the process is subject to ambient conditions which vary daily as well as seasonally. The goal of a sampling program is to adequately represent the quality of sludge; Therefore, both the frequency of sampling and the number of samples taken in any one sampling event must be considered carefully. This section discusses the issue of variability and how sampling frequency and composite sampling can improve the quality of data collected. A sampling plan is recommended for all sampling events to assure representative samples.

Random Variability

Virtually all sewage sludge treatment processes will experience a certain amount of short-term random or cyclic variation in the feed sludge and in process performance. Evaluation of average performance over a 2-week time period is suggested as a reasonable approach to account for these variations. Cyclic variation can be minimized by sampling on randomly selected days and time-of-day in a given week. In the case of Class B fecal coliform analysis ONLY variability is minimized by taking the geometric mean of analytical results. In the case of Class A, all samples must meet the fecal coliform or *Salmonella* sp. numerical limit.

Seasonal Variability

For some sewage sludge treatment processes, performance is poorer during certain parts of the year due to seasonal variations in such factors as temperature, sunshine, and precipitation. For example, aerobic digestion and some composting operations can be adversely affected by low ambient temperature. In such cases, it is critical that process performance be evaluated during the time of year when poorest performance is expected. If a treatment works is evaluated four or more times a year at intervals of 2 or 3 months, there is no problem, because all seasons of the year will be covered. For small treatment works that are evaluated only once or twice a year, it is important to monitor in the time of year where performance is expected to be poorest, to avoid approving a process that is not performing adequately for much of the year. It may also be beneficial to initially conduct sampling more frequently than the required minimum, perhaps on a quarterly basis, in order to determine the range of sludge quality. Process criteria of PSRPs and PFRPs should be discussed by the facility with the regulatory authority, and specific requirements should be included in permit conditions.

Composite Sampling

Composite sampling, or the combination of several grab samples to better represent a large quantity of sludge, is frequently practiced in wastewater treatment. Composites may consist of grab samples taken over time (typically for continuous flow processes) or from random locations in a vessel or pile (typically for batch processes). Since the purpose of composite sampling is to provide representation of a large quantity of sludge, the number and distribution of grab samples, the locations from where they are taken, and the process of combining grab samples to create a composite sample are important to consider.

The following is an example of a sampling procedure for cornpositing a continuous flow process. A small stream of wastewater or sludge is drawn off at rate proportional to the flow of the main stream being sampled and collected as a single sample. Typically, times of collection are for one shift (8 hours) or one day (24 hours). In this case, the accumulated sample represents a volume-average sample over the period of time the sample is drawn. The sample is chilled during the period it is being collected to prevent chemical/microbiological change until it can be brought back to the laboratory for analysis.

Composite sampling from stockpiled solid material involves taking multiple grab samples from a range of locations in the stockpile. Samples should be taken from different interior sections of the pile which may represent material produced in different time periods. Grab samples should all be of the same size so that the composite is an equal representation of all of the grab samples. The grab samples should be mixed thoroughly and a subsample pulled from the mixture.

Composite sampling is useful for any type of sampling, but the protocol must be modified when microbial analy-

ses are intended. Samples must be taken over a shorter period of time so that microbial populations do not undergo significant changes during the sampling event. For example, a composite time-average sample can be obtained by combining a series of small samples taken once every 5 minutes for a period of an hour. A composite sample for bacterial and viral testing could be taken over an hour or less under most circumstances without compromising the results. Composite sampling over 24 hours, or even longer if special precautions are taken, is possible for viable helminth ova provided the ova in the sample are not exposed to thermal or chemical stress (e.g., temperatures above 40°C [104°F] or the addition of certain chemicals such as ammonia, hydroxides, and oxidants). In addition to limiting the sampling period, sterile equipment must be used when taking grab samples or cornpositing the samples for microbiological analysis in order to prevent introducing pathogenic bacteria.

Composite sampling may be possible for samples to be used in some of the procedures to determine whether vector attraction reduction is adequate. It may not be appropriate for those procedures that depend on bacterial respiration (i.e., aerobic or anaerobic digestion). This subject is discussed in Appendix D which presents procedures for three methods to demonstrate reduced vector attraction.

10.7 Regulatory Objectives and Number of Samples that Should be Tested

Overall, it is recommended that numerous samples be taken over a period of 2 weeks in order to represent the average characteristics of a sludge stream. Unfortunately, sampling for microbial and vector attraction reduction parameters is more complicated than sampling for heavy metals because of the time limits and contamination issues involved. In addition, the results of microbial testing must be handled differently. The following is a review of the primary sampling and monitoring issues that relate to particular pathogen and vector attraction reduction parameters.

Class B: Monitoring for Fecal Coliform Densities

Part 503 requires that seven samples be taken to demonstrate compliance with the fecal coliform levels required of Class B biosolids. Under the Class B requirements seven samples also means seven analyses. Seven samples were judged adequate to account for the short-term fluctuations in treated sludge quality and allow determination of average performance. Variance of fecal coliform determinations is known to be high, but analysis (presented below) showed that if seven samples are averaged, the error band about the mean value is sufficiently compressed that treatment works with adequately treated sludge would not have difficulty meeting the standard. If the mean value does not meet the standard, the material is not a Class B biosolids and must be disposed of otherwise until the standard is met.

The regulation requires that the geometric mean fecal coliform density of the seven samples be less than 2 million CFU or MPN per gram of total solids sewage sludge (dry weight basis). If a treatment works were producing a treated sewage sludge with a true mean density of exactly 2 million fecal coliform per gram, measured values of the fecal coliform density would cluster around 2 million per gram, but half would be below and half would be above it. Half the time, the treatment works would appear not to be meeting the requirement. The true mean density must be below 2 million per gram to be confident that the experimentally determined average will be below 2 million per gram. Just how much below depends on the standard error of the average.

Use of at least seven samples is expected to reduce the standard error to a reasonable value. In tests on extended aeration sludges, Farrell et al. (1990) obtained a standard deviation of the logarithm of the fecal coliform density (s) of 0.3 using the membrane filter method. This included the variability in the analysis as well as variability over time (approximately a year). Standard error for the average of seven measurements (S.E.= $s/(n^{1/2})$) is 0.11. Using the normal probability distribution, the true mean must be below 1.30 million if the geometric mean of seven measurements is to be below 2 million 95% of the time (see Table 1 O-l for details of this calculation). If the standard deviation were higher, the true mean would have to be even lower to be reasonably confident that the geometric mean would be below 2 million per gram. Thus, efforts should be made to reduce variability. Steps that can be taken are:

- Reduce the standard error by increasing the number of measurements used to determine the geometric mean.
- Reduce process variability.
- Improve sampling and analytical techniques.

What action to take to reduce the geometric mean depends on the process. For anaerobic or aerobic digestion, some suggested steps are to increase temperature, increase residence time, use a draw-and-fill feeding procedure rather than fill-and-draw or continuous feeding, and increase the time between withdrawal and feeding. After an attempt at improvement, the evaluation should be repeated. If the process continues to fail, more substantial changes to the process may be appropriate.

Class A: Monitoring for Fecal Coliform or Salmonella sp. *Densities*

Part 503 requires that, to qualify as a Class A sludge, sewage sludge must be monitored for fecal coliform or *Salmonella* sp. and have a density of less than 1,000 MPN fecal coliform per gram of total solids sewage sludge (dry weight basis) or *Salmonella* sp. densities below detection limits (3 MPN/4 g). The regulation does not specify the number of samples that have to be taken during a monitoring event. One sample is not enough to properly represent the sewage sludge. It is recommended that multiple

Table 10-I. True Geometric Mean Needed If Standard Fecal Coliform Density of 2 Million CFU Per Gram is to be Rarely Exceeded

Assumptions

- The fecal coliform densities of the sewage sludge are log normally distributed. (The arithmetic mean of the logarithms of the fecal coliform densities is the mean of the distribution. The geometric mean is the antilog of the arithmetic mean of the log values.)
- The goal is to ensure that the measured mean value does not exceed the density requirement more than once in 20 monitoring events.
- The standard deviation of the log density is 0.30.

Calculation

To predict the expected frequency of a measurement using the normal probability distribution, the variable x is converted to the standard measure (u - see below) and its probability of occurrence is obtained from tabulated values of the probability distribution. In this case, the reverse is carried out. A certain probability of occurrence is desired and the value of the standard measure is read from the tables. From the normal distribution table (single-sided), u is 1.645 when P = 0.05 (one in 20),

Where:

P = the proportion of the area under the curve to the right of u relative to the whole area under the curve.

and: u = the standard measure

$u = (\bar{x} - \mu) / S_{\bar{x}}$ (Equation 1)

Where:

μ = true log mean

$\bar{x}$ = log mean of the measurements

$S_{\bar{x}} = s/n^{1/2}$

n = number of measurements that are averaged

s= standard deviation of a single measurement of log mean density

The logarithm of the fecal coliform density requirement (2 million CFU/g) is $\bar{x}$ ($\bar{x}$ = 6.301). This is the number that should not be exceeded more than once in 20 monitoring episodes. Substituting into Equation 1 and calculating μ,

$1.645 = (6.301-\mu)/(0.3/7^{1/2})$

$\mu = 6.114$

Antilog 6.114 = 1.3 million CFU/g.

samples (≥7) be taken over a period of two weeks in order to adequately represent sludge quality. Based on the reliability of the treatment process and historic test results, there may be time when a reduction in this monitoring recommendation is justified. In the case of Class A, analytical results from multiple samples are not averaged together; instead, all results must demonstrate be in compliance with Class A limits.

The measured fecal coliform density provides an estimate of the likelihood of *Salmonella* sp. detection and, if detected, the expected density. Yanko (1987) obtained a good correlation between fecal coliform density and *Salmonella* sp. detections in his extensive investigation of composts derived from sewage sludge. The fraction detected is less than 10% when fecal coliform density is less than 1,000 MPN/g. Yanko also obtained a good correlation between fecal coliform density and *Salmonella* sp. density for those samples for which *Salmonella* sp. were detected. That correlation predicts that, for fecal coliform densities less than 1,000 MPN/g, *Salmonella* sp. densities will be less than 1 .O MPN/g. Thus, at fecal coliform densities 4,000 MPN/g, *Salmonella* sp. detections will be infrequent and, if detected, densities are expected to be below 1 MPN/g.

The Part 503 allows the monitoring of either fecal coliform or *Salmonella* sp. in order to demonstrate compliance with Class A microbiological requirements. The *Salmonella* sp. determination is somewhat similar to the fecal coliform test, but it is much more expensive and requires a high experience level. In all likelihood, the *Salmonella* sp. tests would have to be carried out by a contract laboratory.

The standard deviation for Class A sludges will most likely be lower than for Class B. This is due to the fact that we have many more organisms present in Class B sludges which are not equally distributed within the biosolids. Therefore you have greater variability and hence a higher S.D.

What action to take to further reduce pathogens in case the fecal coliform requirement is not met depends on the process. In general, verification of retention times and temperatures as well as elimination of cross-contamination between feed and treated sludge or opportunities for reintroduction of pathogens into treated sludge are recommended. For aerated deep-pile composting, thicker insulating layers on the pile and longer maturing times are suggested.

Class A: Monitoring for and Demonstration of Enteric Virus and Viable Helminth Ova Reduction

The accuracy of monitoring results demonstrating the absence of enteric viruses and helminth ova is influenced by the variability in the influent to the treatment works and the inherent error in the experimental method. Information

on method error for both enteric viruses and helminth ova is available only on standard deviations calculated from duplicate samples. Goyal et al. (1984) report that, in their comparison of methods for determining enteroviruses, the log standard deviation for the virus determination in sewage sludge was 0.26 (47 degrees of freedom). A review of the work of Reimers et al. (1989) indicates that, in the range of 5 to 100 viable Ascaris ova per 10 grams sewage sludge solids, standard deviation was about half the number of viable ova. This is equivalent to a log density of 0.3, which is about the same *as* for fecal coliform. Thus, there is no unusually high variability in the basic test methods that would require an increased number of samples to minimize this effect.

Deciding how many samples to take for enteric viruses and viable helminth ova is more difficult than for fecal coliform and Salmonella sp. because enteric viruses and viable helminth ova oftenmay not be present in untreated sludge. For this reason, the interpretation of the density determinations for these organisms in treated sludge depends on the quality of the feed sludge. If no enteric viruses or viable helminth ova are detected in the feed sludge, then the absence of these organisms in corresponding samples of treated sludge does not indicate in any way whether the process is or is not capable of reducing these organisms to below detectable limits. The ability of a process to reduce these organisms to below detectable limits is indicated when analysis shows that these organisms were present in the feed sludge and were not present in corresponding samples of treated sludge. One important questions is: What fraction of the total pairs of corresponding samples must show positive in the feed sludge and negative in the treated sludge to provide convincing evidence that the process consistently reduces enteric viruses and viable helminth ova to below detectable levels? This is a difficult question to answer.

Because viable helminth ova are relatively stable microorganisms, compositing is suggested as a way to obtain meaningful representative samples and analytical results. If precautions are taken, such as cooling the sample promptly to close to 0°C (32°F) and destroying or neutralizing any added chemicals such as strong bases that were added as part of the pathogen-reducing process, composites can be collected over a 2-week period. Corresponding composites of feed and treated sludge can be compared, with a much lower likelihood of not finding viable helminth ova in the feed sewage sludge. Because the analytical method itself has a high variance (see above), a minimum of four duplicates of the composite should be tested.

For enteric viruses, the same approach may be used as suggested above for viable helminth ova. Precautions are taken to cool the sample and destroy or neutralize any chemicals added in the pathogen-reducing process. Samples are collected on separate days and are promptly frozen at 0°F (-18°C), or -94°F (-70°C) if samples will be stored for more than 2 weeks. When the samples are to be analyzed, the individual samples are thawed and composited, and viral densities determined.

The density of both viable helminth ova and enteric viruses in processed sludge must be-based on the results of several measurements. Most of these measurements are expected to show below detectable densities. If any one sample is above 1 PFU (for viruses) or 1 viable helminth ovum (for helminths) per 4 grams, the process does not meet the Part 503 operational standard.

Vector Attraction Reduction Tests

Reduction in Volatile Solids

One way to demonstrate reduction in volatile solids requires measurement of volatile solids of the sewage sludge before and after sludge treatment. The sampling point for the "after treatment" measurement can be immediately leaving the processing unit or at the point of use or disposal, provided there has been no significant dilution downstream with inert solids.

Farrell et al. (1996) have determined the standard deviation of the percent volatile solids (%VS) determination for separate samples withdrawn from pilot-scale digesters to be 0.65% (total solids content ranged from 2% to 5%). Conventional statistical procedures (see Davies and Goldsmith, 1972) were used to calculate the standard error of the percent volatile solids reduction (%VSR), which is calculated from the %VS of the untreated and treated sludge. The standard error of the %VSR when calculated by the Van Kleeck equation (see Appendix D) is 2.0% in the range of interest (38% VSR). The 95% confidence limits of the %VSR are ±4%, which is excessive. If the %VSR is the average of seven determinations, the confidence interval is reduced to ±1.5%, which is a more acceptable value.

The most difficult problem with the %VSR determination, as discussed above in Section 10.4, is getting correspondence of the influent sludge with the effluent sludge. If there has been a significant change in an inlet concentration or flow rate, achieving correspondence can require several months of monitoring inlet and outlet volatile solids concentrations. If conditions have been steady and feed compositions have been fluctuating about an average value for a long period, data taken over a 2-week period would be adequate to establish steady state performance.[1] This implies that data have been collected beforehand that demonstrate that sewage sludge composition has reached steady state for a long period before the 2-week sampling period. It appears that regular collection of data for some months before the sampling period is unavoidable to demonstrate steady state performance before the testing period. Fortunately, the total and volatile solids determinations are not costly, and they provide valuable operating information as well.

Total and volatile solids content of a sample do not change significantly over the course of a day, particularly if

[1]Note that, unlike the plug flow case, there should be no displacement in time between comparisons of input and output for fully mixed reactors. Only when there has been a significant change is it necessary to wait a long time before the comparisons can be made.

the sludge is cooled. Time composites collected over a course of a day can **be** used for these determinations. Seven or more determinations are recommended to reduce the error band around the mean to minimize the chance that a process that actually has a greater volatile solids reduction than 38% might show an average that is below this value.

Additional Digestion Tests

The essential measurement in the additional digestion tests for aerobic and anaerobic sludges (see Sections 8.3 and 8.4) is the percent volatile solids content (%VS) from which the percent volatile solids reduction is calculated (%VSR). Using the standard deviation of 0.65% determined by Farrell et al. (see above), the standard error of the %VSR when calculated by the Van Kleeck equation (see Appendix D) is 2.5% in the range of interest (15% VSR). The 95% confidence limits of the %VSR are ± 5%. The tests (see Appendix D) require substantial internal replication which shrinks these confidence limits. Samples should also be taken to account for the variability in the process. The 2-week sampling period suggested for the Class A disinfection microbiological tests may be excessively restrictive if several samples are to be evaluated. The equipment needed for the test is not expensive but the units take up substantial bench space. It is unlikely that a treatment works will want to have more than two sets of test equipment. Since the tests take 30 to 40 days, it is not possible to run more than one set of tests (two in a set) within a monitoring event. It is suggested that these tests be routinely carried out during the year and the results be considered applicable to the monitoring period. It is estimated on a best judgment basis that five tests are needed to account for variability in the feed sludge and in the treatment process itself.

Specific Oxygen Uptake Rate Test

The Oxygen uptake measuring part of the specific oxygen uptake rate test (SOUR, see Appendix D) can be completed in the laboratory or field in a few minutes, so there is no difficulty in completing the test during a monitoring event. The test requires the SOUR determination to be made on two subsamples of a given sample. Farrell et al. (1996) found that, in the target SOUR value of 1.5 mg O_2/hr/g, sludge solids replicates agreed within about ± 0.1 mg O_2/hr/g. Since the test is easy to run, it is suggested that seven tests within the 2-week sampling event will adequately define the SOUR. Labs performing this test should demonstrate that they too can achieve this level of precision for replicates (± 0.1 mg O_2/hr/g). Arithmetic average of the tests should be computed and compared against the Part 503 SOUR value.

Raising the pH to 12

There are two options in the regulation that reduce vector attraction by pH adjustment. In the first, sludge is raised in pH by alkali addition so that pH is 212 for 2 hours after alkali addition and, without further alkali addition, remains at pH 211.5 for an additional 22 hours (see Section 8.7). The second method is for domestic septage. The pH is raised to pH 212 by alkali addition and, without further addition of alkali, remains at 112 for 30 minutes (see Section 8.13). As noted in Section 5.6, the term alkali is used in the broad sense to mean any substance that increases pH.

The pH requirement in the regulation was established using data obtained at room temperature (Counts and Shuckrow, 1975; Ronner and Cliver, 1987), which is presumed to have been 25°C (77°F). Consequently, pH should be measured at 25°C (77°F) or measured at the existing temperature and converted to 25°C (77°F) by use of a temperature-versus-pH conversion table determined experimentally for a treated sludge that meets the pH requirements. The correction is not trivial for alkaline solutions; it is about -0.03 pH units/°C (-0.017 pH unit/°F) for aqueous calcium hydroxide with a pH of about 12, and should not be ignored. Note that temperature-compensated pH meters only adjust instrument parameters and do not compensate for the effect of temperature on the pH of the solution.

pH Adjustment and Septage

Each container of domestic septage being treated with alkali addition must be monitored. The pH is monitored just after alkali addition and a half hour or more after alkali addition. Bonner and Cliver (1987) suggest that alkali (they used slaked lime) be added to the septic tank or to the septic tank truck while domestic septage is being pumped from a septic tank into the tank truck. If slaked lime is used, a dose of 0.35 lb per 10 gallons (4.2 g per liter) is sufficient to raise the pH to 12 for a typical domestic septage of about 1% solids content. The agitation from the high velocity incoming stream of septage distributes the lime and mixes it with the domestic septage. The pH is measured when the truck loading is complete. The truck then moves to the use or disposal site. Agitation generated by the motion of the truck may helps in mixing and distributing the lime however, supplemental mixing in the tank may be needed. The pH is again measured at the use or disposal site. The second pH measurement should be at least a half hour after the addition of lime. The sample may be obtained through the top entry of the tank truck, using, for example, a stainless steel cup welded to a long handle to collect the sample. The pH is most conveniently measured with alkaline pH paper in the pH range of 11 to 13. The pH paper can age and become contaminated. It is best to use strips from two separate containers. If they do not agree, compare with a third batch and reject the one that disagrees with the others. Accuracy of these measurements is within ± 0.1 pH unit. If the pH is below 12, either initially or after 30 minutes, more lime should be added and mixed in. After an additional waiting period of at least 30 minutes, the pH must again be measured to ensure that it is greater than 12.

pH Adjustment and Sewage Sludges

For addition of alkali to sewage sludges, the pH requirement is part of both the PSRP process description (see Section 5.3) and the requirement of a vector attraction option (see Section 8.7). Monitoring is required from 1 to 12 times a year (see Table 3-4 in Chapter 3), and the pro-

cess must meet the prescribed operating conditions throughout the year.

Alkali is sometimes added to liquid sludge and sometimes to dewatered sludge. The pH requirements as stated in the regulation apply in the same way for both liquid and dewatered sludge. For the first measurement of pH in liquid sludge 2 hours after addition of alkali, it is assumed that the alkali and the sludge have been mixed together for a sufficient time to reach equilibrium (not considering the gradual changes that occur over substantial periods of time). Consequently, the pH measurement can be made directly in the liquid sludge. The pH measurement is made preferably with a pH meter equipped with a temperature compensation adjustment and a low-sodium glass electrode for use at pH values over 10. The pH electrode is inserted directly in the sludge for the reading. The second measurement is made 24 hours after addition of alkali. If the sludge is still in the liquid state, the pH measurement is made in the same fashion. However, if the process includes a dewatering step immediately following the alkali addition and the sludge is now a dewatered cake, the cake must be made into a slurry for the pH measurement. Acceptable procedures for preparing the sample and measuring pH are given by EPA (1986). The procedure requires adding 20 mL of distilled water (containing 0.01 M $CaCl_2$) to 10 g of sludge cake, mixing occasionally for half an hour, waiting for the sample to clarify if necessary, and then measuring pH. The important step is the mixing step that allows the alkali-treated dewatered sludge to come into equilibrium with the added water.

Number of Samples

The accuracy of pH meters and of pH paper is within ± 0.1 pH unit. More than one sample is necessary if the domestic septage or sludge is not well mixed. If the lime has been added gradually over the period in which septage is being pumped into a tank trug is considered adequate and a single measurement taken at the top of the tank truck is sufficient. If alkali has been added to liquid sludge in a tank at a treatment plant, tests are easily run to establish how much mixing is required to produce a uniform pH in the sludge. If this adequate mixing time is used, a single sample withdrawn from the tank for pH measurement is sufficient.

If alkali is added to sludge cake, more sampling is suggested. Typically, alkali (usually lime) is added to sludge cake in a continuous process. The sludge from the dewatering process discharges continuously to a mixer, from which it discharges to a pile or to a storage bin. Lime is metered into the mixer in proportion to the sludge flow rate. The flow rate and compositions of the sewage sludge can vary with time. To demonstrate compliance on a given day, several time-composite samples each covering about 5 minutes should be collected, and the pH measured. This procedure should be repeated several times during the course of a 2-week sampling event.

For sludge cake, the composites collected for pH measurement must be reduced in size for the pH measurement.The alkaline-treated sludge may be discharged from the mixing devices in the form of irregular balls that can be up to 5 to 7.6 cm (2 or 3 inches) in diameter. It is important that the biosolids to which the environment will be exposed have been treated to reduce pathogens and vector attraction to the desired level. If the discharged biosolids are ball shaped and the alkali has not penetrated the entire ball, one or both of these goals is not met for the material inside the ball. The entire ball should be at the proper pH. It is suggested that the composite be thoroughly mixed and that a subsample be taken for analysis from the mixed composite. An even more conservative approach is to sample only the interior of the balls.

Percent Solids Greater Than or Equal to 75% and 90%

The monitoring requirement for these vector attraction options (see Sections 8.8 and 8.9) is simply measurement of total solids. This measurement is described in Standard Methods (APHA [1992], Standard Method 2540 G). Standard Methods

states that duplicates should agree within ± 0.5% of their average. For 75% solids, this would be ± 3.8%,. For a continuous process, a time-composite sample can be taken over the course of a day, and duplicate analyses carried out on this composite. This is possible because biological activity essentially ceases at high solids content, and decomposition will not occur. Approximately seven such composites over the course of a 2-week sampling period would provide adequate sampling.

Some drying processes such as drying sludge on sand drying beds are batch processes. In such cases, it may be desirable to ascertain that the sludge meets the vector attraction reduction requirements before removing the sludge from the drying area. This can be done by taking two separate space-composites from the dried sludge, analyzing each of them in duplicate, and removing the sludge only if it meets the required solids content.

Frequently Asked Questions

How many samples should be submitted for each monitoring event for Class A pathogen tests? How many grab samples should be taken for each composite?

The 503 regulations do not specify a minimum number of samples per sampling event for Class A sludge, but it is strongly recommended that enough samples be taken to adequately represent the mass of material which is to be distributed. A minimum of seven samples, as required for Class B fecal coliform testing is recommended, but the number of samples, and the number of grab samples which each composite should represent, depends on the size of the facility and the volume of sludge product that is distributed. A sampling plan should be developed and submitted to the permitting authority for review.

Are you out of compliance for Class A if you take more than one sample, and one result is over the limit?

Yes, In order to meet Class Astandards, all material must meet pathogen standards. Although Class B pathogen standards are based on a geometric mean of analytical results, geometric (or arithmetic) means are not acceptable for compliance with Class A standards. Therefore, if several samples are submitted for analysis during one monitoring event, and one sample is found to be out of compliance with Class A pathogen standards, the entire batch must be considered Class B (assuming it meets Class B standards).

For batch processes, one way to prevent one 'out of compliance' sample from affecting the classification of a large volume of finished product is to maintain smaller separate storage piles and to sample from segregated areas. For example, finished compost could be separated into piles based on when cornposting was completed. If one result shows non-compliance with the Class A standards, but other samples are within the Class A limits, it would be relatively simple to separate out the non-compliance material and reprocess it or distribute it as Class B material.

Continuous flow operations can reduce the probability that one outlying result will cause their process to fail by taking multiple samples over a 24-hour period and cornpositing the samples. The composite sample can then be analyzed in duplicate to provide more data.

Averaging lab results *is* allowable as a means to eliminate laboratory variability however all data must be reported to the permitting authority for review. For example, if a lab runs duplicate fecal coliform analyses on one sample, the results from these analyses can be averaged together for one result. This is not intended to allow facilities to rerun analyses on out of compliance samples in the hope of lowering average results.

Pathogen testing on our Class A sludge product has shown that we consistently reduce Salmonella sp. to below detectable limits, but fecal coliform levels are sometimes over 1000 MPN per gram. Should we be concerned about this? Should we be concerned If the fecal coliform level In our Class A material Is occasionally as high as 990 MPN/gram?

According to the regulations, neither situation is a problem. You are required to comply with either the Salmonella sp. or the fecal coliform standards, not both. However, the level of fecal coliform in the product may indicate that there is incomplete pathogen destruction or some regrowth in your product, in which case you should examine your pathogen and vector attraction reduction processes to ensure that you are complying fully with the requirements and are not contaminating the product. The high fecal coliform counts may also be due to the presence of other, non-fecal coliforms in the sludge. These coliforms, which share some characteristics with fecal coliforms, may be detected in fecal coliform testing. They are particularly likely to appear in compost samples since they tend to be found in woody materials.

In addition, certain processes have been found to leave a residual population of fecal coliform which can repopulate the sludge. It is possible that testing would find fecal coliform over the Class A limits even when the pathogenic bacteria for which fecal coliform are intended to serve as indicators have been reduced below detectable levels. Cornposting and lime treatment are two of these processes. It is therefore recommended that if properly operated Class A facilities yield high populations of fecal coliform in finished solids that Salmonella sp. be used as the indicator organism for these types of facilities.

Can we distribute finished material before getting pathogen test results back? If yes, what do you do if results later show that material was not Class A?

This issue is covered extensively in Section 4.10. Sludge classification is based on the most recent available lab data, and therefore, material generated during a sampling period can be distributed before results from that sampling period are available (based on the results of the previous sampling event). However, it is recommended that materials generated during the sampling period be held on site until results are available in order to prevent a situation in which material is erroneously classified and distributed as Class A.

If composting piles are monitored for temperatures at three different points, do all three points have to meet PFRP at the same time?

All particles of sludge must undergo the PFRP time and temperature regime. For aerated static pile and in-vessel cornposting, the entire pile must meet the temperature requirements concurrently. If one point is found to be below the 55°C level during the temperature monitoring period, the entire pile is considered to be out of compliance, and the three consecutive day PFRP period must start over again. However, if temperatures are taken in distinct piles or cells of an in-vessel system, each section can meet the PFRP requirements separately.

Our facility often stockpiles composted sewage sludge over the winter. In the spring, we may have as much as four months' production of compost on site. How should sampling be conducted?

After material is stored on site, it must be resampled in order to determine if regrowth of pathogens has taken place. The number of samples should correspond to the time period that the stockpile represents and the mandated frequency of sampling based on the facility's size. For example, if a facility is required to sample sludge every month, and there are four months' worth of compost on site, a minimum of four samples (therefore, 4 times 7 or 28 analysis) from appropriate sections of the stockpile must be submitted. Ideally, material will be stored in segregated piles so that each month's production of compost can be sampled separately.

This applies to other long-term sludge storage such as lagoons. The number of samples taken from lagoons should be based on the time period that the lagoon(s) repre-

sent and **the** frequency of sampling that a facility is obligated to follow because of the rate of sludge generation.

What should we do if our process changes or expands?

Permits are granted based on particular operational parameters. Therefore, any projected changes in the operation or expanded flow should be discussed with the permitting authority before changes are made, even if you do not have a permit.

Can we be permitted for operation only during certain months?

If your operation will only meet pathogen or vector attraction reduction standards during part of the year, your permit can contain conditions which allow distribution only during these times. Permits can also be written to take ambient conditions into account; for example, some "low-impact" cornposting facilities are required to retain material over two summers. It may also be practical to limit storage and utilization of particular types of sludges to some seasons.

Can we combine two PSRP processes that individually do not meet the specified process requirements to produce a Class B product? Can time in extended aeration be added to digester time?

The only way to evaluate the effectiveness of pathogen reduction through a combination of two or more PSRP processes is by testing the sludge for fecal coliform density. If sufficient pathogen reduction can be demonstrated consistently, the preparer also may consider applying for a PSRP equivalency for the combined processes in order to eliminate the need for fecal coliform testing.

In general, extended aeration cannot be considered a PSRP or part of a PSRP because raw sewage is continually being added to the aerator and blending with the mixed liquor. Specific cases in which extended aeration is not subject to short-circuiting and is thought to contribute significantly to the pathogen reduction process should be evaluated by testing the resulting sludge for fecal coliform density and by the SOUR test or extended aerobic digestion one for addressing VAR requirements.

If I produce an "exceptional quaiity" (EQ) product and mix the product with topsoil before distribution, does the mix have to be tested for 503 compliance?

Regulations regarding "exceptional quality" material, or material which complies with the highest levels of pathogen and vector attraction reduction as well as heavy metals limits, are based on when the sludge preparer loses control of the material. If the EQ material is still within your control (i.e. on-site or owned by the preparer) when it is mixed, the new product must undergo pathogen and vector attraction reduction processes and be analyzed for Part 503 parameters including pathogens, vector attraction reduction, and heavy metals. This may be problematic for some facilities since a mix of stable compost and soil, for example, is unlikely to meet/undergo PFRP time and temperature requirements. You may have to test the mix for helminth ova and enteric viruses in order to demonstrate compliance with Class A pathogen reduction. If, however, the EQ material has left your control (i.e. is sold to a soil blender), the material falls out of the jurisdiction of the Part 503, and any subsequent blending of the material with other products is not covered by these regulations. Non-EQ materials are always subject to the Pan' 503, and storage or mixing of non-EQ materials with soil, yard waste, or other additives must be followed with re-testing and re-classification. The party responsible for the sludge mixing is considered a sludge preparer and is therefore subject to all Part 503 requirements.

Our sludge product meets vector attraction reduction requirements because the level of total solids In the material is greater than 75 percent. If stored material becomes wet because of rainfall, is the material still in compliance with the requirements?

The vector attraction reduction requirement stipulates that the material be processed to greater than 75 percent (or 90 percent when unstabilized solids are present) total solids. If dried sewage sludge (biosolids) is stored at your facility and becomes wet, It still meets the vector attraction reduction criteria as long as the facility has testing documentation that the biosolids were processed to ≥75 or 90 percent solids prior to the time the material became wet. It is a good management practice however to prevent dried biosolids from getting wet while it is being stored at the facility.

In the case of vector attraction reduction Option 6, it is required that the pH of the sludge be raised to ≥12 for 2 hours and 211.5 for 22 hours. It is not required that the sludge be maintained at the elevated pH once the material has fulfilled the vector attraction reduction requirement. However, it is important to note that the sludge which appears to be stable under the elevated conditions may become odorous and attract vectors if the pH declines. It is recommended that sludge be utilized before the pH drops below 10.5 in order to prevent odors or vector attraction which may result in a public nuisance.

Can Alternative 1 be used to demonstrate pathogen reduction for composting if the compost piles do not attain 55° C for 3 consecutive days?

Alternative 1 is based on similar time/temperature relationships as the cornposting process. Regime A ($D=131{,}700{,}000/10^{0.1400t}$ in which $t \geq 50°$ C and D10.0139 days) can apply to cornposting. The table below shows some points on the time/temperature curve that would comply with the regime.

Time (Days)	Temperature (°C)
0.02 (30 min)	70
0.04 (1 hour)	68
0.08 (2 hours)	66
1	58
2	56
3	55

As shown, it is theoretically possible that a compost pile could comply with Alternative 1 by reaching very high temperatures for a short period of time. **Alternative 1 is based on the assumption that all particles of sludge are attaining these temperatures uniformly.** This may be difficult in a compost pile unless the compost pile is completely enclosed and well insulted. In addition, excessive temperatures in a cornposting process may result in anaerobic conditions and subsequent odors.

Our facility is planning to expand next year, and we would like to implement a new process for pathogen reduction. We will submit our request for equivalency to the PEC this year, but, given the current turn around time for applications, do not expect to have equivalency granted for 2 more years. What should we do in the Interim?

Depending on the class of sludge you are hoping to produce, you have two options. If you are producing **a** Class B sludge, you should continue to do fecal coliform testing in order to demonstrate compliance with the Class B limit of 2 million CFU or MPN per dry gram of sludge. If you are producing a Class A sludge, you could follow Alternative 4 and test the sludge product for helminth ova and enteric viruses as well as either fecal coliform or Salmonella sp.. In either case, an application for equivalency will require data demonstrating pathogen reduction, so this data will be useful in that respect.

You may also wish, in the case of Class A sludge, to test the feed sludge for enteric virus and helminth ova. Adequate demonstration that the process reduces these pathogens on a consistent basis may qualify the process as a PFRP equivalent one (Class A, Alternative 6). You should consult with the permitting authority to determine an acceptable sampling protocol. Demonstration of helminth ova and virus reduction is difficult, particularly if the density of these pathogens in the influent is low or sporadic. The sampling program must demonstrate that actual reduction is taking place, not just that the pathogen density in the treated sludge is low. Once pathogen reduction has been sufficiently demonstrated, testing for enteric viruses and helminth ova are no longer necessary as long as the process is conducted in compliance with specified conditions for PFRP equivalency.

Our facility distributes Class B lime stabilized sludge to farmers who use the sludge on a variety of crops. Is It our responsibility to keep track of how this sludge is used?

You are required to provide the farmers with all sludge quality data as well as regulatory information which will allow them to comply with the appropriate site restrictions. The applicator, the farmer, is then responsible for following the correct site and harvest restrictions. However, given that any problems with land application will most likely affect the public perception of sludge reuse and this may in turn affect your facility, it is recommended that you work closely with farmers to ensure that the regulations are being followed. In addition, the permitting authority may choose to include conditions related to site and harvest restrictions in your permit.

Is there any limit of how long Class B sludge can be stored before it is used?

Part 503 Rule defines storage as "the placement of sewage sludge: on land on which the sewage sludge remains for two years or less." It does not include placement of sewage sludge on the land for treatment. After two years the storage site is considered a final disposal one. The permitting authority may include storage conditions in your permit which mandates usage of the material while it still retains certain characteristics (moisture content) or within **a** certain time period. It is recommended that storage of Class B material be limited to 30 days and be conducted under similar site restrictions as usage of Class B material. For example, public contact and access to the storage site should be restricted.

If the vector attraction reduction requirements have been fulfilled under Option 6, is there any need for the sludge to remain at an elevated pH?

In the case of vector attraction reduction Option 6, it is required that the pH of the sludge be raised to 212 for 2 hours and ≥11.5 for 22 hours. It is not required that the sludge be maintained at the elevated pH once the material has fulfilled the vector attraction reduction requirement. However, it is important to note that sludge that appears to be stable under the elevated conditions may become odorous and attract vectors if the pH declines. It is recommended that sludge be utilized before the pH drops below 10.5 in order to prevent odors or vector attraction that may result in a public nuisance.

References and Additional Resources

Counts, C.A. and A.J. Shuckrow. 1975. Lime stabilized sludge: its stability and effect on agricultural land. Rept. EPA670/2-75-012, pub. U.S. EPA.

Davies, O.L. and P.L. Goldsmith, ad. 1972. Statistical methods in research and production. Essex, England: Longman Group Ltd.

Farrell, J.B., B.V. Salotto, and A.D. Venosa. 1990. Reduction in bacterial densities of wastewater solids by three secondary treatment processes. Res. J. WPCF 62(2): 177-184. .

Farrell, J.B., V. Bhide, and J.E. Smith. 1996. Development of EPA's new methods to quantify vector attraction reduction of wastewater sludges. Water Environment Research, Vol. 68, No. 3.

Goyal, S.M., S.A. Schaub, FM. Wellings, D. Berman, J.S. Glass, C.J. Hurst, D.A. Brashear, C.A. Sorber, B.E. Moore, G. Bitton, P.H. Gibbs, and S.R. Farrah. 1984. Round robin investigation of methods for recovering human enteric viruses from sludge. Applied & Environ. Microbiology 48:531-538.

Kowal, N.E. 1994. Pathogen risk assessment: Status and potential application in the development of Round II regulations. Proceedings of the June 19-20, 1994 Speciality Conference. The Management of Water and Wastewater Solids for the 21st Century: A Global Perspective. Water Environment Federation. Alexandria, VA.

Reimers, R.S., M.D. Little, T.G. Akers, W.D. Henriques, R.C. Badeaux, D.B. McDonnell, and K.K. Mbela. 1989. Persistence of pathogens in lagoon-stored sludge. Rept. No. EPA/600/2-89/015 (NTIS No. PB89-190359/AS). Cincinnati, OH: U.S. EPA Risk Reduction Engineering Laboratory.

Ronner, A.B. and D.O. Cliver. 1987. Disinfection of viruses in septic tank and holding tank waste by calcium hydroxide (Lime). Unpublished report, Small Scale Waste Management Project. Madison, WI: University of Wisconsin.

U.S. EPA. 1986. Test methods for evaluating solid waste: method 9045A, soil and waste pH, Revision 1, Nov. 1990. Washington, D.C.: Office of Solid Waste and Emergency Response, U.S. EPA. (avail. U.S. Supt. of Documents).

Weaver, R.W.; J.S. Angle; and P.S. Bottomley 1994. Methods of Soil Analysis. Part 2. Microbiological and Biochemical properties. Madison, WI Soil Science Society of America.

Yanko, W.A. 1987. Occurrence of pathogens in distribution and marketing municipal sludges. Report No.: EPA/600/1 87/014. (NTIS PB88-154273/AS.) Springfield, VA: National Technical Information Service.

Chapter 11
Role of EPA's Pathogen Equivalency Committee in Providing Guidance Under Part 503

11 .1 Introduction

One way to meet the pathogen reduction requirements of the Part 503 is to treat sewage sludge in a process "equivalent to" the PFRP or PSRP processes listed in Appendix B of the Part 503 regulation (see Tables 4-2 and 5-1 for a list of these processes):

- Under Class A Alternative 6, sewage sludge that is treated in a process equivalent to PFRP and meets the Class A microbiological requirement (see Section 4.3) is considered to be a Class A biosolids with respect to pathogens (see Section 4.9).
- Under Class B Alternative 3, sewage sludge treated by a process equivalent to PSRP is considered to be a Class B biosolids with respect to pathogens (see Section 5.4).

These alternatives provide continuity with the Part 257 regulation, which required that sewage sludge be treated by a PSRP, PFRP, or equivalent process prior to use or disposal. There is one major difference between Part 257 and Part 503 with respect to equivalency. Under Part 257, a process had to be found equivalent in terms of both pathogen reduction and vector attraction reduction. Under Part 503, equivalency pertains only to pathogen reduction. However, like all Class A and B biosolids, sewage sludges treated by equivalent processes must also meet a separate vector attraction reduction requirement (see Chapter 8).

What Constitutes Equivalency?

To be equivalent, a treatment process must be able to consistently reduce pathogens to levels comparable to the reduction achieved by the listed PSRPs or PFRPs. (These levels, described in Section 11.3, are the same levels required of all Class A and B biosolids.) The process continues to be equivalent as long as it is operated under the same conditions (e.g., time, temperature, pH) that produced the required reductions. Equivalency may be site-specific; equivalency applies only to that particular operation run at that location under the specified conditions, and cannot be assumed for the same process performed at a different location, or for any modification of the process. Processes that are able to consistently produce the required pathogen reductions under the variety of conditions that may be encountered at different locations across the country may qualify for a recommendation of national equivalency (a recommendation that the process will be equivalent wherever it is operated in the United States).

Who Determines Equivalency?

The permitting authority is responsible for determining equivalency under Part 503. The permitting authority and facilities are encouraged to seek guidance from EPA's Pathogen Equivalency Committee (PEC) in making equivalency determinations. The PEC makes both site-specific and national equivalency recommendations.

What Are the Benefits of Equivalency?

A determination of equivalency can be beneficial to a facility, because it reduces the microbiological monitoring burden in exchange for greater monitoring of process parameters. For example a facility meeting Class A requirements by sampling for enteric viruses and viable helminth ova in compliance with Alternative 4 may be able to eliminate this monitoring burden if they are able to demonstrate that their treatment process adequately reduces these pathogens on a consistent basis[1]. Similarly, a facility meeting Class B Alternative 1 requirements by analyzing sewage sludge for fecal coliform may be able to eliminate the need for testing if the process is shown to reduce pathogens to the same extent as all PSRP processes. Equivalency is also beneficial to facilities which may have low cost, low technology systems capable of reducing pathogen populations. Options such as long term storage, air drying, or low technology cornposting have been considered by the PEC.

Because equivalency status allows a facility to eliminate or reduce microbiological sampling, it is imperative that the treatment processes deemed equivalent undergo rigorous review to ensure that the Part 503 requirements are met. Obtaining a recommendation of equivalency necessitates a thorough examination of the process and an ex-

[1] A determination of PFRP equivalency will not reduce the monitoring required for Salmonella sp. or fecal coliform because all Class A biosolids, even biosolids produced by equivalent processes, must be monitored for *Salmonella* sp. or fecal coliform (see Section 4.3)

tensive sampling and monitoring program. The time needed to review an application is contingent on the completeness of the initial application. Sewage sludge preparers wishing to apply for equivalency should review this chapter carefully and discuss the issue with the regulatory authority in order to determine if equivalency is appropriate for their situation.

Figure 11-1 indicates when application for equivalency may be appropriate.

Recommendation of National Equivalency

The PEC can also recommend that a process be considered equivalent on a national level if the PEC finds that the process consistently produces the required pathogen reductions under the variety of conditions that may be encountered at different locations across the country. A recommendation of national equivalency can be useful for treatment processes that will be marketed, sold, or used at different locations in the United States. Such a recom-

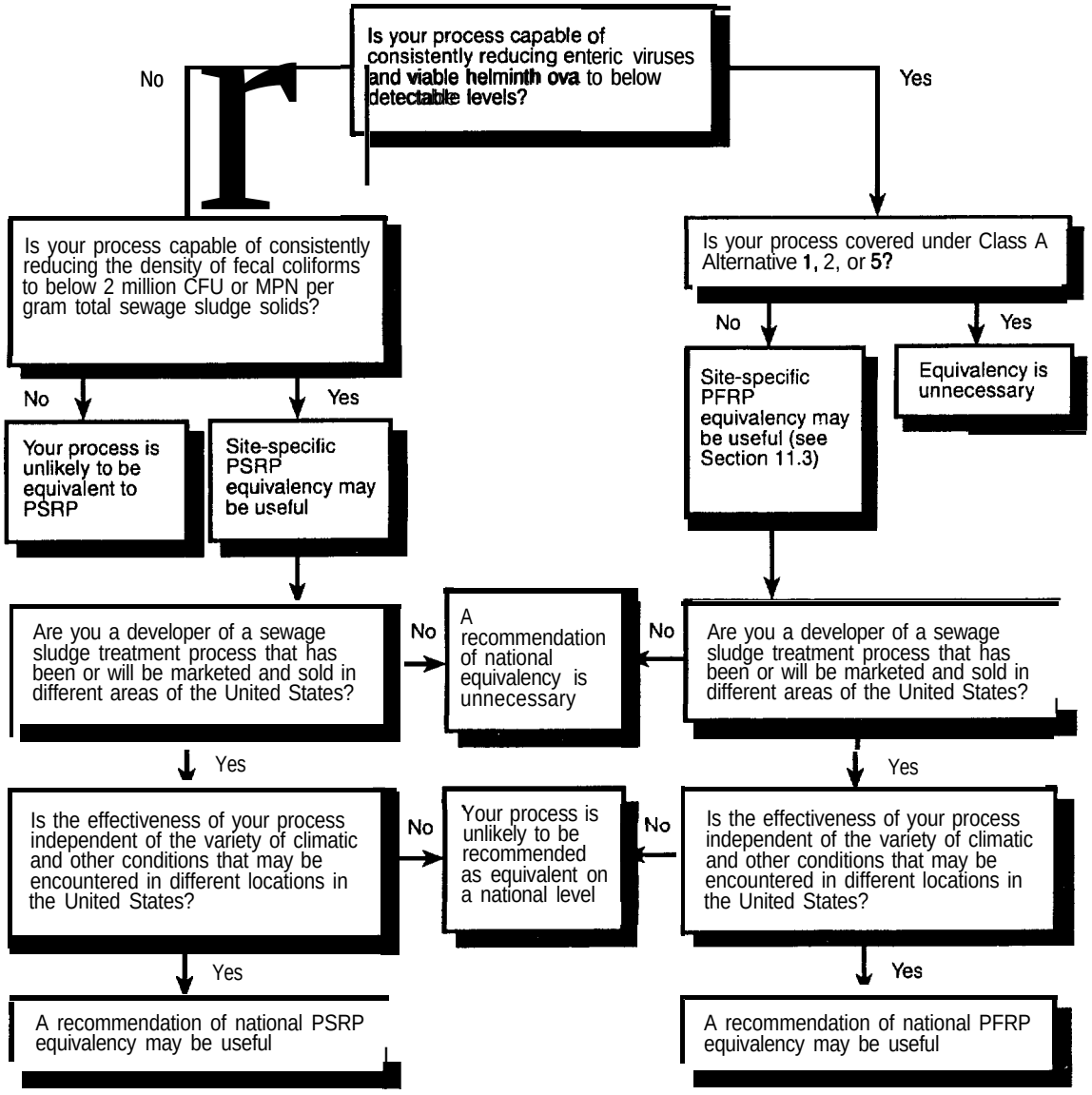


Figure 11-l. When is application for PFRP or PSRP equivalency appropriate?

mendation may be useful in getting PFRP or PSRP equivalency determinations from different permitting authorities across the country.

Role of the Pathogen Equivalency Committee

The U.S. Environmental Protection Agency created the Pathogen Equivalency Committee (PEC) in 1985 to make recommendations to EPA management on applications for PSRP and PFRP equivalency under Part 257 (Whittington and Johnson, 1985). The PEC consists of approximately ten members with expertise in bacteriology, virology, parasitology, environmental engineering, medical and veterinary sciences, statistics, and sewage sludge regulations. It includes representatives from EPA's Research and Development Office, the Office of Water, and the regional offices. The 1993 memorandum included at the end of this chapter describes the role of the PEC.

Guidance and Technical Assistance on Equivalency Determinations

The PEC continues to review and make recommendations to EPA management on applications for equivalency under Part 503. Its members also provide guidance to applicants on the data necessary to determine equivalency, and to permitting authorities and members of the regulated community on issues (e.g., sampling and analysis) related to meeting the Subpart D (pathogen and vector attraction reduction) requirements of Part 503. It is not necessary to consult the PEC with regard to sampling and monitoring programs if a protocol is already approved under one of the Class A alternatives. Figure 11-2 elaborates on the role of the PEC under Part 503.

What's in This Chapter?

This chapter explains how the PEC makes equivalency recommendations and describes how to apply for PEC guidance. The guidance in this chapter may also prove useful for permitting authorities in establishing the information they will need to make equivalency determinations.

11.2 Overview of the PEC's Equivalency Recommendation Process

The first point of contact for any equivalency determination, recommendation, or other guidance is usually the permitting authority. This is the regional EPA office or the State in cases in which responsibility for the Part 503 program has been delegated to the state. Appendix A provides a list of EPA Regional and state Contacts. If PEC involvement is appropriate, the permitting authority will coordinate contact with the PEC.

The PEC considers each equivalency application on a case by-case basis. Applicants submit information on sewage sludge characteristics, process characteristics, climate, and other factors that may affect pathogen reduction or process efficiency as described in Section 11.5. The committee evaluates this information in light of current knowledge concerning sewage sludge treatment and pathogen reduction, and recommends one of five decisions about the process or process sequence:

- It is equivalent to PFRP.
- It is not equivalent to PFRP.
- It is equivalent to PSRP.
- It is not equivalent to PSRP.
- Additional data or other information are needed.

Site-specific equivalency is relevant for many applications; to receive a recommendation for national equivalency, the applicant must demonstrate that the process will produce the desired reductions in pathogens under the variety of conditions that may be encountered at different locations across the country. Processes affected by local climatic conditions or that use materials that may vary significantly from one part of the country to another are unlikely to be recommended as equivalent on a national basis unless specific material specifications and process procedure requirements can be identified.

If the PEC recommends that a process is equivalent to a PSRP or PFRP, the operating parameters and any other conditions critical to adequate pathogen reduction are specified in the recommendation. The equivalency recommendation applies only when the process is operated under the specified conditions.

If the PEC finds that it cannot recommend equivalency, the committee provides an explanation for this finding. If additional data are needed, the committee describes what those data are and works with the permitting authority and the applicant, if necessary, to ensure that the appropriate data are gathered in an acceptable manner. The committee then reviews the revised application when the additional data are submitted.

11.3 Basis for PEC Equivalency Recommendations

As mentioned in Section 11.1, to be determined equivalent, a treatment process must consistently and reliably reduce pathogens in sewage sludge to the same levels achievable by the listed PSRPs or PFRPs. The applicant must identify the process operating parameters (e.g., time, temperature, pH) that result in these reductions.

PFRP Equivalency

To be equivalent to a PFRP, a treatment process must be able to consistently reduce sewage sludge pathogens to below detectable limits. For purposes of equivalency, the PEC is concerned only with the ability of a process to demonstrate that enteric viruses and viable helminth ova have been reduced to below detectable limits. This is because Part 503 requires ongoing monitoring of all Class A biosolids for fecal coliform or *Salmonella* sp. (see Section

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 15 1993

OFFICE OF WATER

MEMORANDUM

SUBJECT: The Role of the **Pathogen Equivalency** Committee Under *the* Part 503 ***Standards*** for ***the Use or Disposal of*** *Sewage* ***Sludge***

FROM: **Michael B. Cook, Director**
Office of Wastewater Enforcement & Compliance

James A. Hanlon, Acting **Director**
Office of Science & Technology

TO: Water Division **Directors**
Regions I - X

PURPOSE

This memorandum explains the role of the Pathogen Equivalency Committee **(PEC)** in providing technical assistance and recommendations regarding pathogen reduction equivalency in implementing the Part 503 ***Standards for the Use or Disposal of Sewage.*** The PEC is an Agency resource available to assist **your** permit writers and regulated authorities. This information should be sent to your Regional Sludge Coordinators, Municipal Construction Managers, Permits and Enforcement Coordinators, and Solid Waste Offices, State Sludge Management Agencies and others concerned with sewage sludge management.

BACKGROUND

The PEC Under Part 257

The Criteria for Classification of Solid Waste Facilities and Practices (44 **FR** 53438, September 13, **1979),** in 40 CFR Part 257 required that sewage sludge disposed on the land be treated by either'a ***Process to Significantly Reduce Pathogens*** (PSRP) or a ***Process to Further Reduce Pathogens (PFRP).*** A list of **PSRPs** and **PFRPs** were included in Appendix II to Part 257.

In 1985, the PEC was formed to provide technical assistance and recommendations on whether sewage sludge treatment processes not included in Appendix II to Part 257 were equivalent to **PSRP or PFRP.** Under Part 257, the PEC provided technical assistance to both the permitting authority and to members of the regulated

Figure 11-2. Role of the PEC under Part 503.

A series of options are provided in the Part 503 regulation for meeting the specific requirements for the two classes of pathogen reduction. One of the Class A alternatives is to treat the sewage sludge by a process equivalent to a PFRP and one of the Class B alternatives is to treat the sewage sludge by a process equivalent to a PSRP. The permitting authority must decide whether a process is equivalent to a PFRP or a PSRP, which is the same approach used under Part 257.

THE PEC UNDER 503

Part 503 provides specific criteria and procedures for evaluating bacterial indicators (Fecal coliforms and Salmonella sp.), enteric virus and viable helminth ova as well as vector attraction reduction. The PEC will continue to support the permitting authority and members of the regulated community under the new Part 503 regulation in evaluating equivalency situations and providing technical assistance in matters such as sampling and analysis. Specifically the PEC:

- will continue to provide technical assistance to the permitting authority and regulated community, including recommendations to the permitting authority about process equivalency. The PEC also will make both site-specific and national (i.e., a process that is equivalent anywhere in the United States where it is installed and operated) recommendations on process equivalency .

- will submit recommendations on process equivalency to the Director, Xealth and Ecological Criteria Division, Office of Science and Technology, who will review those recommendations and then notify the applicant and appropriate permitting authorities of our recommendation.

For site-specific recommendations, requests for PEC review or assistance should be made through the appropriate Federal permitting authority (e.g., the State sludge regulatory authority for delegated programs or the EPA Regional Sludge Coordinator for non-delegated programs). For national recommendations, requests for PEC review or assistance can also be made through the Director, Health and Ecological Criteria Division (WH-586), Office of Science & Technology, U.S. EPA, 401 M St., SW, Washington, D.C. 20460 or directly to the PEC Chairman. The current PEC Chairman is: Dr. James E. Smith, Jr., U.S. EPA, CERI, (Center for Environmental Research Information) 26 W Martin Luther King Dr., Cincinnati, OH 45268 (Tele: 513/569-7355).

Additional information and guidance to supplement the pathogen reduction requirements of Part 503 and the procedures to use to reach the PEC and the assistance provided by the PEC is provided in "Control of Pathogens and Vector Attraction in Sewage

Figure 11-2. Role of the PEC under Part 503 (continued).

community. The PEC membership has included representatives from the Office of Research & Development (ORD), office of Wastewater Enforcement & Compliance (OWEC), and the Office of Science & Technology (OST) with extensive experience in microbiology, sludge process engineering, statistics and regulatory issues. The PEC recommendations regarding the equivalency of processes were forwarded to the Office of Science and Technology, which notified applicants about the PEC's recommendations. Final decisions on equivalency were made by the permitting authority.

The Part 503 Sewage Sludge Standards

The 40 CFR Part 503 Standards for the Use or Disposal of Sevags Sludge were published in the Federal Register on February 19, 1993 (58 FR 9248) under the authority of section 405 of the Clean Water Act, as amended. Part 503 establishes requirements for sewage sludge applied to the land, placed on a surface disposal site, or fired in a sewage sludge incinerator. Along with the 40 CFR Part 258 Municipal Solid Waste (MSW) Landfill Regulation (56 FR 50978, October 9, 1991), which established requirements for materials placed in MSW landfills, the Part 503 requirements for land application of sewage sludge and placement of sewage sludge on a surface disposal site, replaces the requirements for those practices, including the requirement to treat the sewage sludge in either a PSRP or a PFRP, in Part 257.

The Part 503 regulation addresses disease-causing organisms (i.e., pathogens) in sewage sludge by establishing requirements for sewage sludge to be classified either as Class A or Class B with respect to pathogens as an operational standard. Class A requirements are met by treating the sewage sludge to reduce pathogens to below detectable limits, while the Class B requirements rely on a combination of treatment and sits restrictions to reduce pathogens. The site restrictions prevent exposure to the pathogens and rely on Natural Environmental processes to reduce the pathogens in the sewage sludge to below detectable levels. In addition to pathogen reduction, a vector attraction reduction requirement has to be met when sewage sludge is applied to the land or placed on a surface disposal site.

Vector attraction reduction requirements are imposed under Part 503 to reduce the potential for spreading of infectious disease agents by vectors (i.e., flies, rodents, and birds). A series of alternative methods for meeting the vector attraction reduction requirement are provided in the rule.

All sewage sludges that are to be sold or given away in a bag or other container for land application, or applied to lawns or home gardens must meet Class A pathogen control and vector attraction reduction requirements. All sewage sludge intended for land application must meet at least the Class B pathogen control and vector attraction reduction requirements. Surf ace disposal of sewage sludge requires that Class A or Class B requirements, along with one of the vector attraction reduction practices, be met unless the sewage sludge is covered with soil or other material daily.

Figure 11-2. Role of the PEC under Part 503 (continued).

Sludge" (EPA 625/R-92/013), which will-be updated from time to time by the PEC. This document is an update of the 1989 document "Control of Pathogens in Municipal Wastewatsr Sludge" (EPA/625/10-89/006), and is available from CERI.

If there are any questions about this memorandum, please contact Bob Bastiaa from OWEC at 202/260-7378 or Dr. Smith at CERI.

Figure 11-2. Role of the PEC under Part 503 (continued).

4.3) to ensure that Salmonella sp. are reduced to below detectable limits (i.e., to less than 3 MPN per 4 grams total solids sewage sludge [dry weight basis]) and that growth of pathogenic bacteria has not occurred. Thus, to demonstrate PFRP equivalency, the treatment process must be able to consistently show that enteric viruses and viable helminth ova are below the detectable limits, shown below:

There are two ways these reductions can be demonstrated:

- Direct monitoring of treated and untreated sewage sludge for enteric viruses and viable helminth ova
- Comparison of the operating conditions of the process with the operating conditions of one of the listed PFRPs.

The process comparison approach to demonstrating equivalency is discussed in

Section 11.4.

PSRP Equivalency

To be equivalent to PSRPs, a process must consistently reduce the density of pathogenicviruses and bacteria (number per gram of biosolids (dry weight basis)) in mixed sludge from a conventional plant by equal to or greater than 1 log (base 10). Data indicate that, for conventional biological and chemical treatment processes (e.g. digestion and lime treatment) a reduction of 1 log (base 10) in pathogenic virus and bacteria density correlates with a reduction of 1 to 2 logs (base 10) in the density of indicator organisms (Farrell et al., 1985, Farrah et al., 1986). On this basis a 2-log (base 10) reduction in fecal indicator density is accepted as satisfying the requirement to reduce pathogen density by 1 log (base 10) for these types of processes (EPA, 1989c). Specifically, the applicant must demonstrate a 2-log (base 10) reduction (number per gram of biosolids (dry weight basis)) in fecal coliforms.

There is substantial data to indicate that sludge produced by conventional wastewater treatment and anaerobic digestion at 35EC for more than 15 days contains fecal coliforms at average log (base 10) densities (number per gram of biosolids (dry weight basis)) of less than 6.0 (Farrell, 1988). Thus, for processes or combinations of processes that do not depart radically from conventional treatment (gravity thickening, anaerobic or aerobic biological treatment, dewatering, air drying and storage of liquid or sludge cake), or for any process where there is a demonstrated correlation between pathogenic bacteria and virus reduction and indicator organisms reduction, the PEC accepts an average log (base 10) density (no./g. TSS) of fecal coliforms and fecal streptococci of less than 6.0 in the treated sludge as indicating adequate viral and bacterial pathogen reduction. (The average log density is the log of the geometric mean of the samples taken. Calculations of average log density should be based on data from approximately nine sludge samples to account for the natural variability and the variability of the microbiological tests.)

The data submitted must be scientifically sound in order to ensure that the process can reliably produce the required reductions under all the different types of conditions that the process may operate. For example, for processes that may be affected by daily and seasonal variations in the weather, four or more sets of samples taken at different times of the year and during different precipitation conditions (including worst-case conditions) will be needed to make this demonstration.

For national equivalency recommendations, the demonstration must show that the process can reliably produce the desired reductions under the variety of climatic and other conditions that may be encountered at different locations in the United States.

11.4 Guidance on Demonstrating Equivalency for PEC Recommendations

Many of the applicants seeking equivalency do not receive a recommendation from the PEC. The most common reason for this is incomplete applications or insufficient microbiological data. The review process can be both lengthy and expensive, but it can be expedited and simplified if the applicant is aware of the type of data that will be required for the review and submits a complete plan for demonstrating equivalency in a timely fashion.

As described below, equivalency can be demonstrated in one of two ways:

- By comparing operating conditions to existing PFRPs or PSRPs.
- By providing performance and microbiological data.

Comparison to Operating Conditions for Existing PSRPs or PFRPs

If a process is similar to a PSRP or PFRP described in the Part 503 regulation (see Tables 4-2 and 5-1), it may be possible to demonstrate equivalency by providing performance data showing that the process consistently meets or exceeds the conditions specified in the regulation. For example, a process that consistently produces a pH of 12 after 2 hours of contact (the PSRP condition required in Part 503 for lime stabilization) but uses a substance other than lime to raise pH could possibly qualify as a PSRP equivalent. In such cases, microbiological data may not be necessary to demonstrate equivalency.

Process-Specific Performance Data and Microbiologic Data

In all other cases, both performance data and microbiological data (listed below) are needed to demonstrate process equivalency:

- A description of the various parameters (e.g., sewage sludge characteristics, process operating parameters, climatic factors) that influence the microbiological char-

acteristics of the treated sewage sludge (see Section 11.5 for more detail on relevant parameters).

- Sampling and analytical data to demonstrate that the process has reduced microbes to the required levels (see Section 11.3 for a description of levels).
- A discussion of the ability of the treatment process to consistently operate within the parameters necessary to achieve the appropriate reductions.

Sampling and Analytical Methods

Sewage sludge should be sampled using accepted, state-of-the-art techniques for sampling and analyzed using the methods required by Part 503 (see Chapter 9). The sampling program should demonstrate the quality of the sewage sludge that will be produced under a range of conditions. Therefore, sampling events should include a sufficient number of samples to adequately represent product quality, and sampling events should be designed to reflect how the operation might be affected by changes in conditions including climatic and sewage sludge quality variability.

Data Quality

The quality of the data provided is an important factor in EPA's equivalency recommendation. The following steps can help ensure data quality:

- Use of accepted, state-of-the-art sampling techniques (see Chapter 9)
- Obtaining samples that are representative of the expected variation in sewage sludge quality.
- Developing and following quality assurance procedures for sampling.
- Using an independent, experienced laboratory to perform the analysis.

Since processes differ widely in their nature, effects, and processing sequences, the experimental plan to demonstrate that the process meets the requirements for PSRP or PFRP equivalency should be tailored to the process. The permitting authority will evaluate the study design, the accuracy of the data, and the adequacy of the results for supporting the conclusions of the study.

Can Pilot-Scale Data Be Submitted?

Operation of the process at a full scale facility is desirable. However, if a pilot-scale operation truly simulates full-scale operation, testing on this reduced scale is possible. The permitting authority and the PEC should be contacted to discuss this possibility before testing is initiated. In such cases, it is important to indicate that the data were obtained from a pilot-scale operation, and to discuss why and to what extent this simulates full-scale operation. Any data available from existing full-scale operations would be useful.

The conditions of the pilot-scale operation should be at least as severe as those of a full-scale operation. The arrangement of process steps, degree of mixing, nature of the flow, vessel sizing, proportion of chemicals used, etc. are all part of the requirement. Any substantial degree of departure in the process parameters of the full-scale operation that might reduce the severity of the procedure will invalidate any PEC equivalency recommendations and permitting authority equivalency determinations and will require a retest under the new condition.

11.5 Guidance on Application for Equivalency Recommendations

The following outline and instructions are provided as guidance for preparing applications for equivalency recommendations by EPA's Pathogen Equivalency Committee.

Summary Fact Sheet

The application should include a brief fact sheet that summarizes key information about the process. Any important additional facts should also be included.

Introduction

The full name of the treatment works and the treatment process should be provided. The application should indicate whether it is for recommendation of:

- PSRP or PFRP equivalency.
- Site-specific or national equivalency.

Process Description

The type of sewage sludge used in the process should be described, as well as other materials used in the process. Specifications for these materials should be provided as appropriate. Any terms used should be defined.

The process should be broken down into key steps and graphically displayed in a quantified flow diagram of the wastewater and sewage sludge treatment processes. Details of the wastewater treatment process should be provided and the application should precisely define which steps constitute the beginning and end of sewage sludge treatment.*' The earliest point at which sewage sludge treatment can be defined as beginning is the point at which the sewage sludge is collected from the wastewater treatment process. Sufficient information should be provided for a mass balance calculation (i.e., actual or relative volumetric flows and solids concentration in and out of all streams, additive rates for bulking agents or other additives). A description of process parameters should be provided for each step of the process, giving typical ranges and mean values where appropriate. The specific process parameters that should be discussed will depend on the type of process and should include any of the following that affect pathogen reduction or process reliability:

Sewage Sludge Characteristics

- Total and volatile solids content of sewage sludge before and after treatment

- Proportion and type of additives (diluents) in sewage sludge
- Chemical characteristics (as they affect pathogen survival/destruction, e.g., pH)
- Type(s) of sewage sludge (unstabilized vs. stabilized, primary vs. secondary, etc.)
- Wastewater treatment process performance data (as they affect sewage sludge type, sewage sludge age, etc.)
- Quantity of treated sewage sludge
- Sewage sludge age
- Sewage sludge detention time

Process Characteristics

- Scale of the system (e.g., reactor size, flow rate)
- Sewage sludge feed process (e.g., batch vs. continuous)
- Organic loading rate (e.g., kg volatile solids/cubic meter/day)
- Operating temperature(s) (including maximum, minimum, and mean temperatures)
- Operating pressure(s) if greater than ambient
- Type of chemical additives and their loading rate
- Mixing
- Aerobic vs. anaerobic
- Duration/frequency of aeration
- Dissolved oxygen level maintained
- Residence/detention time
- Depth of sewage sludge
- Mixing procedures
- Duration and type of storage (e.g., aerated vs. nonaerated)

Climate

- Ambient seasonal temperature range
- Precipitation
- Humidity

The application should include a description of how the process parameters are monitored including information on monitoring equipment. Process uniformity and reliability should also be addressed. Actual monitoring data should be provided whenever appropriate.

Description of Treated Sewage Sludge

The type of treated sewage sludge (biosolids) should be described, as well as the sewage sludge monitoring program for pathogens (if there is one). How and when are samples taken? For what parameters are the samples analyzed? What protocols are used for analysis? What are the results? How long has this program been in operation?

Sampling Technique(s)

The PEC will evaluate the representativeness of the samples and the adequacy of the sampling techniques. For a recommendation of national PFRP equivalency, samples of untreated and treated sewage sludge are usually needed (see Sections 11.3, 4.6, and 10.4). The sampling points should correspond to the beginning and end of the treatment process as defined previously under Process Description above. Chapters 9 and 10 provide guidance on sampling. Samples should be representative of the sewage sludge in terms of location of collection within the sewage sludge pile or batch. The samples taken should include samples from treatment under the least favorable operating conditions that are likely to occur (e.g., wintertime). Information should be provided on:

- Where the samples were collected from within the sewage sludge mass. (If samples were taken from a pile, include a schematic of the pile and indicate where the subsamples were taken.)
- Date and time the samples were collected. Discuss how this timing relates to important process parameters (e.g., turning over, beginning of drying).
- Sampling method used.
- How any composite samples were compiled.
- Total solids of each sample.
- Ambient temperature at time of sampling.
- Temperature of sample at time of sampling.
- Sample handling, preservation, packaging, and transportation procedures.
- The amount of time that elapsed between sampling and analysis.

Analytical Methods

Identify the analytical techniques used and the laboratory(s) performing the analysis.

Analytical Results

The analytical results should be summarized, preferably in tabular form. A discussion of the results and a summary of major conclusions should be provided. Where appropriate, the results should be graphically displayed. Copies of original data should be provided in an appendix.

Quality Assurance

The application should describe how the quality of the analytical data has been ensured. Subjects appropriate to address are: why the samples are representative; the quality assurance program; the qualifications of the in-house or contract laboratory used; and the rationale for selecting the sampling technique.

Rationale for Why Process Should Be Determined Equivalent

Finally, the application should describe why, in the applicant's opinion, the process qualifies for PSRP or PFRP equivalency. For example, it may be appropriate to describe or review particular aspects of the process that contribute to pathogen reduction, and why the process is expected to operate consistently. Complete references should be provided for any data cited. Applications for a recommendation of national equivalency should discuss why the process effectiveness is expected to be independent of the location of operation.

Appendices

A copy of the complete laboratory report(s) for any sampling and analytical data should be attached as an appendix. Any important supporting literature references should also be included as appendices.

11.6 Pathogen Equivalency Committee Recommendations

Tables 11 .1 and 11.2 list processes that the PEC has recommended for use nationally as equivalent to PSRP or PFRP respectively. Space in the tables limits the detail given for each of the processes. As such individuals having an interest in any of the processes are encouraged to contact either the PEC or the applicant for greater detail on how the process must be operated to be PSRP or PFRP respectively.

Table 11-1. Processes Recommended as Equivalent to PSRP

Applicant	Process	Process Description
N-Viro Engery Systems Ltd., Toledo, Ohio	Alkaline Addition to achieve *Lime Stabilization*	Use of cement kiln dust and lime kiln dust (instead of lime) to treat sludge by raising the pH. Sufficient lime or kiln dust is added to sludge to produce a pH of 12foratleast 12 hours of contact
Synox Corp., Jacksonville, FL	OxyOzonation	Batch process where sludge is acidified to pH 3.0 by sulfuric acid; exposed to 1 lb. Ozone/i 000 gallons of treated sludgte under 60 psig pressure for 60 minutes; depressurized; mixed with 100 mg/l of sodium nitrite and held for ≥ 2 hours; and stored at ≤ pH 3.5. Limitations imposed were for total solids to be ≤ 4%; temperature must be ≥ 20°C; and total solids must be ≤ 6.2% before nitrite addition.

11.7 Current Issues

The PEC is continuing to develop methodologies and protocols for the monitoring of pathogen and vector attraction reduction. Current issues include:

- Establishment of a vector attraction reduction equivalency process
- Conducting round robin laboratory testing for pathogens in sewage sludge and biosolids

In addition, the PEC continues to recommend interpretations of the Part 503 with regard to the sampling and monitoring requirements set forth in this document.

Table 11-2. Processes Recommended as Equivalent to PFRP

Applicant	Process	Process Description
CBI Walker, Inc., Aurora, Illinois	ATP™ Two Stage Sludge Stabilization Process	Sludge is introduced intermittently into a vessel, amounting to 5 to 20% of its volume, where it is heated by both external heat exchange and by the bio-oxidation which results from vigorously mixing air with the sludge (pasteurized) and has a nominal residence time of 18 to 24 hours. Time between feedings of unprocessed sludge can range from 1.2 (@ ~ 65°C) to 4.5 (@ > 60°C) hours. Exiting sludge is heat exchanged with incoming unprocessed sludge. Thus the sludge is cooled before it enters a mesophilic digester. Time and temperature in the first vessel are critical and controlled by the equation below for sludges of ≤ 7% solids, times ≥ 30 minutes, and temperatures ≥ 50°C. Operations of the reaction vessel during the time-temperature period must be either plug flow or batch mode. $D = 50{,}070{,}000 / 10^{0.1400t}$ where D = time required in days; t = temperature in °C
Fuchs Gas und Wassertechnik, Gmbh, Mayen, Germany	Autothermal Thermophilic Aerobic Digestion	ATAD is a two-stage, autothermal aerobic digestion process. The stages are of equal volume. Treated sludge amounting to 1/3 the volume of a stage is removed every 24 hours from the second stage as product. An equal amount then is taken from the firststage and fed to the second stage. Similary, an equal amount of untreated sludge is then fed to the first stage. In the 24 hour period between feedings, the sludge in both stages is vigorously agitated and contacted with air. Bio-oxidation takes place and the heat produced increases the temperature. Sludge temperature in the reactors averages between 56 and 57°C for ≥ a 16 hour period, while the overall hydraulic residence time is 6 days.
International Process Systems, Inc., Glastonbury, Connecticut	Type of Cornposting Process	40 CFR 503.32(a)(7) states that when the within-vessel cornposting method is employed, the sludge is to be maintained at operating condition of 55C or greater for 3 days, for the product to be PFRP. IPS Process' operation is to further be controlled so that the composting mass passes through a zone in the reactor in which the temperature of the compost is at least 55°C throughout the entire zone, and the time of contact in this zone is at least three days.
K-F Environmental Technologies, Inc., Pompton Plains, NJ	Sludge Drying	Sludge is heated to a minimum temperature of 100°C and indirectly dried to below 10% moisture using oil as a heat transfer medium. The final discharge product has exceeded a temperature of 80°C and is granular dry pellet that can be land applied, incinerated or landfilled. In addition the following conditions must be met: *Dewatered sludge cake is dried by direct or indirect contact with hot gases, and moisture content is reduced to 10% or lower. Sludge particles reach temperatures well in excessof 80°C, or the* wet *bulb* temperature *of the gas stream in contact with* the *sludge at the point where it leaves the dryer is in excess of 80°C.*
Lyonnaise des Eaux, Le Pecz-Sur-Seine, France	Two-Phase Thermo-Meso Feed Sequencing Anaerobic Digestion*	Sewage sludge is treated in the absence of air in an acidogenic thermophilic reactor and a mesophilic methanogenic reactor connected in series. The mean cell residence time shall be at least 2.1 days (± 0.05 d) in the acidogenic thermophilic reactor followed by 10.5 days (± 0.3 d) in the mesophilic methanogenic reactor. Feeding of each digester shall be intermittent and occurring 4 time per day every 6 hours. The mesophilic methanogenic reactor shall be fed in priority from the acidogenic thermophilic reactor. Between two consecutive feedings temperature inside the acidogenic thermophilic reactor should be between 49°C and 55°C with 55°C maintained during at least 3 hours. Temperature inside the mesophilic methanogenic reactor shall be constant and at least 37°C.
ATW, Inc. Santa Barbara, CA	Alkaline Stabilization	Manchak process uses quicklime to simultaneously stabilize and pasteurize biosolids. Quicklime, or a combination of quicklime and flyash, is mixed with dewatered biosolids at a predetermined rate in a confined space. An instant exothermic reaction is created in the product wherein the pH is raised in excess of 12 after two hours of contact, in addition, the temperature is raised in excess of 70°C for > 30 minutes.

(continued)

Table 11-2. Continued.

Applicant	Process	Process Description
N-Viro Energy Systems, Ltd., Toledo, OH	Advanced Alkaline stabilization with subsequent accelerated drying	**Alternative 1:** Fine alkaline materials (cement kiln dust, lime kiln dust, quicklime fines, pulcerized lime, *or* hydrated lime) are uniformly mixed by mechanical or aeration mixing into liquid or dewatered sludge to raise the pH to >12 for 7 days. If the resulting sludge is liquid, it is dewatered. The stabilized sludge cake is then air dried (while pH remains >12 for ≥ 7 days) for > 30 days and until the cake is ≥ 65% solids. A solids concentration of ≥ 60% is achieved before the pH drops below 12. The mean temperature of the air surrounding the pile is > 5°C (41°F) for the first 7 days. **Alternative** 2: Fine alkaline materials (cement kiln dust, lime kiln dust, quicklime fines, pulverized lime, or hydrated lime) are uniformly mixed by mechanical or aerationi mixing into liquid or dewater sludge to raise the pH to > 12 for ≥ 72 hours. If the resulting sludge is liquid, it is dewatered. The sludge cake is then heated, while the pH > 12, using exothermic reactionis or other thermal processes to achieve temperatures of ≥ 52°C (126°F) throughout the sludge for ≥ 12 hours. The stabilized sludge is then air dried (while pH > 12 for ≥ 3 days) to ≥ 50% solids.
Synox Corp., Jacksonville, FL	OxyOzonation	Operation occurs in a batch mode and under the following conditions: sludge temperature of > 20°C; sludge solids of c 6% TSS; pH during ozonation of 2.5 - 3.1 and during nitrite contact of 2.6 - 3.5; sludge ORP after ozonation of > 100 mV; nitrite dose of ≥ 670 mg (NO_2)/1 sludge or 16 g (NO_2)/kg sludge solids, whichever is greater is to be mixed into the ozonated sludge. Ozonation takes place in a pressure vessel operating at *60* psig.
Ultraclear, Marlboro, NJ	Microbiological Conditioning and Drying Process (MVCD)	In this process, sludge cake passes through several aerobic-biological type stages (Composting is an example) where different temperatures are maintained for varying times. Stage 1 occurs at 35°C for 7-9 hours; stage 2 occurs at 35-45°C for 8-10 hours; stage 3 occurs at 45-65°C for 7-l 0 hours; and the last stage is pasteurization at 70-80°C for 7-10 hours. In addition one of two conditions described below must be met: **Condltlon 1:** *Dewatered sludge cake* is *dried by direct or indirect contact with hot gases, and moisture content* is *reduced* to 10% *or lower. Sludge particles reach temperatures we// in excess of 80°C, or the wef bulb temperature of the gas stream in contact with the sludge at the point where it leaves the dryer is in excess of 80°C. OR* **Condltlon** *2:* A) *Using the within-vessel, static aerated pile, or windrow composting methods, the sludge is maintained at minimum operating conditions of 40°C for 5 days. For 4 hours during the period the temperature exceeds 55°C; {Note: another PSRP-type process should be substituted for that* of *composting};* and B) *Sludge is maintained for at least 30 minutes at a minimum temperature of 70°C.*

*Currently a site specific recommendation. Undergoing further study for national equivalency

References and Additional Resources

Farrah, S.R., G. Bitton and S.G. Zan. 1986. Inactivation of enteric pathogens during aerobic digestion of wastewater sludge. EPA Pub. No. EPA/600/2-86/047. Water Engineering Research Laboratory, Cincinnati, OH. NTIS Publication No. PB86-183084/A5. National Technical Information Service. Springfield. Virginia.

Farrell, J.B., G. Stern, and A.D. Venosa. 1985. Microbial destructions achieved by full-scale anaerobic digestion. Workshop on control f Sludge pathogens. Series IV. Water Pollution Control Federation. Alexandria, Virginia.

Smith, James E. Jr. and J.B. Farrell. 1996. Current and future disinfection - Federal perspectives. Presented at Water Environment Federal 69th Annual Conference & Exposition.

Whittington, W.A., and E. Johnson. 1985. Application of 40 CFR Part 257 regulations to pathogen reduction preceding land application of sewage sludge or septic tank pumpings. Memorandum to EPA Water Division Directors. U.S. EPA Office of Municipal Pollution Control, November 6.

Chapter 12
References and Additional Resources

APHA.1992. Standard methods for the examination of water and wastewater. 18th ed. Washington, DC: American Public Health Association.

ASTM. 1992a. Annual book of ASTM standards. Philadelphia, PA: American Society for Testing and Materials.

ASTM. 1992b. Standard practice for recovery of viruses from wastewater sludges. Section 11 -Water and Environ. Techn. In ASTM (1992a).

Ahmed, A.U., and D. L. Sorensen. 1995. Kinetics of pathogen destruction during storage of dewatered biosolids. Water Environment Research. 67(2):143 -150.

Ault, S.K. and M.Schott, 1993. Aspergillus, Aspergillosis, and Composting Operations in California, Technical Bulletin No. 1. California Integrated Waste Management Board.

Bastian, R.K. 1997. The biosolids (sludge) treatment, beneficial use, and disposal situation in the USA. European Water Poll. Control. 7(2): 62-79.

Benedict, A.H., and D.A. Calrson. 1973. Temperature acclimation in aerobic bio-oxidation systems. J. WPCF 45(1):10 - 24.

Berg G. and D. Berman.1980. Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. Appl. Environ. Microbiol. 39 (2):361-368.

Bonner, A.B. and D.O. Cliver. 1987. Disinfection of viruses in septic tank and holding tank waste by calcium hydroxide (Lime). Unpublished report, Small Scale Waste Management Project. U. of Wisconsin. Madison, WI.

Casson, L. W., C. A. Sorber, R. H. Palmer, A. Enrico, and P. Gupta. 1992. HIV survivability in wastewater. Water Environ Res. 64:213-215.

Counts, C.A. and A.J. Shuckrow. 1975. Lime stabilized sludge: its stability and effect on agricultural land. Rept. EPA670/2-75-012, U.S. EPA.

Davies, O.L. and P.L. Goldsmith. 1972. Statistical methods in research and production. Longman Group Ltd. Essex, England.

Engineering News Record, August 13, 1987. No AIDS Threat in Sewage. Issue 47

Epstein, E.. 1997.The science of composting. Technomic Publishing Company. Lancaster, PA.

Farrell, J.B., J.E. Smith, Jr., S.W. Hathaway, and R.B. Dean. 1974. Lime stabilization of primary sludges. J. WPCF 46(1):113-122.

Farrell, J.B., G. Stern, and A.D. Venosa. 1985. Microbial destructions achieved by full-scale anaerobic digestion. Workshop on Control of Sludge Pathogens, Series IV. Alexandria, VA: Water Pollution Control Federation.

Farrell, J.B., B.V. Salotto, and A.D. Venosa. 1990. Reduction in bacterial densities of wastewater solids by three secondary treatment processes. Res. J. WPCF 62(2): 177-l 84.

Farrell, J.B. 1993. Fecal pathogen control during composting. pp. 282-300 In: H.A. J. Hoitink and H.M. Keener (eds). Science & Engineering of Composting: Design, Environmental, Microbiological, and Utilization Aspects. Renaissance, Pub., Worthington, OH.

Farrell, J. B., V. Bhide, and J. E. Smith Jr. 1996. Development of EPA's new methods to quantify vector attraction of wastewater sludges. Water Environ. Res. 68 (3): 286-294.

Farzadegan, H. 1991. Proceedings of a Symposium: Survival of HIV in Environmental Waters. Baltimore, MD. National Science Foundation and the Johns Hopkins University.

Feldman, K., 1995. Sampling for Airborne Contaminants. BioCycle 36(8): 84-86

Fisher, W.J. 1984. Calculation of volatile solids destruction during sludge digestion. pp. 514-528 in Bruce, A., (ed). Sewage sludge stabilization and disinfection. Published for Water Research Centre. E. Harwood, Ltd. Chichester, England.

Fox, C.J., P.R. Fitzgerald, and C. Lue-Hing. 1981. Sewage organisms: a color atlas. Metropolitan Water Reclamation District of Greater Chicago, Chicago, Illinois.

(Photos in Chapter 2 reproduced with permission of the Metropolitan Water Reclamation District of Greater Chicago.)

Gerba, C.P., C. Wallis, and J.L. Melmick. 1975. Fate of wastewater bacterial and viruses in soil. J. Irrig. Drain Div. Am. Soc. Civ. Engineers. 101:157-1 74.

Goyal, S.M., S.A. Schaub, F.M. Wellings, D. Berman, J.S. Glass, C.J. Hurst, D.A. Brashear, C.A. Sorber, B.E. Moore, G. Bitton, P.H. Gibbs, and S.R. Farrah. 1984. Round robin investigation of methods for recovering human enteric viruses from sludge. Applied & Environ. Microbiol. 48:531-538.

Gover, N. 1993. HIV in wastewater not a recognized threat, other pathogens can be. National Small Flows Clearinghouse Newsletter. July 1993.

Gupta, P. 1991. HIV Survivability in Wastewater. Proceedings of a Symposium: Survival of HIV in Environmental Waters. Baltimore, MD. National Science Foundation and the Johns Hopkins University.

Haines, J., 1995. Aspergillus in compost: Straw man or fatal flaw? BioCycle, 1995 36 (4): 32-35.

Harding, H.J., R.E. Thomas, D.E. Johnson, and C.A. Sorber. 1981. Aerosols generated by liquid sludge application to land. Rept. No. EPA-600/l -81-028. U.S. EPA, Off ice of Research and Development. Washington, DC.

Haug, R. T. 1993. The practical handbook of compost engineering. Lewis Publishers.

Hay, J. C., 1996. Pathogen destruction and biosolids composting. BioCycle, 37 (6):67-76

Helsel, D.R. 1990. Less than obvious: statistical treatment of data below the detection limit. Environ. Sci. Technol. 24(12): 1767-l 774.

Iacaboni, M.D., J.R. Livingston, and T.J. LeBrun. 1984. Windrow and static pile composting of municipal sewage sludges. Report No.: EPA/600/2-84-122 (NTIS PB84-215748).

Johns Hopkins School of Hygiene and Public Health. 1991. HIV transmission in the environment: What are the risks to the public?s health? Public Health News.

Johnson, R.W., Blatchley, E.R. III, and D.R. Mason. 1994. HIV and the blood borne pathogen regulation: Implications for the wastewater industry. Water Environ. Res. *66:* 684-691.

Keith, L.H., (ed). 1988. Principles of Environmental Sampling. American Chemical Society.

Kenner, B.A. and H.P. Clark. 1974. Detection and enumeration of *Salmonella* and *Pseudomonas aeruginosa.* J. WPCF 46(9):2163-71.

Kent, R.T. and K.E. Payne. 1988. Sampling groundwater monitoring wells: Special quality assurance and quality control considerations. pp. 231-246 *In* Keith, L.H., (ed.) Principles of Environmental Sampling. American Chemical Society.

Kindzierski, W.B., R.E. Roberts, and N.J. Low. 1993. Health effects associated with wastewater treatment, disposal, and reuse. Water Environ. Res. 65: 599-606.

Kowal, N.F. 1985. Health effects of land application of municipal sludge. Pub. No.: EPA/600/1-85/015. Research Triangle Park, NC: U.S. EPA Health Effects Research Laboratory.

Lee, K.M., C.A. Brunner, J.B. Farrell, and A.E. Eralp. 1989. Destruction of enteric bacteria and viruses during two-phase digestion. J. WPCF 61(8): 1422-l 429.

Martin, J.H., Jr., H.E. Bostian, and G. Stem. 1990. Reductions of enteric microorganisms during aerobic sludge digestion. Water. Res. 24(11): 1377-l 385.

Millner, P.D., S.A. Olenchock, E. Epstein, R. Rylander, J. Haines, J. Walker, B.L. Ooi, E. Horne, and M. Maritato 1994. Bioaerosols associated with composting facilities. Compost Sci. and Util. 2(4):6-57.

Moore, B.E., D.E.Camann, G.A.Turk, and C.A. Sorber. 1988. Microbial characterization of municipal wastewater at a spray irrigation site: The Lubbock infection surveillance study. J. WPCF. 60(7): 1222-l 230.

Moore, B.E. 1993. Survival of human immunodeficiency virus (HIV), HIV-infected Lymphocytes, and Poliovirus in Water. Applied and Environ. Microbiol. 59: 1437-l 443.

Newman, M.C. and P.M. Dixon. 1990. UNCENSOR: A program to estimate means and standard deviations for data sets with below detection limit observations. Am. Envir. Laboratory 2(2):26-30.

Obeng, L. Health aspects of water supply and sanitation. In Information and Training for Low-Cost Water Supply and Sanitation. D. Trattles.(ed.) World Bank. Washington, D.C.

Olivieri, V. P., L. Cox, M. Sarao, J. L. Sykora, and P. Gavagahn. 1989. Reduction of selected indicator and pathogenic microorganisms removal during conventional anaerobic sludge digestion. *In* AWWA/WPCF Residuals Management Conference, San Diego, CA.

Ponugoti, Prabhaker R., Mohamed F. Dahab, Rao Surampalli. 1997. Effects of different biosolids treatment systems on pathogen and pathogen indicator reduction. Water Environ. Res. 69:1195-l 206

Reimers, R.S., M.D. Little, T.G. Akers, W.D. Henriques, R.C. Badeaux, D.B. McDonnell, and K.K. Mbela. 1989. Persistence of pathogens in lagoon-stored sludge. Rept. No. EPA/600/2-89/015 (NTIS No. PB89-190359/

AS). Cincinnati, OH: U.S. EPA Risk Reduction Engineering Laboratory.

Schafer, P.L., J.B. Farrell, W.R. Uhte, and B. Rabinowitz. 1994. Pre-pasteurization, European and North American assessment and experience. Pp. 1 O-39 to 1 O-50. In: The Management of Water and Wastewater Solids for the 21 st Century: A Global Perspective. Conference Proceedings, Water Environment Federation.

Scheuerman, P.R., S.R. Farrah, and G. Bitton. 1991. Laboratory studies of virus survival during aerobic and anaerobic digestion of sewage sludge. Water Resources 25:241-245.

Smith Jr, J. E., and J. B. Farrell. 1994. Vector attraction reduction issues associated with the Part 503 regulations and supplemental guidance. *In* Management of Water and Wastewater Solids for the 21 st Century: A Global Perspective . Water Environ. Fed., Washington, D.C.

Smith Jr., J E. and J. B. Farrell. 1996. Current and future disinfection – Federal perspectives. Presented at Water Environment Federation 69th Annual Conference & Exposition. Charlotte, North Carolina

Soares, H. M., B. Cardenas, D. Weir, and M. S. Switzenbaum. 1995. Evaluating pathogen regrowth in biosolids compost. BioCycle, 36(6):70-76.

Sobsey, M.D., and P.A. Shields. 1987. Survival and transport of viruses in soils. Model studies pp. 155-177. In V.C. Rao and J.L. Melnick, (eds). Human viruses in sediments, sludge, and soils. CRC Press, Boca Raton, FL.

Sorber, C.A., and B.E. Moore. 1986. Survival and transport of pathogens in sludge-amended soil, a critical literature review. Report No.: EPA/600/2-87/028. Off ice of Res. and Dev. USEPA.. Cincinnati, OH:

Storey, G.W. and R.A. Phillips. 1985. The survival of parasite eggs throughout the soil profile. Parasitology. 91:585-590.

Switzenbaurm, M. S., L.H. Moss, E.Epstein, A. B. Pincince, J.F. Donovan. 1997. Defining biosolids stability: a basis for public and regulatory acceptance. Water Environ. Res. Foundation. Proj. 94-REM-l.

USDA/U.S. EPA. 1980. Manual for composting sewage sludge by the Beltsville aerated-pile method. Report No.: EPA/600/8-80-022.

U.S. EPA. 1979. Process design manual for sludge treatment and disposal. Report No.: EPA/625/1-79/001. Water Engineering Research Laboratory and Center for Environmental Research Information. USEPA. Cincinnati, OH:

U.S. EPA. 1980. Samplers and sampling procedures for hazardous waste streams. Report No.: EPA/600/2-80/018. Municipal Environmental Research Laboratory. USEPA. Cincinnati, OH:

U.S. EPA. 1983. Enteric virus removal in wastewater treatment lagoon systems (Project Summary, EPA/600/S1-83-012). U.S. EPA/Health Effects Research Laboratory. USEPA. Research Triangle Park, NC.

U.S. EPA. 1985. Health effects of land application of municipal sludge. EPA Pub. No. 600/1-85/01 5. Health Effects Research Laboratory. USEPA. Research Triangle Park, NC.

U.S. EPA. 1984. EPA policy on municipal sludge management. Federal Register 49:24358, June 12, 1984.

U.S. EPA. 1986. Test methods for evaluating solid waste: method 9045A, soil and waste pH, Revision 1, Nov. 1990. Washington, D.C.: Office of Solid Waste and Emergency Response, U.S. EPA. U.S. Supt. of Documents.

U.S. EPA. 1986. Inactivation of enteric pathogens during aerobic digestion of wastewater sludge (Project Summary, EPA/600/SO-86/047). U.S. EPA/Water Engineering Research Laboratory. Cincinnati, OH.

U.S. EPA. 1988. National sewage sludge survey database. National Computer Center. Research. Triangle Park, NC

U.S. EPA. 1989. POTW sludge sampling and analysis guidance document. 2nd edition. EPA 833-B-89-l 00. Office of Wastewater Enforcement and Compliance. Washington, DC.

U.S. EPA. 1989. Technical support document for pathogen reduction in sewage sludge. NTIS No.: PB89-136618. National Technical Information Service. Springfield, VA.

U.S. EPA. 1991. Preliminary risk assessment for viruses in municipal sewage sludge applied to land. Project Summary, EPA/600/SR-92/064. U.S. EPA/Office of Health & Environmental Assessment. Washington, DC.

U.S. EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No.: PB93-11069. National Technical Information Service. Springfield, VA.

U.S. EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No.: PB89-136618. National Technical Information Service. Springfield, VA.

U.S. EPA. 1994. A Plain English guide to the EPA Part 503 Biosolids Rule. EPA/832/R-93/003. Washington, D.C.

U.S. EPA. 1995. Part 503 implementation guidance. EPA 833-R-95-001. Washington, D.C.

U.S, EPA. 1999. Biosolids Management Handbook. U.S. EPA Region VIII, Denver, CO.

WEF/ASCE. 1992. WEF Manual of Practice No. 8, Design of Municipal Wastewater Treatment Plants. Pub. WEF (Alexandria, VA) and ASCE (New York, NY).

WEF/U.S. EPA. 1997. Biosolids: A short explanation and discussion. In Biosolids Fact Sheet Project.

WEF/U.S. EPA. 1997. Can Aids be transmitted by biosolids? in WEF/U.S. EPA Biosolids Fact Sheet Project.

Ward, R.L., G.A. McFeters, and J.G. Yeager. 1984. Pathogens in sludge: Occurrence, inactivation, and potential for regrowth. Sandia National Laboratories, Albuquerque, NM. SAND83-0557, TTC-0428, UC-41. U.S. DOE Contract CEAC04-76DP00789.

Weaver, R.W.; J.S. Angle; and P.S. Bottomley 1994. Methods of Soil Analysis. Part 2. Microbiological and Biochemical properties. Madison, WI Soil Science Society of America.

Whittington, W.A., and E. Johnson. 1985. Application of 40 CFR Part 257 regulations to pathogen reduction preceding land application of sewage sludge or septic tank pumpings. Memorandum to EPA Water Division Directors. U.S. EPA Office of Municipal Pollution Control, November 6.

Yanko, W.A. 1987. Occurrence of pathogens in distribution and marketing municipal sludges. Report No.: EPA/600/1 87/014. (NTIS PB88-154273/AS.) Springfield, VA: National Technical Information Service.

Yeager, J.G. and R.L Ward. 1981. Effects of moisture content on long-term survival and regrowth of bacteria in wastewater sludge. Appl. Environ. Microbiol. 41(5):1117-1122.

Appendix A
EPA Regional and State Sludge Coordinators, Map of EPA Regions, and Listing of EPA Pathogen Equivalency Committee Members

Regional Sludge Coordinators

Thelma Murphy
U.S. EPA Region I
JFK Federal Building - CMU
Boston, MA 02203
(617) 918-l 615 (phone)
(617) 918-l 505 (fax)
MURPHY.THELMA@EPAMAIL.EPA.GOV

Alia Roufaeal
U.S. EPA Region II
Div. of Enforcement and Compliance Assist.
290 Broadway - 20th Floor
New York, New York 10007-l 866
(212) 637-3864
(212) 637-3953
ROUFAEAL.ALIA@EPAMAIL.EPA.GOV

Ann Carkhuff
U.S. EPA Region III
Water Protection Div.
841 Chestnut Street
Philadelphia, PA 19107
(215) 566 5735 (phone)
(215) 566 2301 (fax)
CARKHUFF.ANN@EPAMAIL.EPA.GOV

Madolyn Dominy
U.S. EPA Region IV
100 Alabama Street
Atlanta, GA 30303
(404) 562-9305 (phone)
(404) 562-8692 (fax)
MILLER.VINCE@EPAMAIL.EPA.GOV

John Colletti
U.S. EPA Region V (WN-16J)
Water Division
77 West Jackson Blvd.
Chicago, IL 60604-3590
(312) 886-6106 (phone)
(312) 886-7804 (fax)
COLLETTI.JOHN@EPAMAIL.EPA.GOV

Stephanie Kordzi (6WQ-PO)
U.S. EPA Region VI
Water Quality Management Division
1445 Ross Avenue #1200
Dallas, TX 75202-2733
(214) 665-7520 (phone)
(214) 665-2191 (phone)
KORDZI.STEPHANIE@EPA.GOV

John Dunn
U.S. EPA Region VII
Waste Management Division
726 Minnesota Ave.
Kansas City, KS 66101
(913) 551-7594 (phone)
(913) 551-7765 (fax)
DUNN.JOHN@EPAMAIL.EPA.GOV

Bob Brobst
Biosolids Program manager (P2-W-P)
U.S. EPA Region VIII
999 18th Street, Suite 500
Denver, CO 80202-2466
(303) 312-6129 (phone)
(303) 312-7084 (fax)
BROBST.BOB@EPA.GOV

Lauren Fondahl
U.S. EPA Region IX (WTR-7)
Biosolids Coordinator
Office of Clean Water Act Compliance
75 Hawthorne Street
San Francisco, CA 94105-3901
(415) 744-l 909 (phone)
(415) 744-l 235 (fax)
FONDAHL.LAUREN@EPAMAIL.EPA.GOV

Dick Hetherington
U.S. EPA Region X
NPDES Permits Unit (OW-130)
1200 Sixth Avenue
Seattle, WA 98101
(206) 553-l 941 (phone)
(206) 553-l 280 (fax)
HETHERINGTON.DICK@EPAMAIL.EPA.GOV

State Sludge Coordinators

Region I

Connecticut
Bob Norwood/Warren Herzig
CT DEP
Water Compliance Unit
79 Elm Street
Hartford, CT 06106-l 632
(860) 424-3748 (phone)
(860) 424-4067 (fax)
ROBERT.NORWOOD@PO.STATE.CT.US

Maine
David Wright
Maine DEP
Sludge Residuals Unit
State House, Station 17
Augusta, ME 04333
(207) 287-2651 (phone)
(207) 287-7826 (fax)
DAVID.W.WRIGHT@STATE.ME.US

Massachusetts
Larry Polese
MA DEP
50 Route 20
Millbury, MA 01527
(508) 752-8648 (phone)
(508) 755-9253 (fax)
LARRY.POLESE@STATE.MA.US

New Hampshire
Michael Rainey
Sludge & Septage Management
NH DES
6 Hazen Drive
Concord, NH 03301
(603) 271-2818 (phone)
(603) 271-7894 (fax)
M_RAINEY@DES.STATE.NH.US

Rhode island
Warren Towne, P.E.
Supervising Sanitary Engineer
RI DEM, Office of Water Resources
235 Promenade St.
Providence, RI 02908
(401) 222-6820 (phone)
(401) 222-6177 (fax)

Vermont

Cathy Jamieson
VT Dept. of Environmental Conservation
103 S. Main St., Sewing Bldg.
Waterbury, VT 05676
(802) 241-3831 (phone)
(802) 241-2596 (fax)
CATHYJ@DEC.ANR.STATE.VT.US

Region 2

New Jersey
Mary Jo M. Aiello, Chief
Bureau of Pretreatment and Residuals
Watershed Permitting Element, DWQ
NJ DEP
P.O. Box 029
Trenton, NJ 08625-0029
(609) 633-3823 (phone)
(609) 984-7938 (fax)
MAIELLO@DEP.STATE.NJ.US

New York
Sally J. Rowland, Ph.D., PE
NY Dept. of Environmental Conservation
Division of Solid and Hazardous Materials
50 Wolf Road, Room 212
Albany, New York 12233-7253
(518) 457-3966 (phone)
(518) 457-l 283 (fax)
SJROWLAN@GW.DEC.STATE.NY.US

Puerto Rico
Robert Allada
Water Quality Area
Environmental Quality Board
PO Box 11488
Santurce, Puerto Rico 00916
(787) 767-8073 (phone)

Virgin Islands
Leonard G. Reed, Jr.
Environmental Protection Division
Deparmtnet of Planning & Natural Resources
396-l Foster Plaza
St. Thomas, VI 00802
(340) 777-4577 (phone)

Region 3

Delaware
Steve Rohm
DE DNREC
P.O. Box 1401
89 Kings Highway
Dover, DE 19903
(302) 739-5731 (phone)
(302) 739-3491 (fax)
SROHM@DNREC.STATE.DE.US

District of Columbia
Jeruselem Bekele
Water Quality Control Branch
Department of Health
2100 MLK Jr. Avenue SE #203
Washington, D.C. 20020
(202) 645-6617

Maryland

Hussain Alhija, Chief
Design & Certification Division
Maryland Department of the Environment
2500 Broening Highway
Baltimore, MA 21224
(410) 631-3375
(410) 631-3842

Martha Hynson
MD Dept. of the Environment
2500 Broening Hwy
Baltimore, MD 21224
(410) 631-3375 (phone)
(410) 631-3321 (fax)

Pennsylvania
Cuong Vu
Bureau of Water Quality Protection
P.O. Box 8774
RCSUB 11th Floor
Harrisburg, PA 17105-8774
(717) 787 7381 (phone)
(717) 772-5156 (fax)
VU.CUONG@A1.DEP.STATE.PA.US

Virginia
Cal M. (C.M.) Sawyer
VA Dept. of Health
Division of Wastewater Engineering
Box 2448
Richmond, Virginia 23218
(804) 786-l 755 (phone)
(804) 371-2891 (fax)
CSAWYER@VDH.STATE.VA.US

Lily Choi
VA DEQ
P.O. Box 11143
Richmond, VA 23230-l 143
(804) 698-4054 (phone)
(804) 698-4032 (fax)
YCHOI@DEQ.STATE.VA.US

West Vifginia
Clifford Browning
WV DEP
Office of Water Resources
1201 Greenbrier Street
Charleston, WV 25311
(304) 558-4086 (phone)
(304) 558-5903 (fax)

Region 4

Alabama
L. Cliff Evans
Municipal Branch, Water Division
AL Dept. of Environmental Management
P.O. Box 301463
Montgomery, AL 36130-l 463
(334) 271-7816 (phone)
(334) 279-3051 (fax)
LCE@ADEM.STATE.AL.US

Florida
Maurice Barker
Domestic Wastewater, Section MS #3540
Florida Department of Environmental Protection
Twin Towers Office Bldg, 2600 Blair Stone Road
Tallahassee, Florida 32399-2400
(850) 922-4295 (phone)
(850) 921-6385 (fax)
BARKER-M@DEP.STATE.FL.US

Georgia
Sam Shepard/Nancy Prock
Municipal Permitting Program - Environmental Protection Division
GA DNR
4244 International Pkwy, Suite 110
Atlanta, GA 30354
(404) 656-4708 (phone)
(404) 362-2680 (phone)
(404) 362-2691 (fax)
NANCY-PROCK@MAIL.DNR.STATE.GA.US

Kentucky
Mark Crim/Bob Bickner
Solid Waste Branch, Division of Waste Management
KY Department of Natural Resources and Environmental Protection
Frankfort Office Park
14 Reilly Road
Frankfort, KY 40601
(502) 564-6716 (phone)
(502) 564-4049 (fax)
CRIM@NRDEP.NR.STATE.KY.US
BICKNER@NRDEP.NR.STATE.KY.US

Art Curtis
Facilities Construction Branch, Division of Water
Kentucky Department of Natural Resources and Environmental Protection Cabinet
Frankfort Office Park, 14 Reilly Road
Frankfort, KY 40601
(502) 564-4310 (phone)
(502) 564-4245 (fax)
CURTIS@NRDEP.NR.STATE.KY.US

Mississippi
Glenn Odom, P.E.
MS DEQ
Office of Pollution Control
P.O. Box 10385
Jackson, MS 39289-0385
(601) 961-5159 (phone)
(601) 961-5376 (fax)
GLENN-ODEM@DEQ.STATE.MS.US

North Carolina
Dennis Ramsey
Division of Water Quality
NC Department of Environment and Natural Resources
P.O. Box 29535
512 N. Salisbury Street
Raleigh, NC 27626-0535
(919) 733-5083 ext. 528 (phone)
(919) 733-0719 (fax)
DENNIS_RAMSEY@H2O.ENR.STATE.NC.US

Kim H. Colson
Division of Water Quality
NC Department of Environment and Natural Resources
P.O. Box 29535
512 N. Salisbury Street
Raleigh, NC 27626-0535
(919) 733-5083 (phone)
(919) 733-0719 (fax)
KIM_COLSON@H2O.ENR.STATE.NC.US

Kevin H. Barnett
Division of Water Quality
Non-Discharge Compliance/Enforcement Unit
1617 Mail Service Center
Raleigh, NC 27699-l 617
(919) 733-5083 ext. 529 (phone)
KEVIN.BARNETT@NCMAIL.NET

South Carolina
Michael Montebello
Domestic Wastewater Division
SC Dept. of Health & Environment
2600 Bull Street
Columbia, SC 29201
(803) 734-5226 (phone)
(803) 734-5216 (fax)
MONTEBMJ@COLUMB32.DHEC.STATE.SC.US

Tennessee
John McClurkan/Roger Lemaster
Div. of Water Pollution Control
TN DEC
401 Church Street, Sixth Floor Annex
Nashville, TN 37243-l 534
(615) 532-0625 (phone)
(615) 532-0603 (fax)
JMCCLURKAN@MAIL.STATE.TN.US

Region 5

Illinois
S. Alan Keller
IL EPA
DWPC, Permits Section
P.O. Box 19276
1021 N. Grand Avenue, East
Springfield, IL 62794-9276
(217) 782-0610 (phone)
(217) 782-9891 (fax)
EPA1185@EPA.STATE.IL.US

Indiana
Dennis Lasiter, Chief
Land Use Section
IN DEM
P.O. Box 6015
100 N. Senate Avenue
Indianapolis, IN 46206-6015
(317) 232-8732 (phone)
(317) 232-3403 (fax)
DLASITER@DEM.STATE.IN.US

Michigan
Bob Babcock
Chief, Pretreatment and Biosolids Unit
MI DEQ
Surface WQ Div., Permits Section
Knapp's Off ice Center, Second Floor
300 S. Washington Square
P.O. Box 30273
Lansing, MI 48909-7773
(517) 373 8566 (phone)
(517) 373 2040 (fax)
BABCOCKR@STATE.MI.US

Grace Scott
(517) 335-4107 (phone)
SCOTTG@STATE.MI.US

Minnesota
Jorja DuFresne
WQ Div., Point Source Section
MN Pollution Control Agency
520 Lafayette Road
St. Paul, MN 55155-4194
(612) 296-9292 (phone)
(612) 297-8683 (fax)
JORJA.DUFRESNE@PCA.STATE.MN.US

Ohio
Brad Gallant
Division of Surface Water
Ohio EPA
P.O. Box 1049
1800 Watermark Drive
Columbus, OH 43216-0149
(614) 644-2001 (phone)
(614) 644-2329 (fax)
BRAD@GALLANT@EPA.STATE.OHIO.US

Annette De Havilland
Division of Solid & Infectious Waste Mgmt.
Ohio EPA
P.O. Box 1049
Columbus, Ohio 43216-l 049
(614) 644-2621 (phone)
(614) 728-5315 (fax)
ANNETTE.DEHAVILLAND@EPA.STATE.OH.US

Wisconsin
Greg Kester (WT/2)
WI DNR
Bureau of Watershed Mgmt., Point Source Section
101 South Webster Street
Madison, WI 53707
(608) 267 7611 (phone)
(608) 267 7664 (fax)
KESTEG@DNR.STATE.WI.US

Region 6

Arkansas
Keith Brown, P.E.
Manager, State Permits Branch
Water Division
AR Department of Pollution Control and Ecology
P.O. Box 8913
Little Rock, AR 72219
(501) 682-0648 (phone)
(501) 682-0910 (fax)
BROWNK@ADEQ.STATE.AR.US

Jamal Solaimanian, Ph.D
AR Department of Pollution Control and Ecology
P.O. Box 8913
Little Rock, AR 72219-8913
(501) 682-0648 (phone)
(501) 682-0910 (fax)
JAMAL@ADEQ.STATE.AR.US

Louisiana
J. Kilren Vidrine
Water Pollution Control Division
Louisiana Department of Environmental Quality
P.O. Box 82215
Baton Rouge, LA 70884-2215
(504) 765-0534 (phone)
(504) 765-0635 (fax)
KILRENV@DEQ.STATE.LA.US

Hoa Van Nguyen
Solid Waste Division
Louisiana Department of Environmental Quality
P.O. Box 82178
Baton Rouge, LA 70884-2178
(504) 765-0249 (phone)
(504) 765-0299 (fax)
HOAVAN_N@DEQ.STATE.LA.US

Yolunda Righteous
Solid Waste Division
LA DEQ
P.O. Box 82178
Baton Rouge, LA 70884-2178
(504) 765-0249 (phone)
YOLUNDAR@DEQ.STATE.LA.US

New *Mexico*
Jim Davis
NMED Surface Water Qualitv Bureau
NM Environment Department
P.O. Box 26110
Santa Fe, NM 87502

Oklahoma
Danny Hodges
Water Quality Division
OK Dept. of Environmental Quality
1000 NE Tenth Street
Oklahoma City, OK 73117-l 299
(405) 271-5205 (phone)
(405) 271-7339 (fax)
DANNY.HODGES@DEQMAIL.OK.STATE.US

Texas
Paul Curtis
TX Natural Resource Conservation Commission
P.O. Box 13087
Austin, TX 7871 l-3087
(512) 239-4580 (phone)
(512) 239-4750 (fax)
PCURTIS@TNRCD.STATE.TX.

Region 7

Iowa
Billy Chen
IA Dept. of Water, Air &Waste Mgmt.
Henry A. Wallace Building
900 East Grand
Des Moines, IA 50319
(515) 281-4305 (phone)
(515) 281-8895 (fax)

Kansas
Mark Gerard
Kansas Dept. of Health & Environment
Forbes Field Building 283
Topeka, KS 66620-0001
(785) 296-5520 (phone)
(785) 296-5509 (fax)

Missouri
Ken Arnold
MO DNR
P.O. Box 176
205 Jefferson Street
Jefferson City, MO 65102
(573) 751-6825 (phone)
(573) 526-5797 (fax)

Nebraska
Rudy Fiedler
Permits and Compliance
NE DEQ
Suite 400 The Atrium
1200 N. Street, P.O. Box 98922
Lincoln, NE 68509-8922
(402) 471-4239
(402) 471-2909
DEQ118@MAIL.DEQ.STATE.NE.US

Region 8

Colorado
Lori Tucker
Water Quality Control Division
CO Dept. of Public Health & Environment
4300 Cherry Creek Drive South
Denver, CO 80246-l 530
(303) 692-3613 (phone)
(303) 782-0390 (fax)
LORI.TUCKER@STATE.CO.US

Montana
Paul LeVigne
MT Dept of Environmental Quality
Technical & Financial Assistance Bureau
Metcalf Building
Helena, MT 59620
(406) 444-6697 (phone)
(406) 444 6836 (fax)
PLAVIGNE@MT.GOV

North Dakota
Gary Bracht
Environmental Health Section
Division of Water Quality
ND Dept. of Health
1200 Missouri Ave.
P.O. Box 5520
Bismark, ND 58505-5520
(701) 221-5210 (phone)
(701) 328-5200 (fax)
CCMAIL.GBRACHT@RANCH.STATE.ND.US

South Dakota
Eric Meintsma
SD Dept. of Environment and Natural Resources
Joe Foss Building
523 East Capital
Pierre, SD 57501-3181
(605) 773-3351 (phone)
(605) 773 5286 (fax)
ERICM@DENR.STATE.SD.US

Utah
Mark Schmitz
UT DEQ
Division of Water Quality
28814 1460 Street West
Salt Lake City, UT 84114-4870
(801) 538-6097 (phone)
(801) 538-6016 (fax)
MSCHMITZ@DEQ.STATE.UT.US

Wyoming
Larry Robinson
WY DEQ
Herschler Bldg., 4th Floor West
122 W. 25th Street
Cheyenne, WY 82002
1307) 777-7075 (phone)
(307) 777-5973 (fax)
LROBIN@MISSC.STATE.WY.US

Region 9

Arizona
Nicole Heffington
AZ DEQ
3033 N. Central
Phoenix, AZ 85012
(602) 207-4158 (phone)
(602) 207-2383 (fax)
HEFFINGTON.NICOLE@EV.STATE.AZ.US

Jill Galaway
AZ DEQ
3033 N. Central
Phoenix, AZ 85012
(602) 207-4125 (phone)
(602) 207-2383 (fax)
GALAWAY.JILL@EV.STATE.AZ.US

California
Todd Thompson, P.E.
Division of Water Quality
State Water Resources Control Board
PO Box 944213
Sacramento, CA 94244-2130
(916) 657-0577 (phone)
(916) 657-2388 (fax)
THOMT@DWQ.SWRCB.CA.GOV

Michael Wochnick
CA Integrated Waste Mgmt. Board
Remediation, Closure, and Technical Services Branch
8800 Cal Centre Drive
Sacramento, CA 95826
(916) 255-l 302 (phone)
MWOCHNIC@CIWMB.CA.GOV

Bill Orr
CA Integrated Waste Mgmt. Board
8800 Cal Centre Drive
Sacramento, CA 95826
BORR@MRT.CIWMB.CA.GOV

Hawaii
Dennis Tulang/Gayle Takasaki, Engineer
Wastewater Branch
HI Dept. of Health
P.O. Box 3378
Honolulu, HI 96813
(808) 586-4294 (phone)
(808) 586-4370 (fax)
GTAKASAKI@EHA.HEALTH.STATE.HI.US

Nevada
Bill Coughlin
NV DEP
333 West Nye Lane
Carson City, NV 89706-0866
(702) 687-4670 ext. 3153 (phone)
(702) 6875856 (fax)

Region 10

Alaska
Kris McCum by
Solid Waste Program
AK Dept. of Environmental Conservation
610 University Avenue
Fairbanks, AK 99709-3643
(907) 451-2134 (phone)
(907) 451-2187 (fax)
KMCCUMBY@ENVIRCON.STATE.AK.US

Idaho
Rick Huddleston
DEQ Construction & Permits Bureau
ID DHW
1410 North Hilton
Boise, ID 83706-1253
(208) 373-0501 or (208) 373-0502 (phone)
(208) 373-0576 (fax)
RHUDDLES@DEQ..STATE.ID.US

Oregon
Douglas Peters, Biosolids Coordinator
OR DEQ
Water Quality Policy and Program Development Section
811 SW 6th Avenue
Portland, OR 97204
(503) 229-6442 (phone)
(503) 229-5408 (fax)
PETERS.DOUGLAS@DEQ.STATE.OR.US

Washington
Kyle Dorsey
Biosolids Coordinator
Washington State Department of Ecology
PO Box 47600
Olympia, WA 98504-7600
(360) 407-6107 (phone)
(360) 407-7157 or -6102 (fax)
KDOR461@ECY.WA.GOV

USEPA Pathogen Equivalency Committee Membership - 1999

Robert Bastian (4204)
Senior Scientist - Biologist
USEPA-OWM
401 M Street, SW
Washington, DC 20460
202-260-7378

Robert B. Brobst, PE
Environmental Engineer
Biosolids Program Manager
USEPA-Region 8 (P2-W-P)
999 18th Street, Suite 500
Denver, CO 80202-2466
303-312-6129

Dr. John Cicmanec (MS-G75)
Veterinarian
USEPA-NRMRL-TTSD
26 W Martin Luther King Drive
Cincinati, OH 45268
513-569-7481

Dr. G. Shay Fout (MS-320)
Senior Research Virologist
USEPA-NERL
26 W Martin Luther King Drive
Cincinati, OH 45268
513-569-7387

Dr. Hugh McKinnon (MS-235)
Medical Officer / Doctor
Associate Laboratory Director for Health
USEPA-NRMRL
26 W Martin Luther King Drive
Cincinati, OH 45268
513-569-7689

Mark Meckes (MS-489)
Research Microbiologist
USEPA-NRMRL-WS&WRD
26 W Martin Luther King Drive
Cincinati, OH 45268
513-569-7348

Dr. Frank W. Schaefer, III
Senior Research Parasitologist
USEPA-NERL (MS-320)
26 W Martin Luther King Drive
Cincinati, OH 45268
513-569-7222

Dr. Stephen A. Schaub (4304)
Virologist
USEPA-OST-HECD-HRAB
401 M Street, SW
Washington, DC 20460
202-260-7591

Dr. Jim Smith (MS-G77)
Senior Environmental Engineer & PEC Chair
USEPA-NRMRL-TTSD (CERI)
26 W Martin Luther King Drive
Cincinnati, OH 45268
513-569-7355

Appendix B
Subpart D of the Part 503 Regulation

[Code of Federal Regulations]
[Title 40, Volume 21, Parts 425 to 699]
[Revised as of July 1, 1998]
From the U.S. Government Printing Office via GPO Access
[CITE: 40CFR503.30]

TITLE 40—PROTECTION OF ENVIRONMENT

CHAPTER I-ENVIRONMENTAL PROTECTION AGENCY (Continued)

PART 503—STANDARDS FOR THE USE OR DISPOSAL OF SEWAGE SLUDGE-Table of Contents

Subpart D-Pathogens and Vector Attraction Reduction

Sec. 503.30 Scope.

(a) This subpart contains the requirements for a sewage sludge to be classified either Class A or Class B with respect to pathogens.

(b) This subpart contains the site restrictions for land on which a Class B sewage sludge is applied.

(c) This subpart contains the pathogen requirements for domestic septage applied to agricultural land, forest, or a reclamation site.

(d) This subpart contains alternative vector attraction reduction requirements for sewage sludge that is applied to the land or placed on a surface disposal site.

Sec. 503.31 Special definitions.

(a) Aerobic digestion is the biochemical decomposition of organic matter in sewage sludge into carbon dioxide and water by microorganisms in the presence of air.

(b) Anaerobic digestion is the biochemical decomposition of organic matter in sewage sludge into methane gas and carbon dioxide by microorganisms in the absence of air.

(c) Density of microorganisms is the number of microorganisms per unit mass of total solids (dry weight) in the sewage sludge.

(d) Land with a high potential for public exposure is land that the public uses frequently. This includes, but is not limited to, a public contact site and a reclamation site located in a populated area (e.g, a construction site located in a city).

(e) Land with a low potential for public exposure is land that the public uses infrequently. This includes, but is not limited to, agricultural land, forest, and a reclamation site located in an unpopulated area (e.g., a strip mine located in a rural area).

(f) Pathogenic organisms are disease-causing organisms. These include, but are not limited to, certain bacteria, protozoa, viruses, and viable helminth ova.

(g) pH means the logarithm of the reciprocal of the hydrogen ion concentration.

(h) Specific oxygen uptake rate (SOUR) is the mass of oxygen consumed per unit time per unit mass of total solids (dry weight basis) in the sewage sludge.

(i) Total solids are the materials in sewage sludge that remain as residue when the sewage sludge is dried at 103 to 105 degrees Celsius.

(j) Unstabilized solids are organic materials in sewage sludge that have not been treated in either an aerobic or anaerobic treatment process.

(k) Vector attraction is the characteristic of sewage sludge that attracts rodents, flies, mosquitos, or other organisms capable of transporting infectious agents.

(l) Volatile solids is the amount of the total solids in sewage sludge lost when the sewage sludge is combusted at 550 degrees Celsius in the presence of excess air.

Sec. 503.32 Pathogens.

(a) Sewage sludge-Class A. (1) The requirement in Sec. 503.32(a)(2) and the requirements in either Sec. 503.32(a)(3), (a)(4), (a)(5), (a)(6), (a)(7), or (a)(8) shall be met for a sewage sludge to be classified Class A with respect to pathogens.

(2) The Class A pathogen requirements in Sec. 503.32 (a)(3) through (a)(8) shall be met either prior to meeting or at the same time the vector attraction reduction requirements in Sec. 503.33, except the vector attraction reduction requirements in Sec. 503.33 (b)(6) through (b)(8), are met.

(3) Class A-Alternative 1. (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10 (b), (c), (e), or (f).

(ii) The temperature of the sewage sludge that is used or disposed shall be maintained at a specific value for a period of time.

(A) When the percent solids of the sewage sludge is seven percent or higher, the temperature of the sewage sludge shall be 50 degrees Celsius or higher; the time period shall be 20 minutes or longer; and the temperature and time period shall be determined using equation (2), except when small particles of sewage sludge are heated by either warmed gases or an immiscible liquid.

$$D = \frac{131,700,000}{10^{0.1400t}} \qquad \text{Eq. (2)}$$

Where,
D=time in days.
t=temperature in degrees Celsius.

(B) When the percent solids of the sewage sludge is seven percent or higher and small particles of sewage sludge are heated by either warmed gases or an immiscible liquid, the temperature of the sewage sludge shall be 50 degrees Celsius or higher; the time period shall be 15 seconds or longer; and the temperature and time period shall be determined using equation (2).

(C) When the percent solids of the sewage sludge is less than seven percent and the time period is at least 15 seconds, but less than 30 minutes, the temperature and time period shall be determined using equation (2).

(D) When the percent solids of the sewage sludge is less than seven percent; the temperature of the sewage sludge is 50 degrees Celsius or higher; and the time period is 30 minutes or longer, the temperature and time period shall be determined using equation (3).

$$D = \frac{50,070,000}{10^{0.1400t}} \qquad \text{Eq. (3)}$$

Where,
D=time in days.
t=temperature in degrees Celsius.

(4) Class A-Alternative 2. (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10 (b), (c), (e), or (f).

(ii)(A) The pH of the sewage sludge that is used or disposed shall be raised to above 12 and shall remain above 12 for 72 hours.

(B) The temperature of the sewage sludge shall be above 52 degrees Celsius for 12 hours or longer during the period that the pH of the sewage sludge is above 12.

(C)At the end of the 72 hour period during which the pH of the sewage sludge is above 12, the sewage sludge shall be air dried to achieve a percent solids in the sewage sludge greater than 50 percent.

(5) Class A-Alternative 3. (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella sp. bacteria in sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10 (b), (c), (e), or (f).

(ii)(A) The sewage sludge shall be analyzed prior to pathogen treatment to determine whether the sewage sludge contains enteric viruses.

(B) When the density of enteric viruses in the sewage sludge prior to pathogen treatment is less than one Plaque-forming Unit per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to enteric viruses until the next monitoring episode for the sewage sludge.

(C) When the density of enteric viruses in the sewage sludge prior to pathogen treatment is equal to or greater

than one Plaque-forming Unit per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to enteric viruses when the density of enteric viruses in the sewage sludge after pathogen treatment is less than one Plaque-forming Unit per four grams of total solids (dry weight basis) and when the values or ranges of values for the operating parameters for the pathogen treatment process that produces the sewage sludge that meets the enteric virus density requirement are documented.

(D) After the enteric virus reduction in paragraph (a)(5)(ii)(C) of this section is demonstrated for the pathogen treatment process, the sewage sludge continues to be Class A with respect to enteric viruses when the values for the pathogen treatment process operating parameters are consistent with the values or ranges of values documented in paragraph (a)(5)(ii)(C) of this section.

(iii)(A) The sewage sludge shall be analyzed prior to pathogen treatment to determine whether the sewage sludge contains viable helminth ova.

(B) When the density of viable helminth ova in the sewage sludge prior to pathogen treatment is less than one per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to viable helminth ova until the next monitoring episode for the sewage sludge.

(C) When the density of viable helminth ova in the sewage sludge prior to pathogen treatment is equal to or greater than one per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to viable helminth ova when the density of viable helminth ova in the sewage sludge after pathogen treatment is less than one per four grams of total solids (dry weight basis) and when the values or ranges of values for the operating parameters for the pathogen treatment process that produces the sewage sludge that meets the viable helminth ova density requirement are documented.

(D) After the viable helminth ova reduction in paragraph (a)(5)(iii)(C) of this section is demonstrated for the pathogen treatment process, the sewage sludge continues to be Class A with respect to viable helminth ova when the values for the pathogen treatment process operating parameters are consistent with the values or ranges of values documented in paragraph (a)(5)(iii)(C) of this section.

(6) Class A-Alternative 4. (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge isprepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10 (b), (c), (e), or (f).

(ii) The density of enteric viruses in the sewage sludge shall be less than one Plaque-forming Unit per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10 (b), (c), (e), or (f), unless otherwise specified by the permitting authority.

(iii) The density of viable helminth ova in the sewage sludge shall be less than one per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10 (b), (c), (e), or (f), unless otherwise specified by the permitting authority.

(7) Class A-Alternative 5. (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella, sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or given away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10(b), (c), (e), or (f).

(ii) Sewage sludge that is used or disposed shall be treated in one of the Processes to Further Reduce Pathogens described in appendix B of this part.

(8) Class A-Alternative 6. (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of Salmonella, sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or given away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in Sec. 503.10(b), (c), (e), or (f).

(ii) Sewage sludge that is used or disposed shall be treated in a process that is equivalent to a Process to Further Reduce Pathogens, as determined by the permitting authority.

(b) Sewage sludge-Class B. (l)(i) The requirements in either Sec. 503.32(b)(2), (b)(3), or (b)(4) shall be met for a sewage sludge to be classified Class B with respect to pathogens.

(ii) The site restrictions in Sec. 503.32(b)(5) shall be met when sewage sludge that meets the Class B pathogen requirements in Sec. 503.32(b)(2), (b)(3), or (b)(4) is applied to the land.

(2) Class B-Alternative 1. (i) Seven samples of the sewage sludge shall be collected at the time the sewage sludge is used or disposed.

(ii) The geometric mean of the density of fecal coliform in the samples collected in paragraph (b)(2)(i) of this section shall be less than either 2,000,000 Most Probable Number per gram of total solids (dry weight basis) or 2,000,000 Colony Forming Units per gram of total solids (dry weight basis).

(3) Class B-Alternative 2. Sewage sludge that is used or disposed shall be treated in one of the Processes to Significantly Reduce Pathogens described in appendix B of this part.

(4) Class B-Alternative 3. Sewage sludge that is used or disposed shall be treated in a process that is equivalent to a Process to Significantly Reduce Pathogens, as determined by the permitting authority.

(5) Site restrictions. (i) Food crops with harvested parts that touch the sewage sludge/soil mixture and are totally above the land surface shall not be harvested for 14 months after application of sewage sludge.

(ii) Food crops with harvested parts below the surface of the land shall not be harvested for 20 months after application of sewage sludge when the sewage sludge remains on the land surface for four months or longer prior to incorporation into the soil.

(iii) Food crops with harvested parts below the surface of the land shall not be harvested for 38 months after application of sewage sludge when the sewage sludge remains on the land surface for less than four months prior to incorporation into the soil.

(iv) Food crops, feed crops, and fiber crops shall not be harvested for 30 days after application of sewage sludge.

(v) Animals shall not be allowed to graze on the land for 30 days after application of sewage sludge.

(vi) Turf grown on land where sewage sludge is applied shall not be harvested for one year after application of the sewage sludge when the harvested turf is placed on either land with a high potential for public exposure or a lawn, unless otherwise specified by the permitting authority.

(vii) Public access to land with a high potential for public exposure shall be restricted for one year after application of sewage sludge.

(viii) Public access to land with a low potential for public exposure shall be restricted for 30 days after application of sewage sludge.

(c) Domestic septage. (1) The site restrictions in Sec. 503.32(b)(5) shall be met when domestic septage is applied to agricultural land, forest, or a reclamation site; or (2) The pH of domestic septage applied to agricultural land, forest, or a reclamation site shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for 30 minutes and the site restrictions in Sec. 503.32 (b)(5)(i) through (b)(5)(iv) shall be met.

Sec. 503.33 Vector attraction reduction.

(a)(l) One of the vector attraction reduction requirements in Sec. 503.33 (b)(l) through (b)(lO) shall be met when bulk sewage sludge is applied to agricultural land, forest, a public contact site, or a reclamation site.

(2) One of the vector attraction reduction requirements in Sec. 503.33 (b)(l) through (b)(8) shall be met when bulk sewage sludge is applied to a lawn or a home garden.

(3) One of the vector attraction reduction requirements in Sec. 503.33 (b)(l) through (b)(8) shall be met when sewage sludge is sold or given away in a bag or other container for application to the land.

(4) One of the vector attraction reduction requirements in Sec. 503.33 (b)(l) through (b)(ll) shall be met when sewage sludge (other than domestic septage) is placed on an active sewage sludge unit.

(5) One of the vector attraction reduction requirements in Sec. 503.33 (b)(9), (b)(lO), or (b)(12) shall be met when domestic septage is applied to agricultural land, forest, or a reclamation site and one of the vector attraction reduction requirements in Sec. 503.33 (b)(9) through (b)(12) shall be met when domestic septage is placed on an active sewage sludge unit.

(b)(l) The mass of volatile solids in the sewage sludge shall be reduced by a minimum of 38 percent (see calculation procedures in "Environmental Regulations and Technology-Control of Pathogens and Vector Attraction in Sewage Sludge", EPA-625/R-92/01 3, 1992, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268).

(2) When the 38 percent volatile solids reduction requirement in Sec. 503.33(b)(l) cannot be met for an anaerobically digested sewage sludge, vector attraction reduction can be demonstrated by digesting a portion of the previously digested sewage sludge anaerobically in the laboratory in a bench-scale unit for 40 additional days at a temperature between 30 and 37 degrees Celsius. When at the end of the 40 days, the volatile solids in the sewage sludge at the beginning of that period is reduced by less than 17 percent, vector attraction reduction is achieved.

(3) When the 38 percent volatile solids reduction requirement in Sec. 503.33(b)(l) cannot be met for an aerobically digested sewage sludge, vector attraction reduction can be demonstrated by digesting a portion of the previously digested sewage sludge that has a percent solids of two percent or less aerobically in the laboratory in a bench-scale unit for 30 additional days at 20 degrees Celsius. When at the end of the 30 days, the volatile solids in the sewage sludge at the beginning of that period is reduced

by less than 15 percent, vector attraction reduction is achieved.

(4) The specific oxygen uptake rate (SOUR) for sewage sludge treated in an aerobic process shall be equal to or less than 1.5 milligrams of oxygen per hour per gram of total solids (dry weight basis) at a temperature of 20 degrees Celsius.

(5) Sewage sludge shall be treated in an aerobic process for 14 days or longer. During that time, the temperature of the sewage sludge shall be higher than 40 degrees Celsius and the average temperature of the sewage sludge shall be higher than 45 degrees Celsius.

(6) The pH of sewage sludge shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for two hours and then at 11.5 or higher for an additional 22 hours.

(7) The percent solids of sewage sludge that does not contain unstabilized solids generated in a primary wastewater treatment process shall be equal to or greater than 75 percent based on the moisture content and total solids prior to mixing with other materials.

(8) The percent solids of sewage sludge that contains unstabilized solids generated in a primary wastewater treatment process shall be equal to or greater than 90 percent based on the moisture content and total solids prior to mixing with other materials.

(9)(i) Sewage sludge shall be injected below the surface of the land.

(ii) No significant amount of the sewage sludge shall be present on the land surface within one hour after the sewage sludge is injected.

(iii) When the sewage sludge that is injected below the surface of the land is Class A with respect to pathogens, the sewage sludge shall be injected below the land surface within eight hours after being discharged from the pathogen treatment process.

(10)(i) Sewage sludge applied to the land surface or placed on a surface disposal site shall be incorporated into the soil within six hours after application to or placement on the land.

(ii) When sewage sludge that is incorporated into the soil is Class A with respect to pathogens, the sewage sludge shall be applied to or placed on the land within eight hours after being discharged from the pathogen treatment process.

(11) Sewage sludge placed on an active sewage sludge unit shall be covered with soil or other material at the end of each operating day.

(12) The pH of domestic septage shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for 30 minutes.

Appendix C
Determination of Volatile Solids Reduction by Digestion

Introduction

Under 40 CFR Part 503, the ability of sewage sludge to attract vectors must be reduced when sewage sludge is applied to the land or placed on a surface disposal site. One way to reduce vector attraction is to reduce the volatile solids in the sewage sludge by 38% or more (see Section 8.2 of this document). Typically, volatile solids reduction is accomplished by anaerobic or aerobic digestion. Volatile solids reduction also occurs under other circumstances, such as when sewage sludge is stored in an anaerobic lagoon or is dried on sand beds. To give credit for this extra loss in volatile solids, the regulation allows the untreated sewage sludge to be compared with the treated sewage sludge that leaves the treatment works, which should account for all of the volatile solids reduction that could possibly occur. For most processing sequences, the processing steps downstream from the digester, such as short-term storage or dewatering, have no influence on volatile solids content. Consequently, the appropriate comparison is between the sewage sludge entering the digester and the sewage sludge leaving the digester. The remainder of the discussion is limited to this circumstance, except for the final section of this appendix, which compares incoming sewage sludge with the sewage sludge leaving the treatment works.

The Part 503 regulation does not specify a method for calculating volatile solids reduction. Fischer (1984) observed that the United Kingdom has a similar requirement for volatile solids reduction for digestion (40%), but also failed to prescribe a method for calculating volatile solids reduction. Fischer has provided a comprehensive discussion of the ways that volatile solids reduction may be calculated and their limitations. He presents the following equations for determining volatile solids reduction:

- Full mass balance equation
- Approximate mass balance equation
- "Constant ash" equation
- Van Kleeck equation

The full mass balance equation is the least restricted approach but requires more information than is currently collected at a wastewater treatment plant. The approximate mass balance equation assumes steady state conditions. The "constant ash" equation requires the assumption of steady state conditions as well as the assumption that the ash input rate equals the ash output rate. The Van Kleeck equation, which is the equation generally suggested in publications originating in the United States (WPCF, 1968), is equivalent to the constant ash equation. Fischer calculates volatile solids reduction using a number of examples of considerable complexity and illustrates that different methods frequently yield different results.

Fischer's paper is extremely thorough and is highly recommended for someone trying to develop a deep understanding of potential complexities in calculating volatile solids reduction. However, it was not written as a guidance document for field staff faced with the need to calculate volatile solids reduction. The nomenclature is precise but so detailed that it makes comprehension difficult. In addition, two important troublesome situations that complicate the calculation of volatile solids reduction - grit deposition in digesters and decantate removal - are not explicitly discussed. Consequently, this presentation has been prepared to present guidance that describes the major pitfalls likely to be encountered in calculating percent volatile solids reduction.

It is important to note that the calculation of volatile solids reduction is only as accurate as the measurement of volatile solids content in the sewage sludge. The principal cause of error is poor sampling. Samples should be representative, covering the entire charging and withdrawal periods. Averages should cover extended periods of time during which changes in process conditions are minimal. For some treatment, it is expected that periodic checks of volatile solids reduction will produce results so erratic that no confidence can be placed in them. In this case, adequacy of stabilization can be verified by the method described under Options 2 and 3 in Chapter 8 -periodically batch digest anaerobically digested sewage sludge for 40 additional days at 30EC (86EF) to 37EC (99EF), or aerobically digested sewage sludge for 30 additional days at 20EC (68EF). If the additional VS reduction is less than 17% for the anaerobically digested sewage sludge or less than 15% for the aerobically digested sewage sludge, the sewage sludge is sufficiently stable (see Sections 8.3 and 8.4).

Equations for FVSR

The equations for fractional volatile solids reduction (FVSR) that will be discussed below are the same as those developed by Fischer (1984), except for omission of his constant ash equation. This equation gives identical results to the Van Kleeck equation so it is not shown. Fischer's nomenclature has been avoided or replaced with simpler terms. The material balance approaches are called methods rather than equations. The material balances are drawn to fit the circumstances. There is no need to formalize the method with a rigid set of equations.

In the derivations and calculations that follow, both VS (total volatile solids content of the sewage sludge or decantate on a dry solids basis) and FVSR are expressed throughout as fractions to avoid the frequent confusion that occurs when these terms are expressed as percentages. "Decantate" is used in place of the more commonly used "supernatant" to avoid the use of "s" in subscripts. Similarly, "bottoms" is used in place of "sludge" to avoid use of "s" in subscripts.

Method Full Mass Balance

The full mass balance method must be used when steady conditions do not prevail over the time period chosen for the calculation. The chosen time period must be substantial, at least twice the nominal residence time in the digester (nominal residence time equals average volume of sludge in the digester divided by the average volumetric flow rate. Note: when there is decantate withdrawal, volume of sewage sludge withdrawn should be used to calculate the average volumetric flow rate). The reason for the long time period is to reduce the influence of short-term fluctuations in sewage sludge flow rates or compositions. If input compositions have been relatively constant for a long period of time, then the time period can be shortened.

An example where the full mass balance method would be needed is where an aerobic digester is operated as follows:

- Started with the digester 1/4 full (time zero)
- Raw sewage sludge is fed to the digester daily until the digester is full
- Supernatant is periodically decanted and raw sewage sludge is charged into the digester until settling will not occur to accommodate daily feeding (hopefully after enough days have passed for adequate digestion)
- Draw down the digester to about 1/4 full (final time), discharging the sewage sludge to sand beds

The full mass balance is written as follows:

Sum of total volatile solids inputs in feed streams during the entire digestion period = sum of volatile solids outputs in withdrawals of decantate and bottoms + loss of volatile solids + accumulation of volatile solids in the digester. (1)

Loss of volatile solids is calculated from Equation 1. FVSR is calculated by Equation 2:

$$\text{FVSR} = \frac{\text{loss in volatile solids}}{\text{sum of volatile solids inputs}} \qquad (2)$$

The accumulation of volatile solids in the digester is the final volume in the digester after the drawdown times final volatile solids concentration less the initial volume at time zero times the initial volatile solids concentration.

To properly determine FVSR by the full mass balance method requires determination of all feed and withdrawal volumes, initial and final volumes in the digester, and volatile solids concentrations in all streams. In some cases, which will be presented later, simplifications are possible.

Approximate Mass Balance Method

If volumetric inputs and outputs are relatively constant on a daily basis, and there is no substantial accumulation of volatile solids in the digester over the time period of the test, an approximate mass balance (AMB) may be used. The basic relationship is stated simply:

volatile solids input rate = volatile solids output rate + rate of loss of volatile solids. (3)

The FVSR is given by Equation 2.

No Decantate, No Grit Accumulation (Problem 1)

Calculation of FVSR is illustrated for Problem 1 in Table C-l, which represents a simple situation with no decantate removal and no grit accumulation. An approximate mass balance is applied to the digester operated under constant flow conditions. Because no decantate is removed, the volumetric flow rate of sewage sludge leaving the digester equals the flow rate of sewage sludge entering the digester.

Applying Equations 3 and 2,

$$FY, = BY, + \text{loss} \qquad (4)$$

$$\text{Loss} = 1\ OO(5030) = 2000 \qquad (5)$$

$$\text{FVSR} = \frac{\text{Loss}}{Fy_f} \qquad (6)$$

$$\text{FVSR} = \frac{2000}{(100)\,(50)} = 0.40 \qquad (7)$$

Nomenclature is given in Table C-l. Note that the calculation did not require use of the fixed solids concentrations.

The calculation is so simple that one wonders why it is so seldom used. One possible reason is that the input and output volatile solids concentrations (Y, and Y_p) typically will show greater coefficients of variation (standard deviation divided by arithmetic average) than the fractional volatile solids (VS is the fraction of the sewage sludge solids

Table Cl. Quantitative Information for Example Problems[1,2,3]

Parameter	Symbol	Units	Problem Statement Number 1	2	3	4
Nominal Residence Time	θ	d	20	20	20	20
Time period for averages	—	d	60	60	60	60
Feed Sludge						
Volumetric flow rate	F	m3/d	100	100	100	100
Volatile solids concentration	Y_f	kg/m3	50	50	50	50
Fixed solids concentration	X_f	kg/m3	17	17	17	17
Fractional volatile solids	VS_f	kg/kg	0.746	0.746	0.746	0.746
Mass flow rate of solids	M_f	kg/d	6700	6700	6700	6700
Digested Sludge (Bottoms)						
Volumetric flow rate	B	m3/d	100	100		49.57
Volatile solids concentration	Y_b	kg/m3	30	41.42	41.42	41.42
Fixed solids concentration	X_b	kg/m3	17	15	23.50	23.50
Fractional volatile solids	VS_b	kg/kg	0.638	0.667	0.638	0.638
Mass flow rate of solids	M_b	kg/d	4700	4500		
Decantate						
Volumetric flow rate	D	m3/d	0	0		50.43
Volatile solids concentration	Y_d	kg/m3	—	—	12.76	12.76
Fixed solids concentration	X_d	kg/m3	—	—	7.24	7.24
Fractional volatile solids	VS_d	kg/kg	—	—	0.638	0.638
Mass flow rate of solids	M_d	kg/d	—	—		

Conditions are steady state; all daily flows are constant. Volatile solids are not accumulating in the digester, although grit may be settling out in the digester.

*Numerical values are given at 3 or 4 significant figures. This is unrealistic considering the expected accuracy in measuring solids concentrations nd sludge volumes. The purpose of extra significant figures is to allow more understandable comparisons to be made of the different calculation methods.

[3]All volatile solids concentrations are based on total solids, not merely on suspended solids.

that is volatile-note the difference between VS and Y). If this is the case, the volatile solids reduction calculated by the approximate mass balance method from several sets of Y_f-Y_b data will show larger deviations than if it were calculated by the Van Kleeck equation using VS_f-VS_b data.

Grit deposition can be a serious problem in both aerobic and anaerobic digestion. The biological processes that occur in digestion dissolve or destroy the substances suspending the grit, and it tends to settle. If agitation is inadequate to keep the grit particles in suspension, they will accumulate in the digester. The approximate mass balance can be used to estimate accumulation of fixed solids.

For Problem 1, the balance yields the following:

$$Fx, = BX, + \text{fixed solids loss} \quad (8)$$

$$(100)(17) = (100)(17) + \text{Fixed Solids Loss} \quad (9)$$

$$\text{Fixed Solids Loss} = 0 \quad (10)$$

The material balance compares fixed solids in output with input. If some fixed solids are missing, this loss term will be a positive number. Because digestion does not consume fixed solids, it is assumed that the fixed solids are accumulating in the digester. As Equation 10 shows, the fixed solids loss equals zero. Note that for this case, where input and output sewage sludge flow rates are equal, the fixed solids concentrations are equal when there is no grit accumulation.

Grit Deposition (Problem 2)

The calculation of fixed solids is repeated for Problem 2. Conditions in Problem 2 have been selected to show grit accumulation. Parameters are the same as in Problem 1 except for the fixed solids concentration (X,) and parameters related to it. Fixed solids concentration in the sewage sludge is lower than in Problem 1. Consequently, VS is higher and the mass flow rate of solids leaving is lower than in Problem 1. A mass balance on fixed solids (input rate = output rate + rate of loss of fixed solids) is presented in Equations 11-13.

$$FX, = BX_b + \text{Fixed Solids Loss} \quad (11)$$

$$\text{Fixed Solids Loss} = FX, - BX, \quad (12)$$

$$\text{Fixed Solids Loss} = (100)(7) - (100)(15) = 200 \text{ kg/d} \quad (13)$$

The material balance, which only looks at inputs and outputs, informs us that 200 kg/d of fixed solids have not appeared in the outputs as expected. Because fixed solids are not destroyed, it can be concluded that they are accumulating in the bottom of the digester. The calculation of FVSR for Problem 2 is exactly the same as for Problem 1 (see Equations 4 through 7) and yields the same result. The approximate mass balance method gives the

correct answer for the FVSR despite the accumulation of solids in the digester. As will be seen later, this is not the case when the Van Kleeck equation is used.

Decantate Withdrawal, No Grit Accumulation (Problem 3)

In Problem 3, decantate is withdrawn daily. Volatile and fixed solids concentrations are known for all streams but the volumetric flow rates are not known for decantate and bottoms. It is impossible to calculate FVSR without knowing the relative volumes of these streams. However, they are determined easily by taking a total volume balance and a fixed solids balance, provided it can be assumed that loss of fixed solids (i.e., accumulation in the digester) is zero.

Selecting a basis for F of 100 m^3/d,

Volume balance: $100 = B + D$ (14)

Fixed solids balance: $100 X, + BX, + DX,$ (15)

Because the three Xs are known, B and D can be found. Substituting 100-D for B and the values for the Xs from Problem 3 and solving for D and B,

$$(100)(17) = (100 - D)(23.50) + (D)(7.24) \quad (16)$$

$$D = 40.0 m^3/d, B = 60.0 m^3/d \quad (17)$$

The FVSR can now be calculated by drawing a volatile solids balance:

$$FY, + BY, + DY, + loss \quad (18)$$

$$FVSR = \frac{loss}{FY,} = \frac{FY_f - BY_b - Dy_d}{FY_f} \quad (19)$$

$$FVSR = \frac{(1001(50) - (60)(41.42) - (40)(12.76)}{(100)(50)} = 0\ 40 \quad (20)$$

Unless information is available on actual volumes of decantate and sewage sludge (bottoms), it is not possible to deterrnine whether grit is accumulating in the digester. If it is accumulating, the calculated FVSR will be in error.

When the calculations shown in Equations 18 through 20 are made, it is assumed that the volatile solids that are missing from the output streams are consumed by biological reactions that convert them to carbon dioxide and methane. Accumulation is assumed to be negligible. Volatile solids are less likely to accumulate than fixed solids, but it can happen. In poorly mixed digesters, the scum layer that collects at the surface is an accumulation of volatile solids. FVSR calculated by Equations 18 through 20 will be overestimated if the volatile solids accumulation rate is substantial.

Decantate Withdrawal and Grit Accumulation (Problem 4)

In Problem 4, there is suspected grit accumulation. The quantity of B and D can no longer be calculated by Equations 14 and 15 because Equation 15 is no longer correct. The values of B and D must be measured. All parameters in Problem 4 are the same as in Problem 3 except that measured values for B and D are introduced into Problem 4. Values of B and D calculated assuming no grit accumulation (Problem 3-see previous discussion), and measured quantities are compared below:

	Calculated	Measured
B	60	49.57
D	40	50.43

The differences in the values of B and D are not large but they make a substantial change in the numerical value of FVSR. The FVSR for Problem 4 is calculated below:

$$FVSR = \frac{(100)(50) - (49.57)(41.42) - (50.43)(12.76)}{(100)(50)} = 0.461 \quad (21)$$

If it had been assumed that there was no grit accumulation, FVSR would equal 0.40 (see Problem 3). It is possible to determine the amount of grit accumulation that has caused this change. A material balance on fixed solids is drawn:

$$FX, = BX_b + DX, + \text{Fixed Solids Loss} \quad (22)$$

The fractional fixed solids loss due to grit accumulation is found by rearranging this equation:

$$\frac{\text{Fixed Solids Loss}}{FX_f} = \frac{FX, - BX, - Dx,}{FX_f} \quad (23)$$

Substituting in the parameter values for Problem 4,

$$\frac{\text{Fixed Solids Loss}}{FX_f} = \frac{(100)(17) - (49.57)(23.50) - (50.43)(7.24)}{(100)} = 0.100 \quad (24)$$

If this fixed solids loss of 10 percent had not been accounted for, the calculated FVSR would have been 13% lower than the correct value of 0.461. Note that if grit accumulation occurs and it is ignored, calculated FVSR will be lower than the actual value.

The Van Kleeck Equation

Van Kleeck first presented his equation without derivation in a footnote for a review paper on sewage sludge treatment processing in 1945 (Van Kleeck, 1945). The equation is easily derived from total solids and volatiie solids mass balances around the digestion system. Consider a digester operated under steady state conditions with decantate and bottom sewage sludge removal. A total solids mass balance and a volatile solids mass balance are:

$$M, = M_b + M_d + (\text{loss of total solids}) \quad (25)$$

$$M_f \cdot VS_f = M_b \cdot VS_b + M_d \cdot VS_d + \text{(loss of volatile solids)} \quad (26)$$

where

M_f, M_b, and M_d are the mass of solids in the feed, bottoms, and decantate streams.

The masses must be mass of solids rather than total mass of liquid and solid because VS is an unusual type of concentration unit-it is "mass of volatile solids per unit mass of total solids."

It is now assumed that fixed solids are not destroyed and there is no grit deposition in the digester. The losses in Equations 25 and 26 then comprise only volatile solids so the losses are equal. It is also assumed that the VS of the decantate and of the bottoms are the same. This means that the bottoms may have a much higher solids content than the decantate but the proportion of volatile solids to fixed solids is the same for both streams. Assuming then that VS_b equals VS_d, and making this substitution in the defining equation for FVSR (Equation 2),

$$FVSR = \frac{\text{Loss of vol. solids}}{M_f \times VS_f} = 1 - \frac{(M_b + M_d)VS_b}{M_f \times VS_f} \quad (27)$$

From Equation 25, recalling that we have assumed that loss of total solids equais loss of volatile solids,

$$M_b + M_d + M_f - \text{loss of vol. solids} \quad (28)$$

Substituting for $M_b + M_d$ into Equation 27,

$$FVSR = 1 - \frac{(M_f - \text{loss of vol. solids}) \cdot VS_b}{M_f \cdot VS_f} \quad (29)$$

Simplifying further,

$$1 - (1/VS_f - FVSR) \cdot VS_f \quad (30)$$

Solving for FVSR,

$$FVSR = \frac{VS_f - VS_b}{1 - (VS_f \cdot VS_b)} \quad (31)$$

This is the form of the Van Kleek equation found in WPCF Manual of Practice No. 16 (WPCF, 1968). Van Kleeck (1945) presented the equation in the following equivalent form:

$$FVSR = 1 - \frac{VS_f \times (1 - VS_f)}{VS_f \times (1 - VS_b)} \quad (32)$$

The Van Kleeck equation is applied below to Problems I through 4 in Table C- 1 and compared to the approximate mass balance equation results:

	1	2	3	4
Approximate Mass Balance (AMB)	0.40	0.40	0.40	0.461
Van Kleeck (VK)	0.40	0.318	0.40	0.40

Problem *1:* No decantate and no grit accumulation. Both methods give correct answer.

Problem *2:* No decantate but grit accumulation. VK is invalid and incorrect.

Problem 3: Decantate but no grit accumulation. AMB method is valid. VK method is valid only if VS_f equals VS_b.

Problem *4:* Decantate and grit accumulation. AMB method valid only if B and D are measured. VK method is invalid.

The Van Kleeck equation is seen to have serious shortcomings when applied to certain practical problems. The AMB method can be completely reliable, whereas the Van Kleeck method is useless under some circumstances.

Average Values

The concentrations and VS values used in the equations will all be averages. For the material balance methods, the averages should be weighted averages according to the mass of solids in the stream in question. The example below shows how to average the volatile solids concentration for four consecutive sewage sludge additions.

Addition	Volume	Total Solids Concentration	VS
2	12 m³	72 kg/m³	0.75
3	13 m³	50 60 kg/m³	0.80 0.82
4	10 m³	55 kg/m³	0.77

(33)

Weigh ted by Mass

$$VS\ av = \frac{12 \times 72 \times 0.75 + 8 \times 50 \times 0.82 + 13 \times 60 \times 0.80 + 10 \times 55 \times 0.77}{12 \times 72 + 8 \times 50 + 13 \times 60 + 10 \times 55} = 0.795 \quad (34)$$

Weigh ted by Volume

$$VS\ av = \frac{12 \times 0.75 + 8 \times 0.82 + 13 \times 0.80 + 10 \times 0.77}{12 + 8 + 13 + 10} = 0.783 \quad (35)$$

Arithmetic A verage

$$VS\ av = \frac{0.75 + 0.82 + 0.80 + 0.77}{4} = 0.785 \quad (36)$$

In this example the arithmetic average was nearly as close as the volume-weighted average to the mass-weighted average, which is the correct value.

Which Equation to Use?

Full Mass Balance Method

The full mass balance method allows calculation of volatile solids reduction for all approaches to digestion, even

processes in which the final volume in the digester does not equal the initial volume and where daily flows are not steady. A serious drawback to this method is the need for volatile solids concentration and the volumes of all streams added to or withdrawn from the digester, as well as initial and final volumes and concentrations in the digester. This can be a daunting task, particularly for the small treatment works that is most likely to run digesters in other than steady flow modes. For treatment works of this kind, an "equivalent" method that shows that the sewage sludge has undergone the proper volatile solids reduction is likely to be a better approach than trying to demonstrate 38% volatile solids reduction. An aerobic sewage sludge has received treatment equivalent to a 38% volatile solids reduction if the specific oxygen uptake rate is below a specified maximum. Anaerobically digested sewage sludge has received treatment equivalent to a 38% volatile solids reduction if volatile solids reduction after batch digestion of the sewage sludge for 40 days is less than a specified maximum (EPA, 1992).

Approximate Mass Balance Method

The approximate mass balance method assumes that daily flows are steady and reasonably uniform in composition, and that digester volume and composition do not vary substantially from day to day. Results of calculations and an appreciation of underlying assumptions show that the method is accurate for all cases, including withdrawal of decantate and deposition of grit, provided that in addition to composition of all streams the quantities of decantate and bottoms (the digested sewage sludge) are known. If the quantities of decantate and bottoms are not known, the accumulation of grit cannot be determined. If accumulation of grit is substantial and FVSR is calculated assuming it to be negligible, FVSR will be lower than the true value. The result is conservative and could be used to show that minimum volatile solids reductions are being achieved.

Van Kleeck Method

The Van Kleeck equation has underlying assumptions that should be made clear wherever the equation is presented. The equation is never valid when there is grit accumulation because it assumes the fixed solids input equals fixed solids output. Fortunately, it produces a conservative result in this case. Unlike the AMB method it does not provide a convenient way to check for accumulation of grit. It can be used when decantate is withdrawn, provided VS, equals VS,. Just how significant the difference between these VS values can be before an appreciable error in FVSR occurs is unknown, although it could be determined by making up a series of problems with increasing differences between the VS values, calculating FVSR using the AMB method and a Van Kleeck equation, and comparing the results.

The shortcomings of the Van Kleeck equation are substantial, but the equation has one strong point: The VS of the various sewage sludge and decantate streams are likely to show much lower coefficients of variation (standard deviation divided by arithmetic average) than volatile solids and fixed solids concentration. Reviews of data are needed to determine how seriously the variation in concentrations affect the confidence interval of FVSR calculated by both methods. A hybrid approach may turn out to be advantageous. The AMB method could be used first to determine if grit accumulation is occurring. If grit is not accumulating, the Van Kleeck equation could be used. If decantate is withdrawn, the Van Kleeck equation is appropriate, particularly if the decantate is low in total solids. If not, and if VS, differs substantially from VS,, it could yield an incorrect answer.

Volatile Solids Loss Across All Sewage Sludge Treatment Processes

For cases when appreciable volatile solids reduction can occur downstream from the digester (for example, as would occur in air drying or lagoon storage), it is appropriate to calculate the volatile solids loss from the point at which the sewage sludge enters the digester to the point at which the sewage sludge leaves the treatment works. Under these circumstances, it is virtually never possible to use the approximate mass balance approach, because flow rates are not uniform. The full mass balance could be used in principle, but practical difficulties such as measuring the mass of the output sewage sludge (total mass, not just mass of solids) that relates to a given mass of entering sewage sludge make this also a practical impossibility. Generally then, the only option is to use the Van Kleeck equation, because only the percent volatile solids content of the entering and exiting sewage sludge is needed to make this calculation. As noted earlier, this equation will be inappropriate if there has been a selective loss of high volatility solids (e.g., bacteria) or low volatility solids (e.g., grit) in any of the sludge processing steps.

To make a good comparison, there should be good correspondence between the incoming sewage sludge and the treated sewage sludge to which it is being compared (see Section 10.4). For example, when sewage sludge is digested for 20 days, then dried on a sand bed for 3 months, and then removed, the treated sludge should be compared with the sludge fed to the digester in the preceding 3 or 4 months. If no selective loss of volatile or nonvolatile solids has occurred, the Van Kleeck equation (see Equation 31) can be used to calculate volatile solids reduction.

References

EPA. 1992. Technical Support Document for Part 503 Pathogen and Vector Attraction Reduction Requirements in Sewage Sludge. Office of Water, U.S. EPA, Washington, DC. NTIS No PB93-11069. Natl. Technical Information Service, Springfield, VA.

Fischer, W.J. 1984. Calculation of volatile solids during sludge digestion. In: Bruce, A., ed. Sewage Sludge Stabilization and Disinfection, pp. 499-529. Water Research Centre, E. Horwood Ltd., Chichester, England.

Van Kleeck, L.W. 1945. Sewage Works J., Operation of Sludge Drying and Gas Utilization Units. 17(6): 1240-1255.

Water Pollution Control Federation. 1968. Manual of Practice No. 16, Anaerobic Sludge Digestion. Washington, DC.

Appendix D
Guidance on Three Vector Attraction Reduction Tests

This appendix provides guidance for the vector attraction reduction Options 2, 3, and 4 to demonstrate reduced vector attraction (see Chapter 8 for a description of these requirements).

1. Additional Digestion Test for Anaerobically Digested Sewage Sludge

Background

The additional digestion test for anaerobically digested sewage sludge is based on research by Jeris et al. (1985). Farrell and Bhide (1993) explain in more detail the origin of the time and volatile solids reduction requirements of the test.

Jeris et al. (1985) measured changes in many parameters including volatile solids content while carrying out additional digestion of anaerobically digested sludge from several treatment works for long periods. Samples were removed from the digesters weekly for analysis. Because substantial amount of sample was needed for all of these tests, they used continuously mixed digesters of 18 liters capacity. The equipment and procedures of Jeris et al., although not complex, appear to be more elaborate than needed for a control test. EPA staff (Farrell and Bhide, 1993) have experimented with simplified tests and the procedure recommended is based on their work.

Recommended Procedure

The essentials of the test are as follows:

- Remove, from the plant-scale digester, a representative sample of the sewage sludge to be evaluated to determine additional volatile solids destruction. Keep the sample protected from oxygen and maintain it at the temperature of the digester. Commence the test within 6 hours after taking the sample.

- Flush fifteen 100-mL volumetric flasks with nitrogen, and add approximately 50 mL of the sludge to be tested into each flask. Frequently mix the test sludge during this operation to assure that its composition remains uniform. Select five flasks at random, and determine total solids content and volatile solids content, using the entire 50 mL for the determination. Seal each of the remaining flasks with a stopper with a single glass tube through it to allow generated gases to escape.

- Connect the glass tubing from each flask through a flexible connection to a manifold. To allow generated gases to escape and prevent entry of air, connect the manifold to a watersealed bubbler by means of a vertical glass tube. The tube should be at least 30-cm long with enough water in the bubbler so that an increase in atmospheric pressure will not cause backflow of air or water into the manifold. Maintain the flasks containing the sludge at constant temperature either by inserting them in a water bath (the sludge level in the flasks must be below the water level in the bath) or by placing the entire apparatus in a constant temperature room or box. The temperature of the additional digestion test should be the average temperature of the plant digester, which should be in the range of 30°C to 40°C (86°F to 104°F). Temperature should be controlled within + 0.15°C (0.27°F).

- Each flask should be swirled every day to assure adequate mixing, using care not to displace sludge up into the neck of the flask. Observe the water seal for the first few days of operation. There should be evidence that gas is being produced and passing through the bubbler.

- After 20 days, withdraw five flasks at random. Determine total and volatile solids content using the entire sample for the determination. Swirl the flask vigorously before pouring out its contents to minimize the hold up of thickened sludge on the walls and to assure that any material left adhering to the flask walls will have the same average composition as the material withdrawn. Use a consistent procedure. If holdup on walls appears excessive, a minimal amount of distilled water may be used to wash solids off the walls. Total removal is not necessary, but any solids left on the walls should be approximately of the same composition as the material removed.

- After 40 days, remove the remaining five flasks. Determine total and volatile solids content using the entire sample from each flask for the determination. Use the same precautions as in the preceding step to remove virtually all of the sludge, leaving only material with the same approximate composition as the material removed.

Total and volatile solids content are determined using the procedures of Method 2540 G of Standard Methods (APHA, 1992).

Mean values and standard deviations of the total solids content, the volatile solids content, and the percent volatile solids are calculated. Volatile solids reductions that result from the additional digestion periods of 20 and 40 days are calculated from the mean values by the Van Kleeck equation and by a material balance (refer to Appendix C for a general description of these calculations). The results obtained at 20 days give an early indication that the test is proceeding satisfactorily and will help substantiate the 40-day result.

Alternative approaches are possible. The treatment works may already have versatile bench-scale digesters available. This equipment could be used for the test, provided accuracy and reproducibility can be demonstrated. The approach described above was developed because Farrell and Bhide (1993) in their preliminary work experienced much difficulty in withdrawing representative samples from large digesters even when care was taken to stir the digesters thoroughly before sampling. If an alternative experimental setup is used, it is still advisable to carry out multiple tests for the volatile solids content in order to reduce the standard error of this measurement, because error in the volatile solids content measurement is inflated by the nature of the equation used to calculate the volatile solids reduction.

Variability in flow rates and nature of the sludge will result in variability in performance of the plant-scale digesters. It is advisable to run the additional digestion test routinely so that sufficient data are available to indicate average performance. The arithmetic mean of successive tests (a minimum of three is suggested) should show an additional volatile solids reduction of ≤ 17%.

Calculation Details

Appendix C, Determination of Volatile Solids Reduction by Digestion, describes calculation methods to use for digesters that are continuously fed or are fed at least once a day. Although the additional anaerobic digestion test is a batch digestion, the material balance calculations approach is the same. Masses of starting streams (input streams) are set equal to masses of ending streams (output streams).

The test requires that the fixed volatile solids reduction (FVSR) be calculated both by the Van Kleeck equation and the material balance method. The Van Kleeck equation calculations can be made in the manner described in Appendix C.

The calculation of the volatile solids reduction (and the fixed fractional solids reduction [FFSR]) by the mass balance method shown below has been refined by subtracting out the mass of gas lost from the mass of sludge at the end of the digestion step. For continuous digestion, this loss of mass usually is ignored, because the amount is small in relation to the total digesting mass, and mass before and after digestion are assumed to be the same. Considering the inherent difficulty in matching mass and composition entering to mass and composition leaving for a continuous process, this is a reasonable procedure. For batch digestion, the excellent correspondence between starting material and final digested sludge provides much greater accuracy in the mass balance calculation, so inclusion of this lost mass is worthwhile.

In the equations presented below, concentrations of fixed and volatile solids are mass fractions-mass of solids per unit mass of sludge (mass of sludge includes both the solids and the water in the sludge)- and are indicated by, the symbols lowercase y and x. This is different from the usage in Appendix C where concentrations are given in mass per unit volume, and are indicated by the symbols uppercase y and x. This change has been made because masses can be determined more accurately than volumes in small-scale tests.

In the material balance calculation, it is assumed that as the sludge digests, volatile solids and fixed solids are converted to gases that escape or to volatile compounds that distill off when the sludge is dried. Any production or consumption of water by the biochemical reactions in digestion is assumed to be negligible. The data collected (volatile solids and fixed solids concentrations of feed and digested sludge) allow mass balances to be drawn on volatile solids, fixed solids, and water. As noted, it is assumed that there is no change in water mass- all water in the feed is present in the digested sludge. Fractional reductions in volatile solids and fixed solids can be calculated from these mass balances for the period of digestion. Details of the calculation of these relationships are given by Farrell and Bhide (1993). The final form of the equations for fractional volatile solids reduction (mass balance [m b.] method) and fractional fixed solids reduction (m.b. method) are given below:

$$\text{FVSR(m.b.)} = \frac{Y_f (1 - X_b) - Y_b (1-X_f)}{Y_f (1 - x_b - Y_b)} \quad (1a)$$

$$\text{FFSR(m.b.)} = \frac{x(1-y_b) - x_b (1-Y_f)}{x_f (1-x_b-y_b)} \quad (1b)$$

where:

y = mass fraction of volatile solids in the liquid sludge
x = mass fraction of fixed solids in the liquid sludge
f = indicates feed sludge at start of the test
b = indicates “bottoms” sludge at end of the test

If the fixed solids loss is zero, these two equations are reduced to Equation 2 below:

$$\text{FVSR(m.b.)} = (y, - Y,) / Y, (1-Y,) \quad (2)$$

If the fixed solids loss is not zero but is substantially smaller than the volatile solids reduction, Equation 2 gives surprisingly accurate results. For five sludges batch-digested by Farrell and Bhide (1993), the fixed solids reduc-

tions were about one-third of the volatile solids reductions. When the FVSR(m.b.) calculated by Equation 1 a averaged 15%, the FVSR(m b.) calculated by Equation 2 averaged 14.93%, which is a trivial difference.

The disappearance of fixed solids unfortunately has a relatively large effect on the calculation of FVSR by the Van Kleeck equation. The result is lower than it should be. For five sludges that were batch-digested by Farrell and Bhide (1993), the FVSR calculated by the Van Kleeck method averaged 15%, whereas the FVSR (m.b.) calculated by Equation 1 a or 2 averaged about 20%. When the desired endpoint is an FVSR below 17%, this is a substantial discrepancy.

The additional digestion test was developed for use with the Van Kleeck equation, and the 17% requirement is based on results calculated with this equation. In the future, use of the more accurate mass balance equation may be required, with the requirement adjusted upward by an appropriate amount. This cannot be done until more data with different sludge become available.

2. Specific Oxygen Uptake Rate

Background

The specific oxygen uptake rate of a sewage sludge is an accepted method for indicating the biological activity of an activated sewage sludge mixed liquor or an aerobically digesting sludge. The procedure required by the Part 503 regulation for this test is presented in Standard Methods (APHA, 1992) as Method 2710 B, Oxygen-Consumption Rate.

The use of the specific oxygen uptake rate (SOUR) has been recommend by Eikum and Paulsrud (1977) as a reliable method for indicating sludge stability provided temperature effects are taken into consideration. For primary sewage sludges aerobically digested at 18°C (64°F), sludge was adequately stabilized (i.e., it did not putrefy and cause offensive odors) when the SOUR was less than 1.2 mg O_2/hr/g VSS (volatile suspended solids). The authors investigated several alternative methods for indicating stability of aerobically digested sludges and recommended the SOUR test as the one with the most advantages and the least disadvantages.

Ahlberg and Boyko (1972) also recommend the SOUR as an index of stability. They found that, for aerobic digesters operated at temperatures above 10°C (50°F), SOUR fell to about 2.0 mg O_2/hr/g VSS after a total sludge age of 60 days and to 1 .O mg O_2/hr/g VSS after about 120 days sludge age. These authors state that a SOUR of less than 1.0 mg O_2/hr/g VSS at temperatures above 10°C (50°F) indicates a stable sludge.

The results obtained by these authors indicate that long digestion times-more than double the residence time for most aerobic digesters in use today-are needed to eliminate odor generation from aerobically digested sludges. Since the industry is not being deluged with complaints about odor from aerobic digesters, it appears that a higher SOUR standard can be chosen than they suggest without causing problems from odor (and vector attraction).

The results of long-term batch aerobic digestion tests by Jeris et al. (1985) provide information that is helpful in setting a SOUR requirement that is reasonably attainable and still protective. Farrell and Bhide (1993) reviewed the data these authors obtained with four sewage sludges from aerobic treatment processes and concluded that a standard of 1.5 mg O_2/hr/g TS at 20°C (68°F) would discriminate between adequately stabilized and poorly stabilized sludges. The "adequately digested" sludges were not totally trouble-free, i.e., it was possible under adverse conditions to develop odorous conditions. In all cases where the sludge was deemed to be adequate, minor adjustment in plant operating conditions created an acceptable sludge.

The SOUR requirement is based on total solids rather than volatile suspended solids. This usage is preferred for consistency with the rest of the Part 503 regulation where all loadings are expressed on a total solids basis. The use of total solids concentration in the SOUR calculation is rational since the entire sludge solids and not just the volatile solids degrade and may exert some oxygen demand. Making an adjustment for the difference caused by basing the requirement on TS instead of VSS, the standard is about 1.8 times higher than Eikum and Paulsrud's recommended value and 2.1 times higher than Ahlberg and Boykos' recommendation.

Unlike anaerobic digestion, which is typically conducted at 35°C (95°F), aerobic digestion is carried out without any deliberate temperature control. The temperature of the digesting sludge will be close to ambient temperature, which can range from 5°C to 30°C (41°F to 86°F). In this temperature range, SOUR increases with increasing temperature. Consequently, if a requirement for SOUR is selected, there must be some way to convert SOUR test results to a standard temperature. Conceivably, the problem could be avoided if the sludge were simply heated or cooled to the standard temperature before running the SOUR test. Unfortunately, this is not possible, because temperature changes in digested sludge cause short-term instabilities in oxygen uptake rate (Benedict and Carlson [1973], Farrell and Bhide [1993]).

Eikum and Paulsrud (1977) recommend that the following equation be used to adjust the SOUR determined at one temperature to the SOUR for another temperature:

$$(SOUR)_{T1}/(SOUR)_{T2} = \theta^{(T1-T2)} \quad (3)$$

where:

(SOUR),, = specific oxygen uptake rate at T,

(SOUR),, = specific oxygen uptake rate at T_2

θ = the Streeter-Phelps temperature sensitivity coeffficient

These authors calculated the temperature sensitivity coefficient using their data on the effect of temperature on the rate of reduction in volatile suspended solids with time during aerobic digestion. This is an approximate approach, because there is no certainty that there is a one-to-one relationship between oxygen uptake rate and rate of volatile solids disappearance. Another problem is that the coeffficient depends on the makeup of each individual sludge. For example, Koers and Mavinic (1977) found the value of θ to be less than 1.072 at temperatures above 15°C (59°F) for aerobic digestion of waste activated sludges, whereas Eikum and Paulsrud (1977) determined θ to equal 1.112 for primary sludges. Grady and Lim (1980) reviewed the data of several investigators and recommended that θ = 1.05 be used for digestion of waste-activated sludges when more specific information is not available. Based on a review of the available information and their own work, Farrell and Bhide(1993) recommend that Eikum and Paulsruds' temperature correction procedure be utilized, using a temperature sensitivity coefficient in the range of 1.05 to 1.07.

Recommended Procedure for Temperature Correction

A SOUR of 1.5 mg O_2/hr/g total solids at 20°C (68°F) was selected to indicate that an aerobically digested sludge has been adequately reduced in vector attraction.

The SOUR of the sludge is to be measured at the temperature at which the aerobic digestion is occurring in the treatment works and corrected to 20°C (68°F) by the following equation:

$$SOUR_{,,} = SOUR_{,} \times \theta^{(20-T)} \quad (4)$$

where
θ = 1.05 above 20°C (68°F)
1.07 below 20°C (68°F)

This correction may be applied only if the temperature of the sludge is between 10°C and 30°C (50°F and 86°F). The restriction to the indicated temperature range is required to limit the possible error in the SOUR caused by selecting an improper temperature coefficient.. Farrell and Bhide's (1993) results indicate that the suggested values for θ will give a conservative value for SOUR when translated from the actual temperature to 20°C (68°F).

The experimental equipment and procedures for the SOUR test are those described in Part 2710 B, Oxygen Consumption Rate, of Standard Methods (APHA, 1992). The method allows the use of a probe with an oxygen-sensitive electrode or a respirometer. The method advises that manufacturer's directions be followed if a respirometer is used. No further reference to respirometric methods will be made here. A timing device is needed as well as a 300-mL biological oxygen demand (BOD) bottle. A magnetic mixer with stirring bar is also required.

The procedure of Standard Method 2710 B should be followed with one exception. The total solids concentration instead of the volatile suspended solids concentration is used in the calculation of the SOUR. Total solids concentration is determined by Standard Method 2540 G. Method 2710 B cautions that if the suspended solids content of the sludge is greater than 0.5%, additional stirring besides that provided by the stirring bar be considered. Experiments by Farrell and Bhide (1993) were carried out with sludges up to 2% in solids content without difficulty if the SOUR was lower than about 3.0 mg O_2/g/h. It is possible to verify that rnixing is adequate by running repeat measurements at several stirrer bar speeds. If stirring is adequate, oxygen uptake will be independent of stirrer speed.

The inert mineral solids in the wastewater in which the sludge particles are suspended do not exert an oxygen demand and properly should not be part of the total solids in the SOUR determination. Ordinarily, they are such a small part of the total solids that they can be ignored. If the ratio of inert dissolved mineral solids in the treated wastewater to the total solids in the sludge being tested is greater than 0.15, a correction should be made to the total solids concentration. Inert dissolved mineral solids in the treated wastewater effluent is determined by the method of Part 2540 B of Standard Methods (APHA, 1992). This quantity is subtracted from the total solids of the sludge to determine the total solids to be used in the SOUR calculation.

The collection of the sample and the time between sample collection and measurement of the SOUR are important. The sample should be a composite of grab samples taken within a period of a few minutes duration. The sample should be transported to the laboratory expeditiously and kept under aeration if the SOUR test cannot be run immediately. The sludge should be kept at the temperature of the digester from which it was drawn and aerated thoroughly before it is poured into the BOD bottle for the test. If the temperature differs from 20°C (68°F) by more than ±10°C (±18°F), the temperature correction may be inappropriate and the result should not be used to prove that the sewage sludge meets the SOUR requirement.

Variability in flow rates and nature of the sludge will result in variability in performance of the plant-scale digesters. It is advisable to run the SOUR test routinely so that sufficient data are available to indicate average performance. The arithmetic mean of successive tests-a minimum of seven over 2 or 3 weeks is suggested-should give a SOUR of ≤ 1.5 mg O_2/hr/g total solids.

3. Additional Digestion Test for Aerobically Digested Se wage Sludge

Background

Part 503 lists several options that can be used to demonstrate reduction of vector attraction in sewage sludge. These options include reduction of volatile solids by 38% and demonstration of the SOUR value discussed above (see also Chapter 8). These options are feasible for many, but not all, digested sludges. For example, sludges from extended aeration treatment works that are aerobically di-

gested usually cannot meet this requirement because they already are partially reduced in volatile solids content by their exposure to long aeration times in the wastewater treatment process.

The specific oxygen uptake test can be utilized to evaluate aerobic sludges that do not meet the 38% volatile solids reduction requirement. Unfortunately, this test has a number of limitations. It cannot be applied if the sludges have been digested at temperatures lower than 10°C (50°F) or higher than 30°C (86°F). It has not been evaluated under all possible conditions of use, such as for sludges of more than 2% solids.

A straightforward approach for aerobically treated sludges that cannot meet either of the above criteria is to determine to what extent they can be digested further. If they show very little capacity for further digestion, they will have a low potential for additional biodegradation and odor generation that attracts vectors.Such a test necessarily takes many days to complete, because time must be provided to get measurable biodegradation. Under most circumstances, this is not a serious drawback. If a digester must be evaluated every 4 months to see if the sewage sludge meets vector attraction reduction requirements, it will be necessary to start a regular assessment program. A record can be produced showing compliance. The sludge currently being produced cannot be evaluated quickly but it will be possible to show compliance over a period of time.

The additional digestion test for aerobically digested sludges in Part 503 is based on research by Jeris et al. (1985), and has been discussed by Farrell et al. (EPA, 1992). Farrell and Bhide (1993) explain in more detail the origin of the time and volatile solids reduction requirements of the test.

Jeris et al. (1985) demonstrated that several parameters-volatile solids reduction, COD, BOD, and SOUR-declined smoothly and approached asymptotic values with time as sludge was aerobically digested. Any one of these parameters potentially could be used as an index of vector attraction reduction for aerobic sludges. SOUR has been adopted (see above) for this purpose. Farrell and Bhide (1993) have shown that the additional volatile solids reduction that occurs when sludge is batch digested aerobically for 30 days correlates equally as well as SOUR with the degree of vector attraction reduction of the sludge. They recommend that a sewage sludge be accepted as suitably reduced in vector attraction when it shows less than 15% additional volatile solids reduction after 30 days additional batch digestion at 20°C (68°F). For three out of four sludges investigated by Jeris et al. (1985), the relationship between SOUR and additional volatile solids reduction showed that the SOUR was approximately equal to 1.5 mg O_2/hr/g (the Part 503 requirement for SOUR) when additional volatile solids reduction was 15%. The two requirements thus agree well with one another.

Recommended Procedure

There is considerable flexibility in selecting the size of the digesters used for the additional aerobic digestion test. Farrell and Bhide (1993) used a 20-liter fish tank. A tank of rectangular cross-section is suggested because sidewalls are easily accessible and are easily scraped clean of adhering solids. The tank should have a loose-fitting cover that allows air to escape. It is preferable to vent exhaust gas to a hood to avoid exposure to aerosols. Oil and particle-free air is supplied to the bottom of the digester through porous stones at a rate sufficient to thoroughly mix the sewage sludge. This will supply adequate oxygen to the sludge, but the oxygen level in the digesting sludge should be checked with a dissolved oxygen meter to be sure that the supply of oxygen is adequate. Oxygen level should be at least 2 mg/L. Mechanical mixers also were used to keep down foam and improve mixing.

If the total solids content of the sewage sludge is greater than 2%, the sludge must be diluted to 2% solids with secondary effluent at the start of the test. The requirement stems from the results of Reynolds (1973) and Malina (1966) which demonstrate that rate of volatile solids reduction decreases as the feed solitis concentration increases. Thus, for example, a sludge with a 2% solids content that showed more than 15% volatile solids reduction when digested for 30 days might show a lower volatile solids reduction and would pass the test if it were at 4%. This dilution may cause a temporary change in rate of volatile solids reduction. However, the long duration of the test should provide adequate time for recovery and demonstration of the appropriate reduction in volatile solids content.

When sampling the sludge, care should be taken to keep the sludge aerobic and avoid unnecessary temperature shocks. The sludge is digested at 20°C (68°F) even if the digester was at some other temperature. It is expected that the bacterial population will suffer a temporary shock if there is a substantial temperature change, but the test is of sufficient duration to overcome this effect and show a normal volatile solids reduction. Even if the bacteria are shocked and do not recover completely, the test simulates what would happen to the sludge in the environment. If it passes the test, it is highly unlikely that the sludge will attract vectors when used or disposed to the environment. For example, if a sludge digested at 35°C (95°F) has not been adequately reduced in volatile solids and is shocked into biological inactivity for 30 days when its temperature is lowered to 20°C (68°F), it will be shocked in the same way if it is applied to the soil at ambient temperature. Consequently, it is unlikely to attract vectors.

The digester is charged with about 12 liters of the sewage sludge to be additionally digested, and aeration is commenced. The constant flow of air to the aerobic digestion test unit will cause a

substantial loss of water from the digester. Water loss should be made up every day with distilled water. Solids that adhere to the walls above and below the water line should be scraped off and dispersed back into the sludge daily. The temperature of the digesting sludge should be approximately 20°C (68°F). If the temperature of the labora-

tory is maintained at about 22°C (72°F), evaporation of water from the digester will cool the sludge to about 20°C (68°F).

Sewage sludge is sampled every week for five successive weeks. Before sampling, makeup water is added (this will generally require that air is temporarily shut off to allow the water level to be established), and sludge is scraped off the walls and redistributed into the digester. The sludge in the digester is thoroughly mixed with a paddle before sampling, making sure to mix the bottom sludge with the top. The sample is comprised of several grab samples collected with a ladle while the digester is being mixed. The entire sampling procedure is duplicated to collect a second sample.

Total and volatile solids content of both samples are determined preferably by Standard Method 2540 G (APHA, 1992). Percent volatile solids is calculated from total and volatile solids content. Standard Methods (APHA, 1992) states that duplicates should agree within 5% of their average. If agreement is substantially poorer than this, the sampling and analysis should be repeated.

Calculation Details

Fraction volatile solids reduction is calculated by the Van Kleeck formula (see Appendix C) and by a mass balance method. The mass balance (m.b.) equations become very simple, because final mass of sludge is made very nearly equal to initial mass of sludge by adjusting the volume by adding water. These equations for fractional volatile solids reduction (FVSR) and fractional fixed solids reduction (FFSR) are:

$$FVSR(m.b.) = (y_f - y_b) / y_f \quad (5a)$$

$$FFSR(m.b.) = (x_f - x_b) / x_f \quad (5b)$$

where:

y and x = mass fraction of volatile and fixed solids, respectively (see previous section on"Calculation details" for explanation of "mass fraction")

f and b = subscripts indicating initial and final sludges

This calculation assumes that initial and final sludge densities are the same. Very little error is introduced by this assumption.

The calculation of the fractional fixed solids reduction is not a requirement of the test, but it will provide useful information.

The test was developed from information based on the reduction in volatile solids content calculated by the Van Kleeck equation. As noted in the section on the additional anaerobic digestion test, for batch processes the material balance procedure for calculating volatile solids reduction is superior to the Van Kleeck approach. It is expected that the volatile solids reduction by the mass balance method will show a higher volatile solids reduction than the calculation made by using the Van Kleeck equation.

4. References

Ahlberg, N.R. and B.I. Boyko. 1972. Evaluation and design of aerobic digesters. Jour. WPCF 44(4):634-643.

Benedict, A.H., and D.A. Carlson. 1973. Temperature acclimation in aerobic big-oxidation systems. Jour. WPCF 45(1): 10-24.

Eikum, A., and B. Paulsrud. 1977. Methods for measuring the degree of stability of aerobically stabilized sludges. Wat. Res. 11: 763-770

EPA. 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No.: PB93-11069. Springfield, VA: National Technical Information Service.

Farrell, J.B. and V. Bhide. 1993. Development of methods for quantifying vector attraction reduction. (Manuscript in preparation.)

APHA (American Public Health Association). 1992. Standard methods for the examination of water and wastewater. Greenberg, A.E., L.S. Clesceri, and A.D. Eaton (eds.). APHA, AWWA, and WEF, Washington, DC.

Grady, C.P.L., Jr., and H.C. Lim. 1980. Biological wastewater treatment: theory and applications. Marcel Dekker, New York.

Jeris, J.S., D. Ciarcia, E. Chen, and M. Mena. 1985. Determining the stability of treated municipal sludge. EPA Rept. No. 600/2-85-001 (NTIS No. PB 851-l 471891 AS). U.S. Environmental Protection Agency, Cincinnati, Ohio.

Koers, D.A., and D.V. Mavinic. 1977. Aerobic digestion of waste-activated sludge at low temperatures. Jour. WPCF 49(3): 460-468.

Malina, Jr., J.F. 1966. Discussion, pp. 157-l 60, in paper by D. Kehr, "Aerobic sludge stabilization in sewage treatment plants." Advances in Water Pollution Research, Vol. 2, pp 143-l 63. Water Pollution Control Federation, Washington, DC.

Reynolds, T.D. 1973. Aerobic digestion of thickened waste- Proc. 28th Industr. Waste Conf., Purdue University.

Appendix E
Determination of Residence Time for Anaerobic and Aerobic Digestion

Introduction

The PSRP and PFRP specifications in 40 CFR 257 for anaerobic and aerobic digestion not only specify temperatures but also require minimum mean cell residence times of the sludge in the digesters. The mean cell residence time is the time that the sludge particles are retained in the digestion vessel under the conditions of the digestion. The calculation of residence time is ordinarily simple but it can become complicated under certain circumstances. This appendix describes how to make this calculation for most of the commonly encountered modes for operating digesters.

Approach

The discussion has to be divided into two parts: residence time for batch operation and for plug flow, and residence time for fully mixed digesters. For batch operation, residence time is obvious-it is the duration of the reaction. For plug flow, the liquid-solid mixture that is sludge passes through the reactor with no backward or forward mixing. The time it takes the sludge to pass through the reactor is the residence time. It is normally calculated by the following equation:

$$\theta = V/q \qquad (1)$$

where
- θ = plug flow solids residence time
- V = volume of the liquid in the reactor
- q = volume of the liquid leaving the reactor

Normally the volume of liquid leaving the reactor will equal the volume entering. Conceivably, volume leaving could be smaller (e.g., because of evaporation losses) and residence time would be longer than expected if 1 were based on inlet flow. Ordinarily, either inlet or outlet flow rate can be used.

For a fully mixed reactor, the individual particles of the sludge are retained for different time periods-some particles escape very soon after entry whereas others circulate in the reactor for long periods before escaping. The average time in the reactor is given by the relationship:

$$\theta_n = \frac{\Sigma(\delta s \times \theta)}{\Sigma(\delta s)} \qquad (2)$$

where
- δs = an increment of sludge solids that leaves the reactor
- θ = time period this increment has been in the reactor
- θ_n = nominal average solids residence time

When the flow rates of sludge into and out of the completely mixed vessel are constant, it can be demonstrated that this equation reduces to:

$$\theta_n = \frac{VC_v}{qC_q} \qquad (3)$$

where
- V = reactor volume
- q = flow rate leaving
- C_v = concentration of solids in the reactor
- C_q = concentration of solids in exiting sewage sludge

It is important to appreciate that q is the flow rate leaving the reactor. Some operators periodically shut down reactor agitation, allow a supernatant layer to form, decant the supernatant, and resume operation. Under these conditions, the flow rate entering the reactor is higher than the flow rate of sludge leaving.

Note that in Equation 3, VC_v is the mass of solids in the system and qC_q is the mass of solids leaving. Ordinarily C_v equals C_q and these terms could be canceled. They are left in the equation because they show the essential form of the residence time equation:

$$\theta_n = \frac{\text{mass of solids in the diaester}}{\text{mass flow rate of solids leaving}} \qquad (4)$$

Using this form, residence time for the important operating mode in which sludge leaving the digester is thickened and returned to the digester can be calculated.

In many aerobic digestion installations, digested sludge is thickened with part of the total volume returned to increase residence time and part removed as product. The calculation follows Equation 4 and is identical with the SRT (solids retention time) calculation used in activated sludge process calculations. The focus here is on the solids in the digester and the solids that ultimately leave the system. Applying Equation 4 fcr residence time then leads to Equation 5:

$$\theta n = \frac{VC_v}{pC_p} \quad (5)$$

where

p = flow rate of processed sludge leaving the system

C_p = solids concentration in the processed sludge

The subscript p indicates the final product leaving the system, not the underflow from the thickener. This approach ignores any additional residence time in the thickener since this time is relatively short and not at proper digestion conditions.

Sample Calculations

In the following paragraphs, the equations and principles presented above are used to demonstrate the calculation of residence time for several commonly used digester operating modes:

Case 1

- Complete-mix reactor
- Constant feed and withdrawal at least once a day
- No substantial increase or decrease in volume in the reactor (V)
- One or more feed streams and a single product stream (q)

The residence time desired is the nominal residence time. Use Equation 3 as shown below:

$$\theta_n = \frac{VC_v}{qCq} = \frac{V}{q}$$

The concentration terms in Equation 4 cancel out because C_v equals C_q.

Case 2a

- Complete-mix reactor
- Sludge is introduced in daily batches of volume (Vi) and solids concentration (C_i)
- Vessel contains a "heel" of liquid sludge (V,) at the beginning of the digestion step
- When final volume (V,) is reached, sludge is discharged until V_h remains and the process starts again

Some aerobic digesters are run in this fashion. This problem is a special case involving a batch reaction. Exactly how long each day's feeding remains in the reactor is known, but an average residence time must be calculated as shown in Equation 2:

$$\theta_n = \frac{\Sigma v_i C_i \text{ x time that batch i remains in the reactor}}{\Sigma v_i C_i}$$

The following problem illustrates the calculation:

Let v_h = 30 m³ (volume of "heel")

V_d = 130 m³ (total digester volume)

V_i = each day 10 m³ is fed to the reactor at the beginning of the day

C_i = 12 kg/m³

V, is reached in 10 days. Sludge is discharged at the end of Day 10.

$$\text{Then } \theta_n = \frac{(10 \cdot 12 \cdot 10 + 10 \cdot 12 \cdot 9 + \ldots + 10 \cdot 12 \cdot 1)}{(10.12 + 10.12 + \ldots 10 \cdot 12)}$$

$$\theta_n = \frac{10 \cdot 12 \cdot 55}{10 \cdot 12 \cdot 10} = 5.5 \text{ days}$$

Notice that the volume of the digester or of the "heel" did not enter the calculation.

Case 2b

Same as Case 2a except:

- The solids content of the feed varies substantially from day to day
- Decantate is periodically removed so more sludge can be added to the digester

The following problem illustrates the calculation:

Let V_h = 30 m³, and V_d = 130 m³

Day	vi (m³)	Solids Content (kg/m³)	Decantate (m³)
1	10	10	0
2	10	15	0
3	10	20	0
4	10	15	0
5	10	15	0
6	10	10	0
7	10	20	0
8	10	25	0
9	10	15	10
10	10	10	0
11	10	15	10
12	10	20	0

$$\theta_n = \frac{(10 \cdot 10 \cdot 12 + 10 \cdot 15 \cdot 11 + 10 \cdot 20 \cdot 10 + \ldots + 10 \cdot 10 \cdot 3 + 10 \cdot 15 \cdot 2 + 10 \cdot 20 \cdot 1)}{(10 \cdot 10 + 10 \cdot 15 + 10 \cdot 20 + \ldots + 10 \cdot 10 + 10 \cdot 15 + 10 \cdot 20}$$

$$\theta_n = 11{,}950/1{,}900 + 6.29d$$

The volume of "heel" and sludge feedings equaled 150 m³, exceeding the volume of the digester. This was made possible by decanting 20 m³.

Case 3

Same as Case 2 except that after the digester is filled it is run in batch mode with no feed or withdrawals for several days.

A conservative θ_n can be calculated by simply adding the number of extra days of operation to the On calculated

for Case 2. The same applies to any other cases followed by batch mode operation.

Case 4

- Complete-mix reactor
- Constant feed and withdrawal at least once a day
- No substantial increase or decrease of volume in the reactor
- One or more feed streams, one decantate stream returned to the treatment works, one product stream; the decantate is removed from the digester so the sludge in the digester is higher in solids than the feed

This mode of operation is frequently used in both anaerobic and aerobic digestion in small treatment works.

Equation 3 is used to calculate the residence time:

Let V = 100 m^3
q_1 = 10 m^3/d (feed stream)
C_1 = 40 kg solids/m^3
q = 5 m^3/d (existing sludge stream)
C_v = 60 kg solids/m^3

$$\theta = \frac{100 \times 60}{5 \times 60} = 20 \text{ d}$$

Case 5

- Complete-mix reactor
- Constant feed and withdrawal at least once a day
- Volume in digester reasonably constant
- One or more feed streams, one product stream that is thickened, some sludge is recycled, and some is drawn off as product

This mode of operation is sometimes used in aerobic digesters. Equation 5 is used to calculate residence time.

Let V = 100 m^3
Feed flow rate = 10 m^3/d
Feed solids content = 10 kg/m^3
Flow rate from the digester = 12 m^3/d
Solids content of sludge from the digester = 13.3 kg/m^3
Flow rate of sludge from the thickener = 4 m^3/d
Solids content of sludge from the thickener = 40 kg/m^3
Flow rate of sludge returned to the digester = 2 m^3/d
Flow rate of product sludge = 2 m^3/d

$$\theta_n = \frac{100 \times 13.3}{2 \times 40} = 16.6 \text{ d}$$

The denominator is the product of the flow rate leaving the system (2 m^3/d) and the concentration of sludge leaving the thickener (40 kg/m^3). Notice that flow rate of sludge leaving the digester did not enter into the calculation.

Comments on Batch and Staged Operation

Sludge can be aerobically digested using a variety of process configurations (including continuously fed single- or multiple-stage completely mixed reactors), or it can be digested in a batch mode (batch operation may produce less volatile solids reduction for a primary sludge than the other options because there are lower numbers of aerobic microorganisms in it). Single-stage completely mixed reactors with continuous feed and withdrawal are the least effective of these options for bacterial and viral destruction, because organisms that have been exposed to the adverse condition of the digester for only a short time can leak through to the product sludge.

Probably the most practical alternative to use of a single completely mixed reactor for aerobic digestion is staged operation, such as use of two or more completely mixed digesters in series. The amount of slightly processed sludge passing from inlet to outlet would be greatly reduced compared to singlestage operation. If the kinetics of the reduction in pathogen densities are known, it is possible to estimate how much improvement can be made by staged operation.

Farrah et al. (1986) have shown that the declines in densities of enteric bacteria and viruses follow first-order kinetics. If first-order kinetics are assumed to be correct, it can be shown that a one-log reduction of organisms is achieved in half as much time in a two-stage reactor (equal volume in each stage) as in a one-stage reactor. Direct experimental verification of this prediction has not been carried out, but Lee et al. (1989) have qualitatively verified the effect.

It is reasonable to give credit for an improved operating mode. Since not all factors involved in the decay of microorganisms densities are known, some factor of safety should be introduced. It is recommended then that for staged operation using two stages of approximately equal volume, the time required be reduced to 70% of the time required for single-stage aerobic digestion in a continuously mixed reactor. This allows a 30% reduction in time instead of the 50% estimated from theoretical considerations. The same reduction is recommended for batch operation or for more than two stages in series. Thus, the time required would be reduced from 40 days at 20°C (68°F) to 28 days at 20°C (68°F), and from 60 days at 15°C (59°F) to 42 days at 15°C (59°F). These reduced times are also more than sufficient to achieve adequate vector attraction reduction.

If the plant operators desire, they may dispense with the PSRP time-temperature requirements of aerobic digestion but instead demonstrate experimentally that microbial levels in the product from their sludge digester are satisfactorily reduced. Under the current regulations, fecal coliform densities must be less than or equal to 2,000,000 CFU or MPN per gram total solids. Once this performance is demonstrated, the process would have to be operated between monitoring episodes at time-temperature conditions at least as severe as those used during their tests.

References

Farrah., S.R.., G. Bitton. and S.G. Zan. 1986. Inactivation of enteric pathogens during aerobic digestion of wastewater sludge. EPA Pub. No. EPA/600/2-86/047. Water Engineering Research Laboratory, Cincinnati, OH. NTIS Publication No. PB86-183084/A5. National Technical Information Service, Springfield, Virginia.

Lee, K.M., C.A. Brunner, J.B. Farrell, and A.E. Eralp. 1989. Destruction of enteric bacteria and viruses during two-phase digestion. Journal WPCF 61(6):1421-l 429.

Appendix F
Sample Preparation for Fecal Coliform Tests and Salmonella sp. Analysis

1. Sample Preparation for Fecal Coliform Tests

1.1 Class B Alternative I

To demonstrate that a given domestic sludge sample meets Class B Pathogen requirements under alternative 1, the density of fecal coliform from at least seven samples of treated sewage sludge must be determined and the geometric mean of the fecal coliform density must not exceed 2 million Colony Forming Units (CFU) or Most Probable Number (MPN) per gram of total solids (dry weight basis). The solids content of treated domestic sludge can be highly variable. Therefore, an aliquot of each sample must be dried and the solids content determined in accordance with procedure 2540 G. of the 18th edition of Standard Methods for the Examination of Water and Wastewater (SM).

Sludge samples to be analyzed in accordance with SM 9221 E. Fecal Coliform MPN Procedure and 9222 D. Fecal Coliform Membrane Filter Procedure may require dilution prior to analysis. An ideal sample volume will yield results which accurately estimate the fecal coliform density of the sludge. Detection of fecal coliform in undiluted samples could easily exceed the detection limits of these procedures. Therefore, it is recommended that the following procedures be used (experienced analysts may substitute other dilution schemes as appropriate).

For Liquid Samples:

1. Use a sterile graduated cylinder to transfer 30.0 mL of well mixed sample to a sterile blender jar. Use 270 mL of sterile buffered dilution water (see Section 9050C) to rinse any remaining sample from the cylinder into the blender. Cover and blend for two minutes on low speed. 1 .O mL of this mixture is 0.1 mL of the original sample or 1.0×10^{-1}.

2. Use a sterile pipette to transfer 11 .O mL of the blended sample mixture to 99 mL of sterile buffered dilution in a sterile screw cap bottle and mix by vigorously shaking the bottle a minimum of 25 times. This is dilution "A". 1 .O mL of this mixture is 0.010 mL of the original sample or 1.0×10^{-2}.

3. Use a sterile pipette to transfer 1.0 mL of dilution "A" to a second screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "B". 1 .O mL of this mixture is 0.00010 mL of the original sample or 1.0×10^{-4}.

4. Use a sterile pipette to transfer 1.0 mL of dilution "B" to a sterile screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "C". Go to step 5. for MPN analysis (preferred) or 7. for MF analysis.

5. For MPN analysis, follow procedure 9221 E. in SM. Four series of 5 tubes will be used for the analysis. Inoculate the first series of 5 tubes each with 10.0 mL of dilution "B". This is a 0.0010 mL of the original sample. The second series of tubes should be inoculated with 1 .O mL of dilution "B"(0.00010). The third series of tubes should receive 10.0 mL of "C"(0.000010). Inoculate a fourth series of 5 tubes each with 1 .O mL of dilution "C"(0.0000010). Continue the procedure as described in SM.

6. Refer to Table 9221 .IV.in SM to estimate the MPN index/l00 mL. Only three of the four series of five tubes will be used for estimating the MPN. Choose the highest dilution that gives positive results in all five tubes, and the next two higher dilutions for your estimate. Compute the MPN/g according to the following equation:

$$\text{MPN Fecal Coliform/g} = \frac{10 \times \text{MPN Index/100mL}}{\text{largest volume} \times \text{\% dry solids}}$$

Examples:

In the examples given below, the dilutions used to determine the MPN are underlined. The number in the numerator represents positive tubes; that in the denominator, the total number of tubes planted; the combination of positives simply represents the total number of positive tubes per dilution.

Example	0.0010 mL	0.00010 mL	0.000010 mL	0.0000010 mL	Combination of positives
a	515	5/5	3/5	0/5	5-3-o
b	5/5	3/5	1/5	0/5	5-3-l
c	0/5	1/5	0/5	015	O-1-0

For each example we will assume that the total solids content is 4.0%.

For example a:

The MPN index/l 00 mL from Table 9221.4 is 80. Therefore:

$$MPN/g = \frac{10 \times 80}{0.00010 \times 4.0} = 2.0 \times 10^6$$

For example b:

The MPN index/l 00 mL from Table 9221.4 is 110. Therefore:

$$MPN/g = \frac{10 \times 110}{0.0010 \times 4.0} = 2.8 \times 10^5$$

For example c:

The MPN index/l 00 mL from Table 9221.4 is 2. Therefore:

$$MPN/g = \frac{10 \times 2}{0.0010 \times 4.0} = 5.0 \times 10^3$$

5. Alternately the membrane filter procedure may be used to determine fecal coliform density. This method should only be used if comparability with the MPN procedure has been established for the specific sample medium. Three individual filtrations should be conducted in accordance with SM 9222 D. using 10.0 mL of dilution "C", and 1.0 mL and 10.0 mL of dilution "B". These represent 0.000010, 0.00010, and 0.0010 mL of the original sample. Incubate samples, and count colonies as directed. Experienced analysts are encouraged to modify this dilution scheme(e.g. half log dilutions) in order to obtain filters which yield between 20 and 60 CFU.

6. Compute the density of CFU from membrane filters which yield counts within the desired range of 20 to 60 fecal coliform colonies:

$$\text{coliform colonies/g} = \frac{\text{coliform colonies counted} \times 1\,00}{\text{mL sample} \times \%\ \text{dry solids}}$$

For Solid Samples:

1. In a sterile dish weigh out 30.0 grams of well mixed sample. Whenever possible, the sample tested should contain all materials which will be included in the sludge. For example, if wood chips are part of a sludge compost, some mixing or grinding means may be needed to achieve homogeneity before testing. One exception would be large pieces of wood which are not easily ground and may be discarded before blending. Transfer the sample to a sterile blender. Use 270 mL of sterile buffered dilution water to rinse any remaining sample into the blender. Cover and blend on low speed for two minutes. One milliliter of this sample contains 0.10 g of the original sample.

2. Use a sterile pipette to transfer 11 .O mL of the blender contents to a screw cap bottle containing 99 mL of sterile buffered dilution water and shake vigorously a minimum of 25 times. One milliliter of this sample contains 0.010 g of the original sample. This is dilution "A".

3. Follow the procedures for "Liquid Samples" starting at Step 3.

Examples:

Seven samples of a treated sludge were obtained prior to land spreading. The solids concentration of each sample was determined according to SM. These were found to be:

Sample No.	Solids Concentration (%)
1	3.8
2	4.3
3	4.0
4	4.2
5	4.1
6	3.7
7	3.9

The samples were liquid with some solids. Therefore the procedure for liquid sample preparation was used. Furthermore, the membrane filter technique was used to determine if the fecal coliform concentration of the sludge would meet the criteria for Class B alternative 1. Samples were prepared in accordance with the procedure outlined above. This yielded 21 individual membrane filters (MF) plus controls. The results from these tests are shown in table 1

Table 1. Number of Fecal Coliform Colonies on MF Plates

Sample No.	0.000010 mL Filtration	0.00010 mL Filtration	0.0010 mL Filtration
1	0	1	23
2	2	18	TNTC
3	0	8	85
4	0	5	58
5	0	1	17
6	0	1	39
7	0	1	20

The coliform density is calculated using only those MF plates which have between 20 and 60 blue colonies whenever possible. However, there may be occasions when the total number of colonies on a plate will be above or below the ideal range. If the colonies are not discrete and appear to be growing together results should be reported as "to numerous to count" (TNTC). If no filter has a coliform count falling in the ideal range (20 -60), total the coliform counts on all countable filters and report as coliform colonies/g. For sample number 2 the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{(2 + 18) \times 100}{(0.000010 + 0.00010) \times 4.3} = 4.2 \times 10^6$$

Sample number 3 has two filters which have colony counts outside the ideal range also. In this case both countable plates should be used to calculate the coliform density/g. For sample number 3, the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{(8 + 65) \times 100}{(0.00010 + 0.0010) \times 4.0} = 1.6 \times 10^6$$

Except for sample number 5, all of the remaining samples have at least one membrane filter within the ideal range. For these samples, use the number of colonies formed on that filter to calculate the coliform density. For sample number 1, the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{23 \times 100}{0.0010 \times 3.8} = 6.0 \times 10^5$$

Coliform densities of all the samples were calculated and converted to $\log_0$ values to compute a geometric mean. These calculated values are presented in Table 2.

Table 2. Coliform Density of Sludge Samples

Sample No.	Coliform Density	$\log_{10}$
1	6.0×10^5	5.78
2	4.2×10^6	6.62
3	1.6×10^6	6.20
4	9.0×10^5	5.95
5	4.0×10^5	5.60
6	1.0×10^6	6.00
7	5.1×10^5	5.71

The geometric mean for the seven samples is determined by averaging the $\log_0$ values of the coliform density and taking the antilog of that value.

$$(5.78 + 6.62 + 6.20 + 5.95 + 5.60 + 6.00 + 5.71) / 7 = 5.98$$

The antilog of $5.98 = 9.5 \times 10^5$

Therefore, the geometric mean fecal coliform density is below 2 million and the sludge meets Class B Pathogen requirements under alternative 1.

1.2 Class A Alternative I

Part 503 requires that, to qualify as a Class A sludge, treated sewage sludge must be monitored for fecal coliform (or *Salmonella* sp. and have a density of less than 1,000 MPN fecal coliform per gram of total solids (dry weight basis). The regulation does not specify total number of samples. However, it is suggested that a sampling event extend over two weeks and that at least seven samples be collected and analyzed. The membrane filter procedure may not be used for this determination. This is because the high concentration of solids in such sludges may plug the filter or, render the filter uncountable. The total solids content for each sample must be determined in accordance with procedure 2540 G. of SM.

For Liquid Samples:

1. Follow procedure 9221 E. in SM. Inoculate at least four series of five tubes using ten fold serial dilutions. Prepare the sample as described for "Class B Alternative 1, Liquid Samples," except inoculate each of the first series of tubes with 10.0 mL of the blender contents (the concentration of the enrichment broth must be adjusted to compensate for the volume of added sample). This is equivalent to adding 1 .O mL of sludge to the first series of tubes. Inoculate the remaining tubes and complete the analysis in accordance with SM.

2. Calculate the MPN as directed in Step 4 above.

For Solid Samples:

1. Follow procedure 9221 E. in SM. Inoculate at least four series of five tubes using ten fold serial dilutions. Prepare the sample as described for "Class B alternative 1, Solid Samples," except inoculate each of the first series of tubes with 10.0 mL of the blender contents (the concentration of the enrichment broth must be adjusted to compensate for the volume of added sample). This is equivalent to adding 1 .O g of sludge (wet wieght) to the first series of tubes. Inoculate the remaining tubes and complete the analysis in accordance with SM.

2. Calculate the MPN as directed in step 4 above.

2. Sample Preparation for *Salmonella* sp. Analysis

Salmonella sp. quantification may be used to demonstrate that a sludge meets Class A criteria, instead of analyzing for fecal coliforms. Sludges with *Salmonella* sp. densities below 3 MPN/4 g total solids (dry wieght basis) meet Class A criteria. The analytical method described in Appendix F of this document, describes the procedure used to identify *Salmonella* sp. in a water sample. Similarly, the procedures for analysis of *Salmonella* sp. in SM (Section 9260 D) do not address procedures for sludges, the sample preparation step described here should be used, and the total solids content of each sample must be determined according to method 2540 G in SM.

For Liquid Samples:

1. Follow the same procedure used for liquid sample preparation for fecal coliform analysis described under "Class A Alternative 1". However, the enrichment medium used for this analysis should be dulcitol selenite broth (DSE) as described in Appendix G of this document or dulcitol selenite or tetrathionate broth as described in SM. Only three series of five tubes should be used for this MPN procedure. Use a sterile open tip pipette to transfer 10.0 mL of well mixed sample to each tube in the first series. These tubes should contain 10.0 mL of double strength enrichment broth. Each tube in the second series should contain 10.0 mL of double strength enrichment broth. These tubes should each receive 10.0

mL of the blended mixture. The final series of tubes should contain 10.0 mL of single strength enrichment broth. These tubes should each receive 1.0 mL of the blended mixture. Complete the MPN procedure as described in Appendix G or SM as appropriate.

2. Refer to Table 9221 .IV.in SM to estimate the MPN index/l 00 mL. Calculate the MPN/4 g according to the following equation:

$$\textit{Salmonella}\text{ sp. MPN/4 g} = \frac{\text{MPN Index/l 00mL} \times 4}{\text{\% dry solids}}$$

For example:

If one tube in the first series was identified as being positive for *Salmonella* sp. and no other tubes were found to be positive, from Table 9221 .IV one finds that a l-0-0 combination of positives has an MPN index/l 00 mL of 2. If the percent of dry solids for the sample was 4.0, then:

$$\text{Salmonella sp. MPN/4 g} = \frac{2 \times 4}{4.0} = 2$$

For Solid Samples:

1. Follow the procedure for solid sample preparation for fecal coliform analysis described under Class A Alternative 1 above. However, the enrichment medium used for this analysis should be dulcitol selenite broth (DSE) as described in Appendix G or dulcitol selenite or tetrathionate broth as described in SM, and only three series of five tubes should be used for this MPN procedure. Use aseptic technique to weigh out and transfer 10.0 g of well mixed sample to each screw cap tube in the first series, shake vigorously to mix. These tubes should contain 10.0 mL of double strength enrichment broth. Likewise, each tube in the second series should contain 10.0 mL of double strength enrichment broth. These tubes should receive 10.0 mL of the blended mixture. The final series of tubes should contain 10.0 mL of single strength enrichment broth. These tubes should receive 1 .O mL of the blended mixture. Alternately, because the calculated detection limit is dependant upon the total solids content of the sample, samples with total solids contents >28% can be blended as described above and the blender contents can be used for inoculating the initial series of tubes. When this option is chosen, the final series of tubes will contain 0.1 mL of the blender contents. Complete the MPN procedure as described in Appendix G or SM as appropriate.

2. Refer to Table 9221 .IV.in SM to estimate the MPN index/l 00 mL. Calculate the MPN/4 g according to the following equation:

$$\textit{Salmonella}\text{ sp. MPN/4 g} = \frac{\text{MPN Index/l 00mL} \times 4}{\text{\% dry solids}}$$

Appendix G

1Kenner and Clark (1974) Analytical Method for *Salmonella* sp. Bacteria*

Detection and enumeration of Salmonella and Pseudomonas aerwginosa

BERNARD A. KENNER AND HAROLD P. CLARK

THE FEDERAL WATER POLLUTION CONTROL AMENDMENTS of 1972 [1-4] may well require the quantification and enumeration of pathogens such as ***Salmonella*** species in all classes of waters. The requirements are described by Shedroff .[5]

One of the continuing programs of the Environmental Protection Agency (EPA) is a research project concerned with the development of practical laboratory methods for the isolation, quantification, and enumeration of pathogens from polluted waters. This paper reports a monitoring method developed for the simultaneous isolation and enumeration of ***Salmonella*** species and ***Pseudomonas aeruginosa*** from potable waters, reuse waters, treatment plant effluents, receiving waters, and sludges.

The method described herein, and developed by Kenner,[6] is practical because readily available bacteriological media, chemicals, and equipment are all that are required to obtain the desired results. These results are the establishment of the absence or presence of ***Salmonella*** species (pathogenic hazardous bacteria) and/or ***Pseudomonas aeruginosa*** (potential pathogens) that affect persons who are in a debilitated condition and are very common as infectious agents in hospitals because of their resistance to antibiotic therapy.'-" Potable waters have also been shown to contain ***Ps. aeruginosa***.[6, 10] The sources of these potential pathogens are human and animal feces and wastewaters.[11, 12]

When the monitoring method was used, it was found that 100 percent of municipal wastewaters and treatment plant sludges contained both of these potential pathogens. ***Ps. aeruginosa*** has been found in potable water supplies of large and small municipalities where insufficient residual chlorine is evident. Also important is the fact that these organisms may be found in the absence of fecal coliforms, whereas negative indicator tests may give a false sense of security. It is believed by the authors that these organisms may be better indicators than fecal coliforms of pollution in potable, direct reuse, bathing, and recreational waters.

MATERIALS AND METHODS

The monitoring method uses a multiple tube (MPN) procedure in which dulcitol selenite broth (DSE) [13] is used for primary enrichment medium, and is modified by the use of sodium acid selenite (BBL). The formula is proteose peptone (Bacto), 0.4 percent; yeast extract (Bacto), 0.15 percent; dulcitol, 0.4 percent; BBL, 0.5 percent; Na_2HPO_4, 0.125 percent; and KH_2PO_4, 0.125 percent in distilled water. The constituents are dissolved in a sterile flask, covered with foil, and heated to 88°C in a water bath to obtain a clear sterile medium that does not require adjustment of pH. Productivity for ***Salmo***n& species is enhanced by the addition of an 18-hr, 37°C culture of *Salmonella paratyphi* A (10 percent by volume) in single-strength DSE broth, killed by heating to 88°C.

Concentration of bacteria from large volumes of water is necessary* when potable, direct reuse, receiving waters, and treatment effluents are being monitored.

TABLE I.-Retentive Characteristics of Several Glass Fiber Filter Papers' Compared with Membrane Filters

Filter	Total Bacteria† Filtered	Number Passing Filter	Percentage Retention
Millipore (MF) HAWG 047 HA 0.45 μ, white, grid, 47 mm, Millipore Filter Corp.	1,376	0	100
984H Ultra Glass Fiber Filter, 47 mm, Reeve Angel Corp.	1,229	25	98
GF/F Glass Paper Whatman,‡ 47 mm, Reeve Angel Corp.	2,698	6	99.8
GF/D Glass Paper Whatman,‡ 47 mm, Reeve Angel Corp.	2,622	2,166	17.4
934AH Glass Fiber Filter, 47 mm, Reeve Angel Corp.	1,049	198	81
GF/A Glass Paper Whatman, 47 mm, Reeve Angel Corp.	1,066	680	36

* The 984H Ultra Glass Fiber Filter is flexible when wet, readily allows filtration of large volumes of water, can readily be bent double with forceps, and, when placed into primary enrichment broth, disintegrates when tube is shaken and releases entrapped bacteria.

† Entcric bacteria. *E. coli*, 0.5 × 1-3 μ.

‡ A new paper filter GF/F has better retentive properties than the 984H, and has same properties (tested Oct. 1973).

Concentration is attained by filtration through glass fiber filters * in a membrane filter apparatus. After the desired volume of water is filtered through the ultra filter, the flexible filter is folded double with sterile forceps and inserted into a suitable volume of single-strength DSE medium contained in a test tube located in the first row of the multiple tube setup. The tube should then be shaken to cause filter to disintegrate (Table I and Figure 1). To obtain MPN results per one 1 or per 10 I, 100 ml or 1,000 ml of sample, respectively, are filtered for each tube of DSE medium in the first row of the five-tube MPN setup. Additional dilutions are made by transferring material from tubes in the first row to tubes farther back in the setup.

Obtaining results on a per I-gal (3.8-l) basis requires filtration of 380 ml, and on a per 10-gal (38-1) basis requires filtration of 3,600 ml for each tube in the first row. Where concentration of bacteria is not usually required, as in municipal wastewaters, sludges, or primary effluents, the regular transfer of 10 ml of sample to each tube in the first row of the setup into 10 ml of double-strength DSE is made, 1 ml of sample in 9 ml of single-strength DSE in the second row, and so on. The MPN table in "Standard Methods" [14] is used to read directly the results per volume of sample.

Incubation temperature of 40" ± 0.2°C for 1 and 2 days is critical to obtain optimum recovery of *Salmonella* sp. and *Pseudomonas aeruginosa* when DSE broth is used for primary enrichment. After primary incubation at 40°C, surface loopfuls (scum) (7 mm platinum or nichrome wire loop) are removed from each multiple-tube culture and streaked on each of two sections of a divided plate of Xylose lysine desoxycholate agar (XLD) [15] in order to isolate colonial growth. The numbered plates are inverted and incubated at 37°C for a period not to exceed 24 hr.

Commercial dehydrated XLD agars (BBL and Difco) are satisfactory if they are reconstituted in distilled water in sterile foil-covered flasks and heated to 88" or 92°C, respectively. The agar is then cooled to 55" to 60°C and distributed in sterile petri dishes. This laboratory prefers 10-ml portions in each section of a divided sterile disposable plastic dish (Figure 1).

* Reeve Angel 984H ultra glass fiber filter, 47 mm, Reeve Angel & Co., Inc., Clifton, N. J. Mention of trade names does not constitute endorsement or recommendation by EPA.

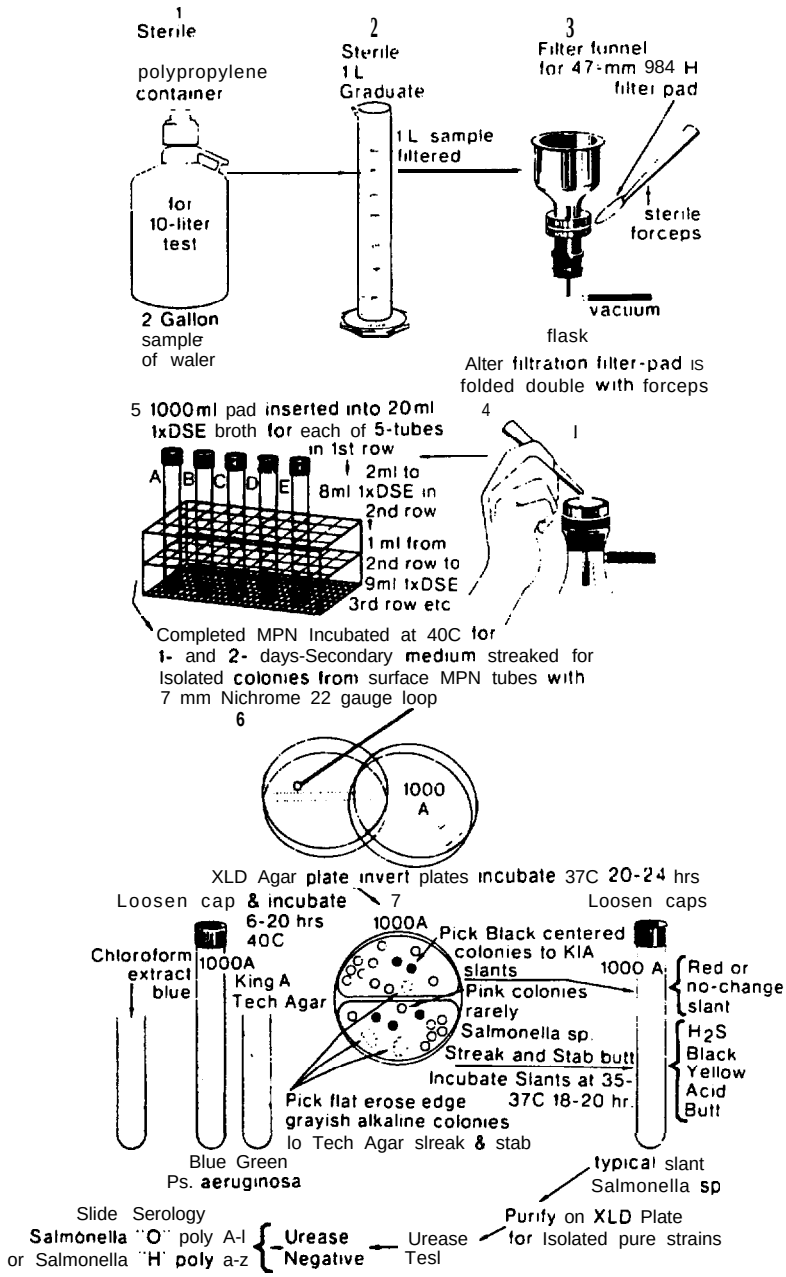


FIGURE I.--Procedure for isolation of pathogens,

Positive incubated XLD plate cultures contain typical clear, pink-edged, black-centered ***Salmonella*** colonies, and flat, mucoid, grayish alkaline, pink erose-edged *Ps. aeruginosa*. The *Salmonella colonies* are picked to Kligler iron agar (KIA) or Triple sugar iron agar slants for typical appearance, purification, and identity tests. *Ps. aeruginosa* colonies are picked to King A agar slants (Tech agar BBL) for obtaining the bluegreen pyocyanin confirmation at 40°C *(Figure* 1).

Typically, *Salmonella* sp. slant cultures (streaked and stabbed), incubated over-

TABLE H.-Advantage of Ultra-filter 984H Use in Monitoring Suspected Waters for *Salmonella* species

Tvpe of Sample	*Salmonella* (no./ 100 ml)	Serotypes Found (no./100 ml)	*Salmonella* (no./gal)	Serotypes Found (no./gal)
Stormwater runoff	4.5	S. *bareilly*[7]	210	S. *kottbus*[10] S. *bareilly*[11]
Stormwater runoff	<3.0	none	7.3	S. *java*[4] S. *muenchen*[2]
Activated sludge effluent	<3.0	none	3.6	S. group G[4]
Municipal wastewater	6.2	Arizona'	1,500	Arizona' S. *anatum*[2] S. *newport*[4] S. *san diego*[7] S. *worthington*[2]
Municipal wastewater	<3.0	none	110	S. *anatum*[3] S. *derby*[1] S. *newport*[3]
Activated sludge effluent	<3.0	none	28	S. *blockley*[7] S. *newport*[3]
Mississippi River water, mile 403.1	43	S. *ohio*[10]	>11,000	S. *ohio*[19] S. *derby'* S. *meleagridis*[6]
Municipal wastewater	3.0	S. *cholerasuis* var. kunzendorf[2]	21	S. *cholerasuis* var. kunzendorf[5] S. *newport*[6]

night at 37°C, give an unchanged or alkaline red-appearing slant; the butt is blackened by H_2S, is acid-yellow, and has gas bubbles, except for rare species. Typical-appearing slant cultures are purified by transferring them to XLD agar plates for the development of isolated colonies. The flat or umbonated-appearing colonies with large black centers and clear pink edges then are picked to KIA slants (streaked and stabbed), incubated at 37°C, and urease tested before the identification procedure (Figure 1). Urease-negative tubes are retained for presumptive serological tests and serotype identification.

Typical Tech agar slant cultures for *Ps. aeruginoca* that are incubated at 40°C overnight turn a bluegreen color from pyocyanin, a pigment produced only by this species. A reddish-blue color is caused by the additional presence of pyorubin. The blue pigment is extractable in chloroform and is light blue in color after a few hours at room temperature. No further tests are necessary. The count is read directly from the MPN table.

JUSTIFICATION FOR PROCEDURES

Choice of primary enrichment medium and secondary isolation agar. Most of the enrichment media described in contemporary literature were designed for the isolation of pathogens from clinical specimens from ill persons or from samples of suspected foods, and they work quite well for those types of samples. When they are used, however, for the isolation of pathogens from polluted waters and other types of environmental samples, such as soils, they do not prove adequate. Enrichment media that were tested and found wanting in regard to detection and selectivity were tetrathionate broth (TT), with and without brilliant green at 41.5°C; selenite cystine broth at 37°C; selenite F broth at 37°C; selenite brilliant green, with and without sulfa, at 37" and 41.5°C; and Cram-negative broth (GN) at 40" and 41.5°C.

None of the media named worked well at 37°C for the isolation of Salmonella sp., and isolation from wastewaters only oc-

TABLE III.-Percentage of Colony Picks from DSE-XLD Combination Positive for *Salmonella* species

Liquid Samples	NO.	Total Picks from XLD	so. Positive	so. Negative	Percentage Positive	Range of *Salmonella* counts/100 ml
Municipal wastewater	15	315	250	65	7Y	3.0–1,500
Stockyard wastewater	1	36	36	0	100	2,100
Rivers						
Mississippi						
Ohio	8	110	a4	26	76	1.5–>300
Stormwater runoffs	2	18	14	4	78	0.2–1.5
Activated sludge biological effluent	20	386	306	80	79	0.1–1,100
Trickling filter effluent	7	183	78	25	76	0.35–140
Package plant effluent	6	41	55	28	66	1.8–620
Package plant sludge	2		37	4	90	43–240
Chlorinated primary outfall	2	17	13	4	76	3-43
Creek 1 mile (1.6 km) below package plant outfall	2	37	16	21	43	4.5–12
Dupont items	2	17	10	7	59	0.26–1.1
Feed	1	20	14	6	70	4.3
Reject	1	16	8	8	50	0.91
Product-negative						
Raw primary sludge	4	80	66	1-l	83	13–700
Primary activated sludge	1	15	13	2	87	23
Anaerobic digester sludge	3	78	65	13	83	79–170
Anaerobic digester sludge (28 days)	1	9	3	6	33	2
Activated secondary sludge	6	189	155	34	82	11–>11,000
Total	84	1,570	1,223	347	average 78	

curred by chance and was purely qualitative. Of the above-named media used in preliminary tests, selenite brilliant green sulfa broth (SBGS) at 41.5°C gave the best isolation of *Salmonella* sp. from wastewaters (with and without the addition of S. *typhimurium* in known numbers). Of thirteen wastewater samples tested in SBGS at 41.5°C, six contained *Salmonella* or 46 percent were positive. With DSE broth at 40°C, 28 of 28, or 100 percent of wastewater samples, gave positive results,

Studies were not continued on SBGS medium when it was noted that some lots of commercially available SBGS seemed to be selective for *Salmonella* sp. while others were not. The medium was then prepared according to the original formula [16] with six different lots of brilliant green (certified), only one of which was selective. The use of brilliant green agar as a selective medium is subject to the same variability, according to Read and Reyes.[17]

The main reasons for rejection of TT, with and without brilliant green, and for selenite broth's using brilliant green agar and XLD agar as secondary media are not only fewer isolations of *Salmonella* sp., but also the poor selectivity of these combinations when they are used for monitoring polluted waters. These combinations' poor selectivity at 41.5°C is apparent in the results of Dutka and Bell,[15] where the TT broth-XLD combination yielded 26 percent confirmation of colonial picks, and selenite broth-BGA and selenite broth-XLD gave 55 and 56 percent confirmations, respectively. The authors had similar results. The GN-XLD combination was poorest for water samples at 40" and 41.5°C, yielding less than 10 percent isolations from wastewaters.

Effect of incubation temperature on isolation of *Salmonella* sp. In a study of 26 wastewater samples that was conducted with the DSE multiple tube setups at three

TABLE IV.-Serotypes of *Salmonella* Found in Polluted Waters

Serotype	No. of Strains	Rank in Water Isolations	Rank in Human Occurrence*
1 *typhimurium**	375	1	1 and 6†
2 *derby*	287	2	12
3 *cubana*	223	3	20
4 *chester*	203	4	19
5 *newport*	188	5	4
6 *kottbus*	158	6	—‡
7 *blockley*	157	7	11
8 *infantis*	141	8	9
9 *enteritidis*	128	9	5
10 *anatum*	127	10	13
11 *heidelberg*	110	11	6
12 *manhattan*	97	12	14
13 *paratyphi* B	91	13	17
14 *illinois*	77	14	—‡
15 *thompson*	63	15	3
16 *livingstone*	52	16	—‡
17 *montevideo*	47	17	16
18 *muenchen*	45	18	18
19 *oranienberg*	44	19	15
20 *san diego*	44	19	18
21 *bareilly*	42	20	23
22 *tshiongwe*	41	21	—
23 *orion*	41	21	—‡
24 *senftenberg*	39	22	25
25 *schwarzengrund*	37	23	—‡
26 *lexington*	33	24	—‡
27 *cholerasuis*	30	25	—‡
28 *binza*	29	26	24
29 *cholerasuis* var. kunzendorf	29	26	—‡
Other serotypes:			
30 *albany*	20	31	—‡
31 *benfica*	10	40	—‡
32 *braenderup*	13	38	—‡
33 *brancaster*	1		—‡
34 *bredeney*	8		23
35 *california*	2		—‡
36 *drypool*	14	37	—‡
37 *friedenau*	4		—
38 *give*	25	29	22
39 *grumpensis*	10	40	—‡
40 *haifa*	2		—‡
41 *hartford*	2		—‡
42 *havana*	16	35	—‡
43 *indiana*	10	40	—‡
44 *java*	15	36	7
45 *javiana*	13	38	8
46 *litchfield*	17	34	21
47 *lomita*	26	28	—‡
48 *meleagridis*	18	33	25
49 *mission*	14	37	—‡
50 *newington*	2		—‡
51 *newlands*	8		—‡
52 *norwich*	14	37	—‡
53 *ohio*	19	32	—‡
54 *preston*	2		—
55 *reading*	26	28	—‡
56 *rubislaw*	15	36	—‡
57 *saint paul*	21	30	10
58 *schleissheim*	12	39	—
59 *simsbury*	9		—‡
60 *taksony*	16	35	—‡
61 *tennessee*	12	39	20
62 *typhi-suis* var. voldagsen	2		—
63 *uzaramo*	28	27	—‡
64 *wil*	3		—
65 *worthington*	10	40	24
Sub total	3,417		
Arizona	151		
Incomplete serology	232		
Total	3,800		

* Rank in human occurrence Table I, Martin and Ewing.[19]

† Separation of *S. typhimurium* and var. copenhagen not done after initial identifications.

‡ Serotypes occurring in humans, 1965–1971, Center for Disease Control, Salmonella Surveillance, Annual Summary 1971, Table IX, U. S. DHEW, PHS DHEW Publ. No. (HSM) 73-8184 (Oct. 1972).

different temperatures, it was found that 100 percent of the samples contained Salmonella sp. and *Ps. aeruginosa* at 40°C. At 41.5°C, however, only 50 percent or 13 of the samples yielded *Salmonella* sp., and at 37°C only 8 percent or 2 of the samples yielded *Salmonella* sp.

Effect of enhancement of DSE broth with a killed culture of S. *paratyphi* A. In a study of 84 samples of activated sludge effluents, trickling filter effluents, package plant effluents, and stream waters, DSE broth enhanced with a killed culture of *S. paratyphi* A in DSE broth (10 percent by volume) yielded isolations in 64 samples or 74 percent isolated Salmonella sp., compared with 48 samples or 57 percent isolations when the DSE broth was used without enhancement. An improved isolation of 17 percent was achieved with enhanced DSE broth.

Ultra-filter. The advantages of ultra-filter use in testing water samples are illustrated in Table II.

RESULTS AND DISCUSSION

Of importance to those who must use bacteriological tests to obtain *Salmonella* sp. and *Ps. aeruginosa* counts from waters is the amount of work that must be done to secure accurate results. Table III presents the percentage of colony picks made with the described method that proved to be *Salmonella* sp. If there are black-centered colonies on the XLD plates, more than 75 percent of the picks will prove to be *Salmonella* sp.; thus, the method leads to less unproductive work. When other methods were used, the authors have at times had to pick 50 black-centered colonies to obtain only 5 *Salmonella* sp. strains. This type of unproductive work has given the search for pathogens in the environment an undeserved bad reputation, and it has caused some to give up.

In Table II it may readily be seen that in many cases the fault with many tests has been the testing of an insufficient volume of sample. Many people think that it involves too much work, and that only espensive fluorescent antibody techniques will work. The problem is, however, to

TABLE V.-Percentage of Various Types of Water Samples Positive for *Salmonella* species

Type of Sample	Number of Samples	Number Positive	Number Negative	Percentage Positives
Municipal wastewaters	28	28	0	100.0
Municipal primary effluents (chlorinated)	9	5	4	56.0
Activated sludge effluents (clarified)	40	29	11	72.5
Activated sludge effluents Before chlorination	5	4	1	80.0
Afresidual,15amin,contact0 mg/l	8	0	8	0.0
Trickling filter effluents	26	15	11	57.7
Package plant effluents	15	7	8	46.7
Creek 1 mile (1.6 km) below package plant	3	3	0	100.0
Ohio River above Cincinnati public landing	20	9	11	45.0
Wabash River	4	3	1	75.0
Mississippi River	4	3	1*	75.0
Streams collective	31	18	13	58.0
Stormwater runoff alter heavy rain	6	3†	3‡	50.0
Farm wells	4	0	4	0.0
Home cisterns suburban	5	2	3	40.0
Septic tank sludges	6	3	3	5U.0
Totals	183	114	69	

* Municipal intake.
† Positive by per-gallon technique.
‡ Negative by per-100 ml technique.

concentrate the bacteria in a 10-gal (38-l) sample or a 100-gal (380-I) sample of potable or reuse water to obtain results, and still not require even more expensive filtration or centrifugation equipment. It also seems unrealistic to test only extremely small samples of the water being examined, because they may not be representative.

Table IV contains a list of *Salmonella* serotypes isolated from polluted waters and ranked according to the frequency of serotype isolations. It will be noted that all of the serotypes except S. *typhi* were isolated from environmental samples by the monitoring method, and that only 6 of the 65 serotypes reported were not reported as occurring in humans in the U. S. over the period from 1965 to 1971.

Table V summarizes the percentage of various types of water samples positive for *Salmonella* sp. Of interest is the fact that 100 percent of the municipal wastewaters tested contain *Salmonella* sp., that 56 percent of chlorinated primary effluents tested contain the pathogens, and that 100 percent of chlorinated secondary effluents are negative for pathogens. There are more studies scheduled for testing of secondary and tertiary effluents to obtain minimal chlorine residuals. Calabro et *al.*[20] reported that more than 50 attempts at isolating *Salmonella* Sp. from septic tank samples using SBGS-BGSA combinations were unsuccessful.

Table VI summarizes the isolation of *Ps. aeruginosa* from potable water supply, that is, wells, cisterns, and small municipal water supply. It should be noted that fecal coliforms were not detected in most of these samples. Fecal streptococci counts were higher than fecal coliform counts where both tests were used. *Ps. aeruginosa* were present in all but three of the tests, and *Salmonella* sp. were isolated from two different cistern samples.

It is of importance to the user of pathogen tests that the test be quantitative. In initial studies on the DSE-XLD combination, it was important to know if the enrichment broth would support the growth of a wide

TABLE VI.-Isolation of *Pseudomonas aeruginosa* from Potable **Water** Supply

Type of Sample	*Ps. aeruginosa* Isolation	Indicators/LOO ml		
		Total Coliforms	Fecal Coliforms	Fecal Streptococci
Well 8/16/7 1	+	4	—	—
Well 8/25/7 I	+	22	—	—
Well 3/27/72	+	—	<1	—
Well 3/27/72 (chlorinated)	+	—	<1	—
Well 8/23/72	+	—	0.25	62
Well 10/ 4/72	+	—	<2	46
Suburban cisterns				
8/ 4/72	+	—	180	—
10/ 9/72*	+	—	15	156
11/ 6/72*	+	—	<2	22
11/ 6/72	+	—	<2	2
11/26/72	+	—	3	28
Municipal applies				
Population served 54,700				
3/17/71	+	—	<1	—
6/21/71	+	—	<1	—
7/19/71	+	—	<1	—
6/19/72	+	—	0.26	—
10/ 9/72	+	—	<1	<1
5/ 8/72	0	—	<1	—
Population served **14,000**				
5/ 8/72	0	—	<1	—
10/24/72	+	—	<1	18
Population served < **10,000**				
11/27/72	0	—	<1	<1

* *Salmonella* sp. **also present in samples.**

range of ***Salmonella*** serotypes. Laboratory cultures of ***S. paratyphi*** A, ***S. typhimurium, S. bredeney, S. oranienberg, S. pullorum, S. anatum, S. give,*** and S. *worthington were* tested in three enrichment broths. The time required to isolate each of the above cultures from an estimated 10 to 20 organisms/100 ml in buffer water was 48 to 72 hr for ***S. paratyphi*** A in TT broth, 24 hr for DSE broth, and 36 to 48 hr for SBGS broth. The rest of the cultures were isolated in estimated numbers in 14 to 24 hr in TT and DSE broths. In SBGS broth, ***S. typhimurium, S. bredeney, S. anatum, S.*** give, and S. *worthington* required 36 to 48 hr incubation, and ***S. pullorum*** and S. ***oranienberg*** required 48 to 72 hr incubation.

It is impossible to know if 100 percent of ***Salmonella*** sp. in a polluted water sample are isolated. In tests where laboratory cultures have been added in low numbers to wastewater and treatment effluent samples, all of the numbers added were detected, as well ***as*** the ***Salmonella*** sp. that were naturally occurring. The higher the quality of the water (for example, secondary or tertiary treatment effluent, or even potable waters), the better the possibility of isolation of all the ***Salmonella*** serotypes present, as well as Ps. ***aeruginosa,*** a potential pathogen.

SUMMARY

A practical laboratory method is presented for the simultaneous isolation and enumeration of ***Salmonella*** sp. and ***Pseudomonas aeruginosa*** from all classes of waters, including potable water supplies, with a minimum of interfering false positive isolations. The method allows for the testing

of large volumes of high quality waters, wherein the absence of indicator bacteria (that is, total coliforms, fecal coliforms, and fecal streptococci), may give a false sense of security because of the low volumes of water usually tested. Justification for each step of the procedural method is presented.

ACKNOWLEDGMENTS

Credits. The technical assistance of **Pauline C.** Haley in performing the necessary serology for identifying many of the ***Salmonella*** serotypes reported is gratefully acknowledged.

Authors. Bernard A. Kenner is supervisory research microbiologist, and Harold P. Clark is biological technician, Waste Identification and Analysis Activity of the Advanced Waste Treatment Research Laboratory, Natonal Environmental Research Center, U. S. Environmental Protection Agency, Cincinnati, Ohio.

REFERENCES

1. Federal Water Pollution Control Amendments, PL 92-500, 86 Stat. 816, 33 U. S. Code Sec. 1151 et seq. (1972).
2. FWPCA, Section 504 as amended (1972).
3. FWPCA, Section 307 (a) (1972).
4. FWPCA, Section 311 (1972).
5. Quality Assurance Division, Office of Research and Monitoring, U. S. EPA, "Proc. 1st Microbiology Seminar on Standardization of Methods." EPA-R4-73-022 (Mar. 1973).
6. Kenner, B. A., et *al.*, "Simultaneous Quantitation of ***Salmonella*** species and ***Pseudomonas oeruginosa.*** I. Polluted Waters. II. Persistence of Pathogens in Sludge Treated Soils. III. Analysis of Waste Treatment Sludges for ***Salmonella species*** as a Surveillance Tool." U. S. EPA, National Environmental Research Center, Cincinnati, Ohio (Sept. 1971).
7. Moody, M. ***R., et al.***, "*Pseudomonas aeruginosa* in a Center for Cancer Research. I. Distribution of Intraspecies Types from Human and Environmental Sources." ***Jour. Inf. Diseases, 125. 95 (1972).***
8. Edmonds, P., *et al.*, "Epidemiology of *Pseudomonas* ***aeruginosa*** in a Burns Hospital. Surveillance by a Combined Typing System." ***Appl. Microbiol., 24, 219 (1972).***
9. "New Hospital Controls Urged to Stem Pseudomonas Rise." ***Clin. Lab. Forum*** (Eli Lilly), 2, 1 (May-June 1970).
10. Reitler, R., and Seligmann, R., ***"Pseudomonas aeruginosa*** in Drinking Water." *Jour. Appl. Bacteriol.*, 20, 145 ***(1957).***
11. Ringen, L. M., and Drake, C. H., ***"A Study*** of the Incidence of ***Pseudomonas*** *aeruginosa* from Various Natural Sources." ***Jour. Bacteriol., 64, 841 (1952).***
12. Drake, C. H., "Evaluation of Culture Media for the Isolation and Enumeration of ***Pseudomonas aeruginoso." Health Lab. Sci., 3,*** 10 ***(1966).***
13. Raj, H., "Enrichment Medium for Selection of Salmonella from Fish Homogenate." *Appl. Microbiol.*, 14, 12 (1966).
14. "Standard Methods for the Examination of Water and Wastewater." 13th Ed., Amer. Pub. Health Assn., New York, N. Y. (1971).
15. Taylor, W. I., "Isolation of Shigellae. I. Xylose Lysine Agars; New hledia for Isolation of Enteric Pathogens." ***Tech. Bull. Reg. Med. Technol., 35,*** 161 (1965).
16. Osborne, W. W., and Stokes, J. L., "A Modified Selenite Brilliant-Green Medium for the Isolation of Salmonella from Egg Products." ***Appl. Microbiol., 3, 295 (1955).***
17. Read, R. B., Jr., and Reyes, A. L., "Variation in Plating Efficiency of Salmonellae on Eight Lots of Brilliant Green Agar." *Appl. Microbiol.*, 16, 746 (1968).
18. Dutka, B. J., and Bell, J. B., "Isolation of Salmonellae from Moderately Polluted Waters." ***Jour. Water Poll. Control Fed.,*** 45, 316 ***(1973).***
19. hlartin, W. J., and Ewing, W. H., "Prevalence of Serotypes of Salmonella." *Appl. Microbiol.*, 17, 111 (1969).
20. Calabro, J. F., ***et al."*** Recovery of Gram Negative Bacteria with Hektoen Agar." ***Jour.*** Water ***Poll. Control Fed., 44, 491 (1972).***

Appendix H
Method for the Recovery and Assay of Total Culturable Viruses from Sludge

1 Introduction

1.1. Scope

This chapter describes the method that must be followed to produce Class A sludge when virus monitoring under 40 CFR Part 503 is required. The method is designed to demonstrate that sludges meet the requirement that human enteric viruses (i.e., viruses that are transmitted via the fecal-oral route) are less than one plaque-forming unit (PFU) per 4 g of total dry solids.

1.2. Significance

More than 100 different species of pathogenic human enteric viruses may be present in raw sludge. The presence of these viruses can cause hepatitis, gastroenteritis and numerous other diseases. Hepatitis A virus and caliciviruses are the primary human viral pathogens of concern, but standard methods for their isolation and detection have not been developed. The method' detailed in this chapter detects total culturable viruses, which primarily include the enteroviruses (e.g., polioviruses, coxsackieviruses, echoviruses) and reoviruses.

1.3. Safety

The sludges to be monitored may contain pathogenic human enteric viruses. Laboratories performing virus analyses are responsible for establishing an adequate safety plan and must decontaminate and dispose of wastes according to their safety plan and all applicable regulations. Aseptic techniques and sterile materials and apparatus must be used throughout the method.

2. Sample Collection

For each batch of sludge that must be tested for viruses, prepare a composite sample by collecting ten representative samples of 100 mL each (1,000 mL total) from different locations of a sludge pile or at different times from batch or continuous flow processes. Combine and mix thoroughly all representative samples for a composite. Batch samples that cannot be assayed within 8 hours of collection must be frozen; otherwise, they should be held at 4°C until processed. If representative samples must be frozen before they can be combined, then thaw, combine and mix them thoroughly just prior to assay. Then remove a 50 mL portion from each composite sample for solids determination as described in section 3. The remaining portion is held at 4°C while the solids determination is being performed or frozen for later processing if the assay cannot be initiated within 8 hours.

3. Determination of Total Dry Solids[2]

3.1. Weigh a dry weighing pan that has been held in a desiccator and is at a constant weight. Place the 50 mL sludge portion for solids determination into the pan and weigh again.

3.2. Place the pan and its contents into an oven maintained at 103-l 05°C for at least one hour.

3.3. Cool the sample to room temperature in a desiccator and weigh again.

3.4. Repeat the drying (1 h each), cooling and weighing steps until the loss in weight is no more than 4% of the previous weight.

3.5. Calculate the fraction of total dry solids (T) using the formula:

$$R = \frac{P}{400} x 100$$

where A is the weight of the sample and dish after drying, B is the weight of the sample and dish before drying, and C is the weight of the dish. Record the fraction of dry solids (T) as a decimal (e.g., 0.04).

4. Total Culturable Virus Recovery from Sludge

4.1. In froducfion

Total culturable viruses in sludge will primarily be associated with solids. Although the fraction of virus associated with the liquid portion will usually be small, this frac-

'Method D4994-89, ASTM (1992)

*Modified from EPA/600/4-84/013(R7), September 1989 Revision (section 3). This and other cited EPA publications may be requested from the Biohazard Assessment Research Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, USA 45268.

tion may vary considerably with different sludge types. To correct for this variation, samples will first be treated to bind free virus to solids. Virus is then eluted from the solids and concentrated prior to assay.

4.2. Conditioning of Suspended Solids

Conditioning of sludge binds unadsorbed total culturable viruses present in the liquid matrix to the sludge so/ids.

Each analyzed composite sample (from the portion remaining after so/ids determination) must have an initial total dry solids content of at least 16 g. This amount is needed for positive controls and for storage of a portion of the sample at -70°C as a backup in case of procedural mistakes or sample cytotoxicity.

4.2.1. Preparation

(a) Apparatus and Materials

(a.1) Refrigerated centrifuge capable of attaining 10,000 (g and screw-capped centrifuge bottles with 100 to 1000 m L capacity.

Each bottle must be rated for the relevant centrifugal force.

(a.2) A pH meter with an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(a.3) Magnetic stirrer and stir bars.

(b) Media and Reagents

Analytical Reagent or ACS grade chemicals (unless specified otherwise) and deionized, distilled water (dH_2O) should be used to prepare all reagents. All water used must have a resistance of greater than 0.5 megohms-cm, but water with a resistance of 18 megohms-cm is preferred.

(b.1) Hydrochloric acid (HCl) — 1 and 5 M.

Mix 10 or 50 mL of concentrated HCl with 90 or 50 m L of dH_2O, respective/y.

(b.2) Aluminum chloride ($AlCl_3 . 6H_2O$) — 0.05 M.

Dissolve 12.07 g of aluminum chloride in a final volume of 7000 mL of dH_2O. Autoclave at 121°C for 15 minutes.

(b.3) Sodium hydroxide (NaOH) — 1 and 5 M.

Dissolve 4 or 20 g of sodium hydroxide in a final volume of 100 mL of dH_2O, respectively.

(b.4) Beef extract (Difco Product No. 0115-i 7-3 or equivalent).

Prepare buffered 10% beef extract by dissolving 10 g beef extract, 1.34 g Na_2HPO_4 ! $7H_20$ and 0.12 g citric acid in 100 mL of dH_2O. The pH should be about 7.0. Dissolve by stirring on a magnetic stirrer. Autoclave for 15 minutes at 121°C.

Do not use paste beef extract (Difco Laboratories Product No. 0126) for virus elution. This beef extract tends to elute cytotoxic materials from sludges.

(b.5) HOCl — 0.1%

Add 19 mL of household b/each (Clorox, The Clorox Co., or equivalent) to 981 mL of dH_2O and adjust the pH of the solution to 6-7 with 1 M HCl.

(b.6) Thiosulfate — 2% and 0.02%

Prepare a stock solution of 2% thiosulfate by dissolving 20 g of thiosulfate in a total of 1 liter of dH_20. Sterilize the solution by autoclaving at 121°C for 75 minutes. Prepare a working solution of 0.02% thiosulfate just prior to use by mixing 1 mL of 2% thiosulfate with 99 mL of sterile dH_2O.

4.2.2. Conditioning Procedure

— Figure 1 gives a flow diagram for the procedure to condition suspended solids.

(a) Calculate the amount of sample to condition.

Use a graduated cylinder to measure the volume. If the volumes needed are not multiples of 100 mL (100, 200, 300 mL, etc.), add sterile water to bring the volume to the next multiple of 100 mL. Each sample should then be aliquoted into 100 mL portions before proceeding. Samples must be mixed vigorously just before aliquoting because solids begin to settle out as soon as the mixing stops. Each aliquot should be placed into a 250 mL beaker containing a stir bar.

CAL/T/ON: A/ways avoid the formation of aerosols by slowly pouring samples down the sides of vessels.

(a.1) Calculate the amount needed to measure the endogenous total culturable virus in a composite sludge sample using the formula:

$$Xts = \frac{12}{T}$$

where Xts equals the milliliters of sample required to obtain 12 g of total solids and T equals the fraction of total dry solids (from section 3).[3]

(a.2) Calculate the amount needed for a recovery control for each sludge composite from the formula:

$$Xpc = \frac{4}{T}$$

where Xpc equals the milliliters of sample required to obtain 4 g of total solids.

Add exact/y 400 plaque forming units (PFU) of poliovirus to the recovery control sample.

---

[3]This formula is based upon the assumption that the density of the liquid in sludge is 1 g/mL. If the fraction of total dry solids is too low (e.g., less than 0.02), then the volume of sludge collected must be increased.

USPENDED SOLIDS (PER 100 mL)

↓ Mix suspension on magnetic stirrer.
Add 1 mL of 0.05 M Al Cl $_3$.

ALTED SOLIDS SUSPENSION

↓ Continue mixing suspension.
Adjust pH of salted suspension to 3.5 ± 0.1 with 5 M HC1.
Mix vigorously for 30 minutes.

H-ADJUSTED SOLIDS SUSPENSION

↓ Centrifuge salted, pH-adjusted suspension at 2,500 xg for 15 minutes at6 4°C.
Discard supernatant.
Retain solids.

OLIDS

Figure 1. Flow diagram of method for conditioning suspended solids.

(a.3) Place 30 mL of 10% buffered beef extract and 70 mL of dH_2O into a 250 mL beaker with stir bar to serve as a negative process control.

(a.4) Freeze any remaining composite sample for backup purposes.

(b) Perform the following steps on each 100 mL aliquot from steps 4.2.2a.1 to 4.2.2a.3.

(b.1) Place the beaker on a magnetic stirrer, cover loosely with aluminum foil, and stir at a speed sufficient to develop vortex. Add 1 mL of 0.05 M $AlCl_3$ to the mixing aliquot.

The final concentration of $AlCl_3$ in each aliquot is approximately 0.0005 M.

(b.2) Place a combination-type pH electrode into the mixing aliquot. Adjust the pH of the aliquot to 3.5 ± 0.1 with 5 M HCI. Continue mixing for 30 minutes.

The pH meter must be standardized at pH 7 and 4. When solids adhere to an electrode, clean it by moving up and down gent/y in the mixing aliquot.

After adjusting the pH of each sample, rinse the electrode with dH_2O and sterilize it with 0.7% HOCl for five minutes. Neutralize the HOC/ by submerging the electrode in sterile 0.02% thiosulfate for one to five minutes.

The pH of the aliquot should be checked at frequent intervals. If the pH drifts up, readjust it to 3.5 Å 0.1 with 5 M HCl. If the pH drifts down, readjust it with 5 M NaOH. Use 1 M acid or base for small adjustments. Do not allow the pH to drop below 3.4.

(b.3) Pour the conditioned aliquot into a centrifuge bottle and centrifuge at 2,500 xg for 15 minutes at 4°C.

To prevent the transfer of the stir bar into the centrifuge bottle when decanting the aliquot, ho/d another stir bar or magnet against the bottom of the beaker. So/ids that adhere to the stir bar in the beaker may be removed by manipulation with a pipette. It may be necessary to pour the aliquot back and forth several times from the centrifuge bottle to the beaker to obtain a// the so/ids in the bottle. If a large enough centrifuge bottle is available, the test sample aliquots may be combined into a sing/e bottle at this step. If there is more than one recovery control aliquot, they may also be combined into another centrifuge bottle.

(b.4) Decant the supernatant into a beaker and discard. Replace the cap onto the centrifuge bottle. Elute the solids by following the procedure described in section 4.3.

4.3. Elution of Viruses from Solids

4.3.1. Apparatus and Materials

In this and following sections only apparatus and materials which have not been described in previous sections are listed.

(a) Membrane filter apparatus for sterilization — 47 mm diameter Swinnex filter holder and 60 mL slip-tip syringe (Millipore Corp. Product No. SXOO 047 00 and Becton Dickinson Product No. 1627 or equivalent).

(b) Disc filters, 47 mm diameter — 3.0, 0.45, and 0.2 m pore size filters (Mentec America, Filterite Div., Duo-Fine series, Product No. 8025-030, 8025-034 and 8025-037 or equivalent). Filters may be cut to the proper diameter from sheet filters.

Disassemble a Swinnex filter holder. Place the filter with a 0.25 m pore size on the support screen of the filter holder and stack the remaining filters on top in order of increasing pore size. Reassemble and tighten filter holder. Wrap filter stack in foil and sterilize by autoclaving at 121 °C for 15 min.

Filters stacked in tandem as described tend to clog more slowly when turbid material is filtered through them. Prepare several filter stacks.

4.3.2. Elution Procedure

A flow diagram of the virus elution procedure is given in Figure 2.

(a) Place a stir bar and 100 mL of buffered 10% beef extract into the centrifuge bottle containing the solids (from section 4.2.2b.4).

If the test and control samples are divided into more than one centrifuge bottles, the solids should be combined at this step.

(b) Place the centrifuge bottle on a magnetic stirrer, and stir at a speed sufficient to develop a vortex for 30 min at room temperature.

To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop vortex.

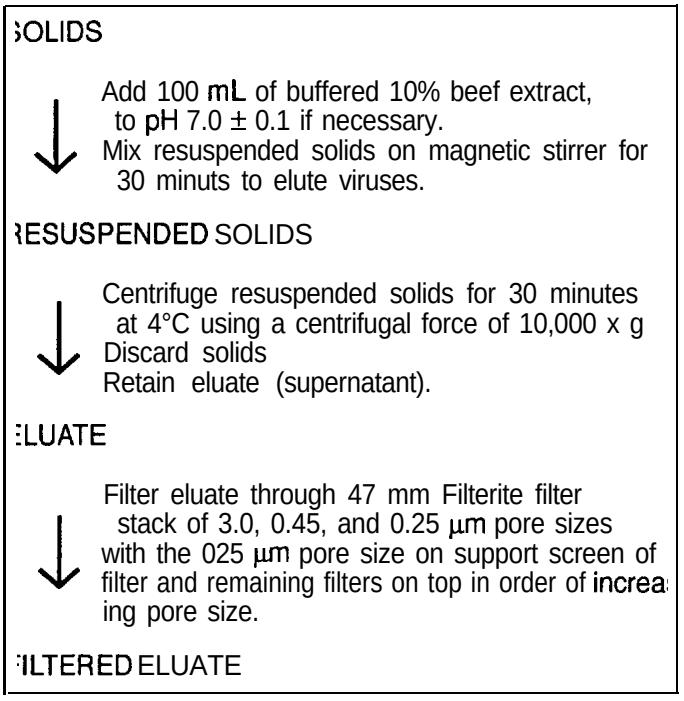


Figure 2. Flow diagram of method for elution of virus from solids.

(c) Remove the stir bar from each bottle with a long sterile forceps or a magnet retriever and centrifuge the solids-eluate mixture at 10,000 (g for 30 minutes at 4°C. Decant supernatant fluid (eluate) into a beaker and discard the solids.

Determine if the centrifuge bottle is appropriate for the centrifugal force that will be applied.

Cen trifuga tion at 1 0, 000 (g is normally required to clarify the sludge samples sufficiently to force the resulting supematant through the filter stacks.

(d) Place a filter holders that contains filter stacks (from section 4.3.1 b) onto a 250 mL Erlenmeyer receiving flask. Load 50 mL syringes with the supernatants from step 4.3.2c. Place the tip of the syringe into the filter holder and force the supernatant through the filter stacks into 250 mL receiving flasks.

Prior to use, pass 15 mL of 3% beef extract through each filter holder to minimize non-specific adsorption of viruses. Prepare 3% beef extract by mixing 4.5 mL of 10% beef extract and 10.5 mL of dH_2O. Take care not to break off the tip of the syringe and to minimize pressure on the receiving flask because such pressure may crack or topple the flask. If the filter stack begins to clog badly, empty the loaded syringe into the beaker containing unfiltered eluate, fill the syringe with air, and inject air into filter stack to force residual eluate from the filters. Continue the filtration procedure with another filter holder and filter stack. Discard contaminated filter holders and filter stacks. This procedure may be repeated as often as necessary to filter the entire volume of supernatant. Disassemble each filter holder and examine the bottom 0.25 m filters to be certain they have not ruptured. If a bottom filter has ruptured, repeat the step with new filter holders and filter stacks.

Proceed immediately to section 4.4.

4.4 Organic Flocculation

This organic flocculation concentration procedure (Katzenelson et al., 1976) is used to reduce the number of cell cultures needed for assays by concentrating total culturable viruses in the eluate. The step significantly reduces costs associated with labor and materials.

Floc formation capacity of the beef extract reagent must be pretested, Because some beef extract lots may not produce sufficient floc, each new lot must be pretested to determine virus recovery. This may be performed by spiking 100 mL of dH_2O with a known amount of poliovirus in the presence of a 47 mm nitrocellulose filter. This sample should be conditioned using section 4.2 above to bind virus to the filter. Virus should then be eluted from the filter using the procedure in section 4.3, and concentrated and assayed using the following procedures. Any lot of beef extract not giving a overall recovery of at least 50% should not be used.

4.4.1 Media and Reagents

In this and following sections only media and reagents which have not been described in previous sections are listed.

(a) Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 7H_2O$) — 0.15 M.

Dissolve 40.2 g of sodium phosphate in a final volume of 1000 mL. Autoclave at 121 °C for 75 minutes.

4.4.2 Virus Concentration Procedure

A flow diagram for the virus concentration procedure is given in Figure 3.

(a) Pour the filtered eluates from the test sample, recovery control and negative process control from section 4.3.24 into graduated cylinders, and record their volumes. Transfer the samples into separate 600 mL beakers and cover them loosely with aluminum foil.

(b) For every 3 mL of beef extract eluate, add 7 mL of dH_2O to the 600 mL beakers. Add stir bars to each beaker.

The concentration of beef extract is now 3%. This dilution is necessary because 10% beef extract often does not process well by the organic flocculation concentration procedure.

(c) Record the total volume of the diluted eluates. Place the beakers onto a magnetic stirrer, cover loosely with aluminum foil, and stir at a speed sufficient to develop vortex.

ILTERED ELUATE

↓ Add sufficient volume of dH_2O TO Filtered eluate to reduce concentration of beef extract from 10% to 3%. Record total volume of the diluted beef extract.

ILUTED, FILTERED ELUATE

↓ Mix diluted eluate on a magnetic stirrer. Adjust the pH of the eluate to 3.5 ± 0.1 with 1 M HC1. A precipitate (floc) will form. Continue mixing for 30 minutes.

LOCCULATED ELUATE

↓ Centrifuge flocculated eluate at 2,500 xg for 15 minutes at 4°C. Discard supernatant. Retain floc.

'LOC FROM ELUATE

↓ Add 0.15 M Na_2HPO_4 to floc, using 1/20th of the recorded volume of the diluted 3% beef extract. Mix suspended floc on magnetic stirrer until floc dissolves. Adjust to a pH of 7.0 to 7.5.

ISSOLVED FLOC

↓ See section 5 for virus assay procedure.

SSAY DISSOLVED FLOC FOR VIRUSES

Figure 3. Flow diagram of method for concentration of viruses from beef extract.

To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop vortex.

(d) For each diluted, filtered beef extract, insert a sterile combination-type pH electrode and then add 1 M HCl slowly until the pH of the extract reaches 3.5 ± 0.1. Continue to stir for 30 minutes at room temperature.

The pH meter must be standardized at pH 4 and 7. Sterilize the electrode by treating it with 0.1% HOC/ for five minutes. Neutralize the HOC/ by treating the electrode with 0.02% sterile thiosulfate for one to five minutes.

A precipitate will form. If the pH is accidentally reduced below 3.4, add 1 M NaOH until it reaches 3.5 ± 0.7. Avoid reducing the pH below 3.4 because some inactivation of virus may occur.

(e) Pour the contents of each beaker into 1,000 mL centrifuge bottles. Centrifuge the precipitated beef extract suspensions at 2,500 (g for 15 minutes at 4°C. Pour off and discard the supernatants.

To prevent the transfer of the stir bar into a centrifuge bottle, ho/d another stir bar or magnet against bottom of the beaker when decanting contents.

(f) Place stir bars into the centrifuge bottles that contains the precipitates. To each, add a volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ equal to exactly 1120 of the volume recorded in step 4.4.2c. Place the bottles onto a magnetic stirrer, and stir slowly until the precipitates have dissolved completely.

Support the bottles as necessary to prevent toppling. Avoid foaming which may inactivate or aerosolize viruses. The precipitates may be partially dissipated with sterile spatulas before or during the stirring procedure.

(g) Measure the pH of the dissolved precipitates.

If the pH is above or below 7.0-7.5, adjust to that range with either 1 M HCl or 1 M NaOH.

(h) Freeze exactly one half of the dissolved precipitate test sample (but not the positive and negative controls) at -70°C. This sample will be held as a backup to use should the sample prove to be cytotoxic. Record the remaining test sample volume (this volume represents 6 g of total dry solids). Refrigerate the remaining samples immediately at 4°C until assayed in accordance with the instructions given in section 5 below.

If the virus assay cannot be undertaken within eight hours, store the remaining samples at -70°C.

5. Assay for Plaque-forming Viruses[4]

5.1 Introduction

This section outlines procedures for the detection of viruses in sludge by use of the plaque assay system. The system uses an agar medium to localize virus growth following attachment of infectious virus particles to a cell monolayer. Localized lesions of dead cells (plaques) developing some days after viral infection are visualized with the vital stain, neutral red, which stains only live cells. The number of circular unstained plaques are counted and reported as plaque forming units, whose number is proportional to the amount of infectious virus particles inoculated.

The detection methodology presented in this section is geared towards laboratories with a small-scale virus assay requirement. Where the quantities of cell cultures, media and reagents set forth in the section are not sufficient for processing the test sample concentrates, the prescribed measures may be increased proportionally to meet the demands of more expansive test regimes.

'Modified for EPA/600/4-84/013(R11), March 1988 Revision

5.2 *Plaque Assay Procedure*

5.2.1 Apparatus and materials.

(a) Waterbath set at 50 ± 1 °C.

Used for maintaining the agar temperature (see section 5.2.2j).

5.2.2 Media and Reagents.

(a) ELAH — 0.65% lactalbumin hydrolysate in Earle's base.

Dissolve 6.5 g of tissue culture, highly soluble grade lactalbumin hydrolysate (Gibco BRL Product No. 11800 or equivalent) in 1 L of Earle's base (Gibco BRL Product No. 14015 or equivalent) prewarmed to 50-60%C. Sterilize ELAH through a 0.22 m filter stack and store for up to two months at 4°C.

(b) Wash medium — Add 1 mL of penicillin-streptomycin stock (see section 6.4.2e.1 for preparation of antibiotic stocks), 0.5 mL of tetracycline stock and 0.2 mL of fungizone stock per liter to ELAH immediately before washing of cells.

(c) HEPES — 1 M (Sigma Chemical Product No. H-3375 or equivalent).

Prepare 50 m L of a 1 M solution by dissolving 11.92 g of HEPES in a final volume of 50 m L dH_2O. Sterilize by autoclaving at 121°C for 15 min.

(d) Sodium bicarbonate ($NaHCO_3$) — 7.5% solution.

Prepare 50 m L of a 7.5% solution by dissolving 3.75 g of sodium bicarbonate in a final volume of 50 mL dH_2O. Sterilize by filtration through a 0.22 m filter.

(e) Magnesium chloride ($MgCl_2$! $6H_2O$) — 1 .O% solution.

Prepare 50 mL of a 1 .O% solution by dissolving 0.5 g of magnesium chloride in a final volume of 50 mL dH_2O. Sterilize by autoclaving at 121 °C for 15 min.

(f) Neutral red solution — 0.333%, 100 mL volume (GIBCO BRL Product No. 630-5330 or equivalent).

Procure one 100 m L bottle.

Some neutral red solutions are cytotoxic. All new solutions should be tested prior to their use for assaying sludge samples, Testing may be performed by assaying a stock of poliovirus with known titer using this plaque assay procedure.

(g) Bacto skim milk (Difco Laboratories Product No. 0032-01 or equivalent).

Prepare 100 mL of 10% skim milk in accordance with directions given by manufacturer.

(h) Preparation of Medium 199.

The procedure described is forpreparation of 500 mL of Medium 199 (GIBCO BRL Product No. 400-1100 or equivalent) at a 2X concentration. This procedure will prepare sufficient medium for at least fifty 6 oz glass bottles or eighty 25 cm^2 plastic flasks.

(h.1) Place a three inch stir bar into a one liter flask. Add the contents of a 1 liter packet of Medium 199 into the flask. Add 355 mL of dH_2O. Rinse medium packet with three washes of 20 mL each of dH_2O and add the washes to the flask.

Note that the amount of dH_2O is 5% less than desired final volume of medium.

(h.2) Mix on a magnetic stirrer until the medium is completely dissolved. Filter the reagent under pressure through a filter stack (see section 6.2.6).

Test each lot of medium to confirm sterility before the lot is used (see section 6.5). Each batch may be stored for two months at 4°C.

(i) Preparation of overlay medium for plaque assay.

The procedure described is for preparation of 100 mL of overlay medium and will prepare sufficient media for at /east ten 6 oz glass bottles or twenty 25 oz plastic f/asks when mixed with the agar prepared in section 5.2.2j.

(i.1) Add 79 mL of Medium 199 (2X concentration) and 4 mL of serum to a 250 mL flask.

(i.2) Add the following to the flask in the order listed, with swirling after each addition: 6 mL of 7.5% $NaHCO_3$, 2 mL of 1% $MgCl_2$, 3 mL of 0.333% neutral red solution, 4 mL of 1 M HEPES, 0.2 mL of penicillin-streptomycin stock (see section 6.4.2e for a description of antibiotic stocks), 0.1 mL of tetracycline stock, and 0.04 mL of fungizone stock.

(i.3) Place flask with overlay medium in waterbath set at 36 ± 1°C.

(j) Preparation of overlay agar for plaque assay.

(j.l) Add 3 g of agar (Sigma Chemical Product No. A-9915 or equivalent) and 100 m L of dH_2O to a 250 mL flask. Melt by sterilizing the agar solution in an autoclave at 121 °C for 15 min.

(j.2) Cool the agar to 50°C in waterbath set at 50 ± 1°C.

(k) Preparation of agar overlay medium.

(k.1) Add 2 mL of 10% skim milk to overlay medium prepared in section 5.2.2i.

(k.2) Mix equal portions of overlay medium and agar by adding the medium to the agar flask.

To prevent solidification of the liquified agar, limit the portion of agar overlay medium mixed to that volume which can be dispensed in 10 min.

5.2.3 Procedure for Inoculating Test Samples.

Section 6.6 provides the procedures for the preparation of cell cultures used for the virus assay in this section.

Cell cultures used for virus assay are generally found to be at their most sensitive level between the third and sixth days after initiation. Those older than seven days should not be used.

(a) Decant and discard the growth medium from previously prepared cell culture test vessels.

To prevent splatter, a gauze-covered beaker may be used to collect spent medium.

The medium is changed from one to four hours before cultures are to be inoculated and carefully decanted so as not to disturb the cell monolayer.

(b) Replace discarded medium with an equal volume of wash medium (from section 5.2.2b) on the day the cultures are to be inoculated.

To reduce shock to cells, prewarm the maintenance medium to 36.5 ± 1 °C before placing it onto the cell monolayer.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the maintenance medium to the side of cell culture test vessel opposite the cell monolayer.

(c) Identify cell culture test vessels by coding them with an indelible marker. Return the cell culture test vessels to a 36.5 ± 1 °C incubator and hold at that temperature until the cell monolayers are to be inoculated.

(d) Decant and discard the wash medium from cell culture test vessels.

Do not disturb the cell monolayer.

(e) Inoculate BGM cultures with the test sample and positive and negative process control samples from section 4.4.2h. Divide each sample onto a sufficient number of BGM cultures to ensure that the inoculum volume is no greater than 1 mL for each 40 cm^2 of surface area. Use Table 1 as a guide for inoculation size.

Avoid touching either the cannula or the pipetting device to the inside rim of the cell culture test vessels to avert the possibility of transporting contaminants to the remaining culture vessels.

If the samples are frozen, thaw them rapidly by placing them in warm wafer. Samples should be shaken during the thawing process and removed from the warm wafer as soon as the last ice crystals have dissolved.

(e.1) Inoculate BGM cultures with the entire negative process control sample using an inoculum volume per vessel that is appropriate for the vessel size used.

Table 1. Guide for Virus Inoculation, Suspended Cell Concentration and Overlay Volume of Agar Medium

Vessel Type	Volume of Virus Inoculum (mL)	Volume of Agar Overlay Medium (mL)	Total Number of Cells
1 oz glass bottle'	*V=0.8' P' C* 0.1	5	1×10^7
25 cm^2 plastic flask	0.1-0.5	10	2×10^7
6 oz glass bottle	0.5-l .0	20	4×10^7
75 cm^2 plastic flask	1 B-2.0	30	$6 \times 10'$

'Size is given in oz only when it is commercially designated in that unit.

(e.2) Inoculate two BGM cultures with an appropriate volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ preadjusted to pH 7.0-7.5 and spiked with 20-40 PFU of poliovirus. These cultures will serve as a culture sensitivity control.

(e.3) Remove a volume of the test sample concentrate exactly equal to 1/6th (i.e., 1 g of total dry solids) of the volume recorded in section 4.4.2h. Spike this subsample with 20-40 PFU of poliovirus. Inoculate the subsample onto one or more BGM cultures using a inoculum volume per vessel that is appropriate for the vessel size used. These cultures will serve as controls for cytotoxicity (see section 5.2.5b).

(e.4) Inoculate BGM cultures with the entire recovery control sample using an inoculum volume per vessel that is appropriate for the vessel size used.

(e.5) Record the volume of the remaining 5/6th portion of the test sample. This remaining portion represents a total dry solids content of 5 g. Inoculate the entire remaining portion (even if diluted to reduce cytotoxicity) onto BGM cultures using an inoculum volume per vessel that is appropriate for the vessel size used. Inoculation of the entire volume is necessary to demonstrate a virus density level of less than 1 PFU per 4 g total dry solids.

(f) Rock the inoculated cell culture test vessels gently to achieve uniform distribution of inoculum over the surface of the cell monolayers. Place the cell culture test vessels on a level stationary surface at room temperature (22-25%) so that the inoculum will remain distributed evenly over the cell monolayer.

(g) Incubate the inoculated cell cultures at room temperature for 80 min to permit viruses to adsorb onto and infect cells and then proceed immediately to section 5.2.4.

It may be necessary to rock the vessels every 15-20 min during the 80 min incubation to prevent cell death in the middle of the vessels from dehydration.

5.2.4 Procedure for Overlaying Inoculated Cultures with Agar

If there is a likelihood that a test sample will be toxic to cell cultures, the cell monolayer should be treated in accordance with the method described in section 5.2.5b.

(a) To each cell culture test vessel, add the volume of warm (42-46°C) agar overlay medium appropriate for the cell surface area of the vessels used (see Table 1).

The preparation of the overlay agar and the agar overlay medium must be made far enough in advance so that they will be at the right temperature for mixing at the end of the 80 min inoculation period.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the agar overlay medium to the side of the cell culture test vessel opposite the cell monolayer.

(b) Place cell culture test vessels, monolayer side down, on a level stationary surface at room temperature (22-25°C) so that the agar will remain evenly distributed as it solidifies. Cover the vessels with a sheet of aluminum foil, a tightly woven cloth, or some other suitable cover to reduce the light intensity during solidification and incubation. Neutral red can damage or kill tissue culture cells by light-induced crosslinking of nucleic acids.

Care must be taken to ensure that all caps on bottles and f/asks are tight; otherwise, the gas sea/ will not be complete and an erroneous virus assay will result.

Agar will fully solidify within 30 min.

(c) After 30 min, invert the cell culture test vessels and incubate them covered in the dark at 36.5 ± 1°C.

5.2.5. Plaque Counting Technique

(a) Count, mark and record plaques in cell culture test vessels on days one, two, three, four after adding the agar overlay medium. Plaques should be counted quickly using a lightbox (Baxter Product No. 85080-l or equivalent) in a darkened room. Most plaques should appear within 1 week.

Depending on the virus density and virus types present in the inoculated sample, rescheduling of virus counts at plus or minus one day may be necessary. Virus titers are calculated from the total plaque count. Note that not all plaques will be caused by viruses.

(b) Determine if samples are cytotoxic by macroscopic examination of the appearance of the cell culture monolayer (compare negative, positive and recovery controls from section 5.2.3e with spiked and unspiked test samples) after one to four days of incubation at 36.5 ± 1 °C. Samples show cytotoxicity if cell death is observed on test and recovery control samples prior to its development on positive controls. Cytotoxicity should be suspected when the agar color is more subdued, generally yellow to yellow-brown. This change in color results in a mottled or blotchy appearance instead of the evenly diffused "reddish" color observed in "healthy" cell monolayers. Cytotoxicity may also cause viral plaques to be reduced in number or to be difficult to distinguish from the surrounding monolayer. To determine if this type of cytotoxicity is occurring, compare the two types of positive controls (section 5.2.3e). If samples are cytotoxic, do not proceed to the next steps. Re-assay a small amount of the remaining sample using 1:2, 1:4 and 1:8 dilutions. Then re-assay the remaining sample as specified in section 5.2.3 using the dilution which removes cytotoxicity and the specified number of flasks times the reciprocal of the dilution.

A small amount of sample may be tested for cytotoxicity prior to a full assay.

(c) Examine cell culture test vessels as in step 5.2.5a on days six, eight, twelve and sixteen.

If no new plaques appear at 1 6 days, proceed with step 5.2.6; otherwise continue to count, mark and record plaques every two days until no new plaques appear between *counts and then proceed with step 5.2.6.*

Inoculated cultures should always be compared to uninoculated control cultures so that the deterioration of the cell monolayers is not recorded as plaques.

If negative process controls develop plaques or if positive controls fail to develop plaques, stop a// assays until the source of the problem is corrected.

Samples giving plaque counts that are greater than 2 plaques per cm² should be diluted and replated.

5.2.6. Virus Plaque Confirmation Procedure

The presence of virus in plaques must be confirmed for all plaques obtained from sludge samples. Where more than ten plaques are observed, it is allowable to confirm at least ten well-separated plaques per sample or 10% of the plaques in a sample, whichever is greater. Flasks may be discarded after samples are taken for plaque confirmation.

(a) Apparatus, Materials and Reagents

(a.1) Pasteur pipettes, disposable, cotton plugged — 229 mm (9 inches) tube length and rubber bulb — 1 mL capacity.

Flame each pipette gently about 2 cm from end of the tip until the tip bends to an approximate angle of 45%. Place the pipettes into a 4 liter beaker covered with aluminum foil and sterilize by autoclaving or by dry heat.

(a.2) 16 x 150 mm Cell Culture Tubes Containing BGM Cells.

See section 6.6 for the preparation of cell culture tubes.

(a.3). Tissue culture roller apparatus — 1/5 rpm speed (New Brunswick Scientific Product No. TC-1 or equivalent) with culture tube drum for use with roller apparatus (New Brunswick Scientific Product No. ATC-TT16 or equivalent).

(a.4) Freezer vial, screw-capped (with rubber insert) or cryogenic vial — 0.5-l dram capacity.

(b) Procedure for obtaining viruses from plaque.

In addition to plaques from sludge samples, perform the procedure on at least three negative regions of negative process control f/asks and at /east three plaques from positive control f/asks.

(b.1) Place a rubber bulb onto the upper end of a cotton-plugged Pasteur pipette and then remove the screw-cap or stopper from a plaque bottle.

(b.2) Squeeze the rubber bulb on the Pasteur pipette to expel the air and penetrate the agar directly over the edge of a plaque with the tip of the pipette. Gently force the tip of the pipette through the agar to the surface of the vessel, and scrape some of the cells from the edge of the plaque.

Repeatedly scratch the surface and use gent/e suction to insure that virus-cell-agar plug enters the pipette.

(b.3) Remove the pipette from the plaque bottle and tightly replace the cap or stopper.

(c) Procedure for inoculating cell cultures with agar plugs from negative control samples and from plaques.

(c.1) Prepare plaque conformation maintenance medium by adding 5 mL of serum and 5 mL of dH_2O per 90 mL of wash medium (section 5.2.2b) on day samples are to be tested.

(c.2) Pour the spent medium from cell culture tubes and discard the medium. Replace the discarded medium with 2 mL of the plaque conformation maintenance medium. Label the tubes with sample and plaque isolation identification information.

To prevent splatter, a gauze-covered beaker may be used to collect spent medium.

To reduce shock to cells, warm the maintenance medium to 36.5 ± 1 °C before p/acing on cell monolayer.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the maintenance medium to the side of cell culture test tube opposite the cell monolayer. Note that cells will be on/y on the bottom inner surface of the culture tube relative to their position during incubation.

(c.3) Remove the cap from a cell culture tube and place the tip of a Pasteur pipette containing the agar plug from section 5.2.6b.3 into the maintenance medium in the cell culture tube. Force the agar plug from the Pasteur pipette by gently squeezing the rubber bulb. Withdraw and discard the pipette, and replace and tighten down the screw-cap on the culture tube.

Tilt cell culture tube as necessary to facilitate the procedure and to avoid scratching the cell sheet with the pipette.

Squeeze bulb repeated/y to wash contents ofpipetfe info the maintenance medium.

(c.4) Place the cell culture tubes in the drum used with the tissue culture roller apparatus. Incubate the cell cultures at 36.5 ± 1°C while rotating at a speed of 1/5 rpm. Examine the cells daily microscopically for 1 week for evidence of cytopathic effects (CPE).

CPE may be identified as cell disintegration or as changes in cell morphology. Rounding-up of infected cells is a typical effect seen with enteric virus infections. However, uninfected cells round up during mitosis and a sample should not be considered positive unless there are significant clusters of rounded-up cells over and beyond what is observed in the uninfected controls. *If there is any doubt about the presence of CPE or if CPE appears late (i.e., on day 6 or 7), the conformation process should be repeated by transferring 0.2 mL of the medium in the culture tube to a freshly prepared tube.*

Incubation of BGM cells in roller apparatus for periods greater than 1 week is not recommended as cells under these conditions fend to die-off if he/d longer.

If tubes receiving agar plugs from negative controls develop CPE or tubes receiving agar plugs from positive controls fail to develop CPE, stop all assays until the source of the failure is identified and corrected.

Tubes developing CPE may be stored in a -70°C freezer for additional optional tests (e.g., the Lim Benyesh-Melnick identification procedure.[5]

(c.5) Determine the fraction of confirmed plaques (C) for each sludge sample tested. Calculate "C" by dividing the number of tubes inoculated with agar plugs from plaques that developed CPE by the total number of tubes inoculated (i.e., if CPE was obtained from 17 of 20 plaques, C = 0.85).

5.2.7 Calculation of virus titer.

If more than one composite sample was assayed, average the titer of all composite samples and report the average titer and the standard deviation for each lot of sludge tested.

(a) If the entire remaining portion of a test sample was inoculated onto BGM cultures as described in section 5.2.3e.5, calculate the virus titer (V) in PFU per 4 g of total dry solids according to the formula:

$$V = 0.8 \times P \times C$$

where P is the total number of plaques in all test vessels tor that sample and C equals the fraction of confirmed plaques.

(b) If the sample was diluted due to high virus levels (e.g., when the virus density of the input to a process is

[5]For more information see EPA/600/4-84/013(R12), May 1986 Revision

being determined; see section 5.2.5c), calculate the virus titer (V) in PFU per 4 g total dry solids with the formula:

$$V = 0.8 \times \frac{P}{1} \times D \times C$$

where P is the total number of plaques in all test vessels for dilution series, I is the volume (in mL) of the dilution inoculated, **D** is reciprocal of the dilution made on the inoculum before plating, S is the volume of the remaining portion of the test sample (as recorded in section 5.2.3e.5) and C is the fraction of confirmed plaques.

5.2.8 Calculate the percent of virus recovery (R) using the formula:

$$R = \frac{P}{400} \times 100$$

where P is the total number of plaques on all test vessels inoculated with the recovery control.

6. Cell Culture Preparation and Maintenance[6]

6.1. Introduction

This section outlines procedures and media for culturing the Buffalo green monkey (BGM) cell line and is intended for the individual who is experienced in cell culture preparation. BGM cells are a continuous cell line derived from African Green monkey kidney cells. The characteristics of this line were described by Barron et *al.* (1970). Use of BGM cells for recovering viruses from environmental samples was described by Dahling et *al.* (1974). The media and methods recommended are the results of the BGM cell line optimization studies by Dahling and Wright (1986). The BGM cell line can be obtained by qualified laboratories from the Biohazard Assessment Research Branch, National Exposure Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio, USA 45268. Although BGM cells will not detect all enteric viruses that may be present in sludges, the use of this cell line alone is sufficient to meet the requirements of 40 CFR Part 503.

6.2. Apparatus and Materials

6.2.1. Glassware, Pyrex (Corning Product No. 1395 or equivalent).

Storage vessels must be equipped with airtight closures.

6.2.2. Autoclavable inner-braided tubing with metal quick- connect connectors or with screw clamps for connecting tubing to equipment to be used under pressure.

Quick-connect connectors can be used only after equipment has been properly adapted.

6.2.3. Positive pressure air, nitrogen or 5% CO, source equipped with pressure gauge.

Pressure sources from laboratory air lines and pumps must be equipped with an oil filter. The source musf not deliver more pressure to the pressure vessel than is recommended by manufacturer.

6.2.4. Dispensing pressure vessel — 5 or 20 liter capacity (Millipore Corp. Product No. XX67 OOP 05 and XX67 OOP 20 or equivalent).

6.2.5. Disc filter holders — 142 mm or 293 mm diameter (Millipore Corp. Product No. YY30 142 36 and YY30 293 16 or equivalent).

Use only pressure type filter holders.

6.2.6. Sterilizing filter stacks — 0.22 m pore size (Millipore Corp. Product No. GSWP 142 50 and GSWP 293 25 or equivalent). Fiberglass prefilters (Millipore Corp. Product No. AP15 142 50 or AP15 293 25 and AP20 142 50 or AP20 293 25 or equivalent).

StackAP20 and AP15 prefilters and 0.22 μm membrane filter into a disc filter holder with AP20 prefilter on top and 0.22 m membrane filter on bottom.

Always disassemble the filter stack after use to check the integrity of the 0.22 m filter. Refilter any media filtered with a damaged stack.

6.2.7. Positively-charged cartridge filter — 10 inch (Zeta plus TSM, Cuno Product No. 45134-01-600P or equivalent). Holder for cartridge filter with adaptor for 10 inch cartridge (Millipore Corp. Product No. YY16 012 00 or equivalent).

6.2.8. Culture capsule filter (Gelman Sciences Product No. 12140 or equivalent).

6.2.9. Cell culture vessels — Pyrex, soda or flint glass or plastic bottles and flasks or roller bottles (e.g., Brockway Product No. 1076-09A, 1925-02, Corning Product No. 25100-25, 2511 O-75, 25120-I 50, 25150-I 750 or equivalent).

Vessels must be made from clear glass or plastic to allow observation of the cultures and be equipped with airtight closures. Plastic vessels must be treated by the manufacturer to allow cells to adhere properly.

6.2.10. Screw caps, black with rubber liners (Brockway Product No. 24-414 for 6 oz bottles[7] or equivalent).

[6]Modified from EPA/600/4-84/013(R9), January 1987 Revision

[7]Size is given in oz only when it is commercially designated in that unit.

Caps for larger culture bottles usually supplied with bottles.

6.2.11. Roller apparatus Bellco Product No. 7730 or equivalent).

6.2.12. Incubator capable of maintaining the temperature of cell cultures at 36.5 ± 1 °C.

6.2.13. Waterbath, equipped with circulating device to assure even heating at 36.5 ± 1 °C.

6.2.14. Light microscope, with conventional light source, equipped with lenses to provide 40X, 100X, and 400X total magnification.

6.2.15. Inverted light microscope equipped with lenses to provide 40X, 100X, and 400X total magnification.

6.2.16. Cornwall syringe pipettors, 2, 5 and 10 mL sizes (Cut-tin Matheson Scientific Product No. 221-861, 221-879, and 221-887 or equivalent).

6.2.17. Brewer-type pipetting machine (Curtin Matheson Scientific Product No. 138-l 07 or equivalent).

6.2.18. Phase contrast counting chamber (hemocytometer) (Cur-tin Matheson Scientific Product No. 158-501 or equivalent).

6.2.19. Conical centrifuge tubes, sizes 50 mL and 250 mL.

6.2.20. Rack for tissue culture tubes (Bellco Product No. 2028 or equivalent).

6.2.21. Bottles, aspirator-type with tubing outlet, size 2,000 mL.

Bottles for use with pipetting machine.

6.2.22. Storage vials, size 2 mL.

Via/s must withstand temperatures to -70°C.

6.3. Media and Reagents

6.3.1. Sterile fetal calf, gammagobulin-free newborn calf or iron-supplemented calf serum, certified free of viruses, bacteriophage and mycoplasma (GIBCO BRL or equivalent).

Test each lot of serum for cell growth and toxicity before purchasing. Serum should be stored at -20°C for long-term storage. Upon thawing, each bottle should be heat-inactivated at 56°C for 30 min and stored at 4°C for short term use.

6.3.2. Trypsin, 1:250 powder (Difco Laboratories Product No. 0152-l 5-9 or equivalent) or trypsin, 1:300 powder (BBL Microbiology Systems Product No. 12098 or equivalent).

6.3.3. Sodium (tetra) ethylenediamine tetraacetate powder (EDTA), technical grade, (Fisher Scientific Product No. S657-500 or equivalent).

6.3.4. Thioglycollate medium (Difco Laboratories Product No. 0257-01-g or equivalent).

6.3.5. Fungizone (amphotericin B, Sigma Chemical Product No. A-9528 or equivalent), Penicillin G (Sigma Chemical Product No. P- 3032 or equivalent), dihydrostreptomycin sulfate (ICN Biomedicals Product No. 100556 or equivalent), and tetracycline (ICN Biomedicals Product No. 103011 or equivalent).

Use antibiotics of at /east tissue culture grade.

6.3.6. Eagle's minimum essential medium (MEM) with Hanks' salts and L-glutamine, without sodium bicarbonate (GIBCO BRL Product No. 41 O-l 200 or equivalent).

6.3.7. Leibovitz's L-l 5 medium with L-glutamine (GIBCO BRL Product No. 430-1300 or equivalent).

6.3.8. Trypan blue (Sigma Chemical Product No. T-6146 or equivalent).

Note: This chemical is on the EPA list of proven or suspected carcinogens.

6.3.9. Dimethyl sulfoxide (DMSO; Sigma Chemical Product No. D-2650 or equivalent).

6.3.10. Mycoplasma testing kit (Irvine Scientific Product No. T500-000 or equivalent).

6.4 Preparation of Cell Culture Media

6.4.1. General Principles

(a) Equipment care — Carefully wash and sterilize equipment used for preparing media before each use.

(b) Disinfection of work area — Thoroughly disinfect surfaces on which the medium preparation equipment is to be placed. Many commercial disinfectants do not adequately kill total culturable viruses. To ensure thorough disinfection, disinfect all surfaces and spills with either a solution of 0.5% (5 g per liter) I, in 70% ethanol or 0.1% HOCI.

(c) Aseptic technique — Use aseptic technique when preparing and handling media or medium components.

(d) Dispensing filter-sterilized media-To avoid post-filtration contamination, dispense filter-sterilized media into storage containers through clear glass filling bells in a microbiological laminar flow hood. If a hood is unavailable, use an area restricted solely to cell culture manipulations.

(e) Coding media -Assign a lot number to and keep a record of each batch of medium or medium components

prepared. Place the lot number, the date of preparation, the expiration date, and the initials of the person preparing the medium on each bottle.

(f) Sterility test — Test each lot of medium and medium components to confirm sterility as described in section 6.5 before the lot is used for cell culture.

(g) Storage of media and medium components — Store media and medium components in clear airtight containers at 4°C or -20°C as appropriate.

(h) Sterilization of $NaHCO_3$-containing solutions — Sterilize media and other solutions that contain $NaHCO_3$ by positive pressure filtration.

Negative pressure filtration of such solutions increases the pH and reduces the buffering capacity

6.4.2. Media Preparation Recipes

(a) Sources of cell culture media.

Commercially prepared liquid cell culture media and medium components are available from several sources. Cell culture media can also be purchased in powder form that requires only dissolution in dH_2O and sterilization. Media from commercial sources are quality controlled. The conditions specified by the supplier for storage and expiration dates should be strictly observed. However, media can also be prepared in the laboratory directly from chemicals. Such preparations are labor intensive, but allow quality control of the process at the level of the preparing laboratory.

(b) Procedure for the preparation of EDTA-trypsin.

The procedure described is for the preparation of 10 liters of EDTA-trypsin reagent. It is used to dislodge cells attached to the surface of culture bottles and f/asks. This reagent, when stored at 4°C, retains its working strength for at least four months. The amount of reagent prepared should be based on projected usage over a four month period.

(b.1) Add 30 g of trypsin (1:250) or 25 g of trypsin (1:300) and two liters of dH_2O to a six liter flask containing a three inch stir bar. Place the flask onto a magnetic stirrer and mix the trypsin solution rapidly for a minimum of one hour.

Trypsin remains cloudy.

(b.2) Add four liters of dH_2O and a three-inch stir bar into 20 liter clear plastic carboy. Place the carboy onto a magnetic stirrer and stir at a speed sufficient to develop a vortex while adding the following chemicals: 80 g NaCl, 12.5 g EDTA, 50 g dextrose, 11.5 g $Na_2HPO_4 \cdot 7H_2O$, 2.0 g KCl, and 2.0 g KH_2PO_4.

Each chemical does not have to be completely dissolved before adding the next one.

(b.3) Add four more liters of dH_2O to carboy.

Continue mixing until all chemicals are completely dissolved.

(b.4) Add the two liters of trypsin from step 6.4.2b.1 to the prepared solution in step 6.4.2b.3 and mix for a minimum of one hour. Adjust the pH of the EDTA-trypsin reagent to 7.5 - 7.7.

(b.5) Filter reagent under pressure through a disc filter stack and store the filtered reagent in tightly stoppered or capped containers at 4°C.

The cartridge prefilter (section 6.2.7) can be used in line with the culture capsule sterilizing filter (section 6.2.8) as an alternative to a filter stack (section 6.2.6).

(c) Procedure for the preparation of MEM/L-15 medium.

The procedure described is for preparation of 10 liters of MEM/L-15 medium.

(c.1) Place a three inch stir bar and four liters of dH_2O into 20 liter carboy.

(c.2) Place the carboy onto a magnetic stirrer. Stir at a speed sufficient to develop a vortex and then add the contents of a five liter packet of L-15 medium to the carboy. Rinse the medium packet with three washes of 200 mL each of dH_2O and add the rinses to the carboy.

(c.3) Mix until the medium is evenly dispersed.

L-15 medium may appear cloudy as it need not be totally dissolved before proceeding to step 6.4.2c.4.

(c.4) Add three liters of dH_2O to the carboy and the contents of a five liter packet of MEM medium to the carboy. Rinse the MEM medium packet with three washes of 200 mL each of dH_2O and add the rinses to the carboy. Add 800 mL of dH_2O and 7.5 g of $NaHCO_3$ and continue mixing for an additional 60 min.

(c.5) Transfer the MEM/L-15 medium to a pressure can and filter under positive pressure through a 0.22 µm sterilizing filter. Collect the medium in volumes appropriate for the culturing of BGM cells (e.g., 900 mL in a 1 liter bottle) and store in tightly stoppered or capped containers at 4°C.

Medium may be stored for periods of up to two months.

(d) Procedure for preparation of trypan blue solution.

The procedure described is for the preparation of 100 mL of trypan blue solution. It is used in the direct determination of the viable cell counts of the BGM stock cultures. As trypan blue is on the EPA suspect carcinogen list, particular care should be taken in its preparation and use so as to avoid skin contact or inhalation. The wearing of rubber gloves during preparation and use is recommended.

(d.1) Add 0.5 g of trypan blue to 100 mL of dH_2O in a 250 mL flask. Swirl the flask until the trypan blue is completely dissolved.

(d.2) Sterilize the solution by autoclaving at 121°C for 15 minutes and store in a screw-capped container at room temperature.

(e) Procedure for preparation of stock antibiotic solutions.

If not purchased in sterile form, stockantibiotic solutions must be filter-sterilized by the use of 0.22 µm membrane filters. It is important that the recommended antibiotic levels not be exceeded when planting cells as the cultures are particular/y sensitive to excessive concentrations at this stage.

Antibiotic stock solutions should be placed in screw-capped containers and stored at -20°C until needed. Once thawed, they may be refrozen; however, repeated freezing and thawing of these stock solutions should be avoided by distributing them in quantities that are sufficient to support a week's cell culture work.

(e.1) Preparation of penicillin-streptomycin stock solution.

The procedure described is for preparation of ten 10 mL aliquots ofpenicillin-streptomycin stock solution at concentrations of 1,000,000 units of penicillin and 1,000,000 g of streptomycin per 10 mL unit. The antibiotic concentrations listed in step 6.4.2e. 1.7 may not *correspond to the concentrations obtained from other lots or from a different source.*

(e.1 .l.) Add appropriate amounts of penicillin G and dihydrostreptomycin sulfate to a 250 mL flask containing 100 mL of dH_2O. Mix the contents of the flasks on magnetic stirrer until the antibiotics are dissolved.

For penicillin suppled at 1435 units per mg, add 7 g of the antibiotic,

For streptomycin supplied at 740 mg per g, add 14 g of the antibiotic.

(e.l.2) Sterilize the antibiotics by filtration through 0.22 m membrane filters and dispense in 10 mL volumes into screw-capped containers.

(e.2) Preparation of tetracycline stock solution. Add 1.25 g of tetracycline hydrochloride powder and 3.75 g of ascorbic acid to a 125 mL flask containing 50 mL of dH_2O. Mix the contents of the flask on a magnetic stirrer until the antibiotic is dissolved. Sterilize the antibiotic by filtration through a 0.22 µm membrane filter and dispense in 5 mL volumes into screw-capped containers.

(e.3) Preparation of amphotericin B (fungizone) stock solution. Add 0.125 g of amphotericin B to a 50 mL flask containing 25 mL of d. dH_2O. Mix the contents of the flask on a magnetic stirrer until the antibiotic is dissolved. Sterilize the antibiotic by filtration through 0.22 µm membrane filter and dispense 2.5 mL volumes into screw-capped containers.

6.5 Procedure for Verifying Sterility of Liquids

There are many techniques available for verifying the sterility of liquids such as cell culture media and medium components. The two techniques described below are standard in many laboratories. The capabilities of these techniques are limited to the detection of microorganisms that grow unaided on the test medium utilized. Viruses, mycoplasma, and microorganisms that possess fastidious growth requirements or that require living host systems will not be detected. Nonetheless, with the exception of a few special contamination problems, the test procedures and microbiological media listed below should prove adequate. Do not add antibiotics to media or medium components until after sterility of the antibiotics, media and medium components has been demonstrated. The BGM cell line used should be checked every six months for mycoplasma contamination according to test kit instructions. Cells that are contaminated should be discarded.

6.5.1. Procedure for Verifying Sterility of Small Volumes of Liquids. Inoculate 5 mL of the material to be tested for sterility into 5 mL of thioglycollate broth. Shake the mixture and incubate at 36.5 ± 1°C. Examine the inoculated broth daily for seven days to determine whether growth of contaminating organisms has occurred.

Vessels that contain thioglycollate medium must be tightly sealed before and after medium is inoculated.

6.52. Visual Evaluation of Media for Microbial Contaminants. Incubate media at 36.5 ± 1 °C for at least one week prior to use. Visually examine and discard any media that lose clarity.

A clouded condition that develops in the media indicates the occurrence of contaminating organisms.

6.5.3. Procedures for Preparation and Passage of BGM Cell Cultures

A laminar flow biological safety cabinet should be used to process cell cultures. If a biological safety cabinet is not available, cell cultures should be prepared in controlled facilities used for no other purposes. Viruses or other microorganisms must not be transported, handled, or stored in cell culture transfer facilities.

6.6.1. Vessels and Media for Cell Growth

(a) The BGM cell line grows readily on the inside surfaces of glass or specially treated, tissue culture grade plastic vessels. 16 to 32 oz (or equivalent growth area) flat-sided, glass bottles, 75 or 150 cm^2 plastic cell culture flasks, and 690 cm^2 glass or 850 cm^2 plastic roller bottles are usually used for the maintenance of stock cultures. Flat-sided bottles and flasks that contain cells in a stationary position are incubated with the flat side (cell monolayer side) down. If available, roller bottles and roller apparatus units are preferable to flat-sided bottles and flasks because roller

cultures require less medium than flat-sided bottles per unit of cell monolayer surface. Roller apparatus rotation speed should be adjusted to one-half revolution per minute to ensure that cells are constantly bathed in growth medium.

(b) Growth and maintenance media should be prepared on the day they will be needed. Prepare growth medium by supplementing MEM/L-15 medium with 10% serum and antibiotics (100 mL of serum, 1 mL of penicillin-streptomycin stock, 0.5 mL of tetracycline stock and 0.2 mL of fungizone stock per 900 mL of MEM/L-15). Prepare maintenance medium by supplementing MEM/L-15 with antibiotics and 2% or 5% serum (20 or 50 mL of serum, antibiotics as above for growth medium and 70 or 50 mL of dH_2O, respectively).

6.6.2. General Procedure for Cell Passage

Pass stock BGM cell cultures at approximately seven day intervals using growth medium.

(a) Pour spent medium from cell culture vessels, and discard the medium.

To prevent splatter, a gauze-covered beaker may be used to collect spent medium.

Before discarding, autoclave all media that have been in contact with cells or that contain serum.

(b) Add to the cell cultures a volume of warm EDTA-trypsin reagent equal to 40% of the volume of medium replaced.

*See Table 2 for the amount of reagents required for com-*monly used vessel types.

To reduce shock to cells, warm the EDTA-trypsin reagent to 36.5 ± 1 °C before placing if on cell monolayers. Dis-pense the EDTA-trypsin reagent *directly* onto the *cell* mono-*layer.*

Table 2. Guide for Preparation of BGM Stock Cultures

Vessel Type	EDTA-Trypsin Volume (mL)[1]	Media Volume (mL)[2]	Total No. Cells to Plate per Vessel
16 oz glass flat bottles[3]	10	25	2.5×10^6
32 oz glass flat bottles	20	50	5.0×10^6
75 cm^2 plastic flat flask	12	30	3.0×10^6
150 cm^2 plastic flat flask	24	60	6.0×10^6
690 cm^2 glass roller bottle	40	100	7.0×10^7
850 cm^2 plastic roller bottle	50	120	8.0×10^7

[1]The volume required to remove cells from vessels.
[2]Serum requirements: growth medium contains 10% serum; maintenance medium contains 2-5% serum. Antibiotic requirements: penicillin-streptomycin stock solution, 1 .O mL/ liter; tetracycline stock solution, 0.5 ml/liter; fungizone stock solution, 0.2 ml/liter.
[3]Size is given in oz only when it is commercially designated in that unit.

(c) Allow the EDTA-trypsin reagent to remain in contact with the cells at either room temperature or at 36.5 ± 1°C until cell monolayer can be shaken loose from inner surface of cell culture vessel (about five min).

If necessary, a sterile rubber policeman (or scraper) may be used to physically remove the *cell sheet from the bottle. However, this procedure should be used only as a last resort because of the risk of cell culture contamination inherent in such manipulations. The EDTA-trypsin reagent should remain in contact with the cells no longer than necessary as prolonged contact can alter or damage the cells.*

(d) Pour the suspended cells into centrifuge tubes or bottles.

*To facilitate collection and resuspension of cell pellets, use tubes or bottles with conical bottoms. Centrifuge tubes and bottles used for this purpose must be able to with-*stand the g-force applied.

(e) Centrifuge cell suspension at 1,000 (g for 10 min to pellet cells. Pour off and discard the supernatant.

Do not exceed this speed as cells may be damaged or destroyed.

(f) Suspend the pelleted cells in growth medium (see section 6.6.1 b) and perform a viable count on the cell suspension according to procedures in section 6.7.

*Resuspend pelleted cells in sufficient volumes of medium to allow thorough mixing of the cells (to reduce sampling error) and to minimize the significance of the loss of the 0.5 mL of cell suspension required for the cell counting procedure. The quantity of medium used for resuspending pelleted cells varies from 50 to several hundred mL, de-*pending upon the volume of the individual laboratory's need *for cell cultures.*

(g) Dilute the cell suspension to the appropriate cell concentration with growth medium and dispense into cell culture vessels with either a Cornwall-type syringe or Brewer-type pipetting machine dispenser.

Calculate the dilution factor requirement using the cell count established in *section 6.7* and the cell *and volume parameters given in Tab/e 2 for stock cultures and in Table 3 for virus assay cultures.*

As a general rule, the BGM cell line can be split at a 1:3 ratio. *However, a more suitable inoculum is obtained if low passages of the line (passages 100-150) are split at a 1:2 ratio and higher passages (generally above passage 250) are split at a 1:4 ratio. To plant two hundred 25 cm^2 cell culture f/asks week/y from a low-level passage of the line would require the preparation of six roller bottles (surface area 690 cm^2 each): two to prepare the six roller bottles and four to prepare the 25 cm^2 flasks.*

Table 3. Guide for Preparation of Virus Assay Cell Cultures

Vessel Type	Volume of Medium (mL)[1]	Final Cell Count per Bottle
1 oz glass bottle[2]	4	9.0×10^5
25 cm^2 plastic flask	10	3.5×10^6
6 oz glass bottle	15	5.6×10^6
75 cm^2 plastic flask	30	1.0×10^7
16 mm (150mmtubes	2	4.0×10^4

'Serum requirements: growth medium contains 10% serum; maintenance medium contains 2-5% serum. Antibiotic requirements: penicillin-streptomycin stock solution, 1 .O ml/liter; tetracycline stock solution, 0.5 ml/liter; fungizone stock solution, 0.2 ml/liter.
[2]Size is given in oz only when it is commercially designated in that unit.

(h) Except during handling operations, maintain BGM cells at 36.5 ± 1 °C in airtight cell culture vessels.

6.6.3. Procedure for Changing Medium on Cultured Ceils

Cell monolayers normally become 95 to 100% confluent three to four days after seeding with an appropriate number of cells, and growth medium becomes acidic. Growth medium on confluent stock cultures should then be replaced with maintenance medium containing 2% serum. Maintenance medium with 5% serum should be used when monolayers are not yet 95% to 100% confluent but the medium in which they are immersed has become acidic. The volume of maintenance medium should equal the volume of discarded growth medium.

6.7. Procedure for Performing Viable Cell Counts

With experience a fair/y accurate cell concentration can be made based on the volume of packed cells. However, viable cell counts should be performed periodically as a quality control measure.

6.7.1. Add 0.5 mL of cell suspension (or diluted cell suspension) to 0.5 mL of 0.5% trypan blue solution in a test tube.

To obtain an accurate cell count, the optimal total number of cells per hemocytometer section should be between 20 and 50. This range is equivalent to between 6.0 (10^5 and 1.5 (10^6 cells per mL of cell suspension. Thus, a dilution of 1:10 (0.5 mL of cells in 4.5 mL of growth medium) is usually required for an accurate count of a cell suspension.

6.7.2. Disperse cells by repeated pipetting.

Avoid introducing air bubbles into the suspension, because air bubbles may interfere with subsequent filling of the hemocytometer chambers.

6.7.3. With a capillary pipette, carefully fill a hemocytometer chamber on one side of a slip-covered hemocytometer slide. Rest the slide on a flat surface for about one min to allow the trypan blue to penetrate the cell membranes of nonviable ceils.

Do not under or over fill the chambers.

6.7.4. Under 100X total magnification, count the cells in the four large corner sections and the center section of the hemocytometer chamber.

Include in the count cells lying on the lines marking the top and left margins of the sections, and ignore cells on the lines marking the bottom and right margins. Trypan blue is excluded by living cells. Therefore, to quantify viable cells, count only cells that are clear in color. Do not count cells that are blue.

6.7.5. Calculate the average number of viable cells in each mL of cell suspension by totaling the number of viable cells counted in the five sections, multiplying this sum by 4000, and where necessary, multiplying the resulting product by the reciprocal of the dilution.

6.8. Procedure for Preservation of BGM Cell Line

An adequate supply of BGM cells must be available to replace working cultures that are used only periodically or become contaminated or lose virus sensitivity Cells have been held at -70°C for more than 15 years with a minimum loss in cell viability

6.6.1. Preparation of Cells for Storage

The procedure described is for the preparation of 100 cell culture via/s. Cell concentration per mL must be at /east 1×10^6.

Base the actual number of vials to be prepared on usage of the line and the anticipated time interval requirement between cell culture start-up and full culture production.

(a) Prepare cell storage medium by adding 10 mL of DMSO to 90 mL of growth medium (see section 6.6.1 b). Sterilize cell storage medium by passage through an 0.22 m sterilizing filter.

Collect sterilized medium in 250 mL flask containing a stir bar.

(b) Harvest BGM cells from cell culture vessels as directed in section 6.6.2. Count the cells according to the procedure in section 6.7 and resuspend them in the cell storage medium at a concentration of 1 (10^6 cells per mL.

(c) Place the flask containing suspended cells on a magnetic stirrer and slowly mix for 30 min. Dispense 1 mL volumes of cell suspension into 2 mL vials.

6.8.2. Procedure for Freezing Cells

The freezing procedure requires slow cooling of the cells with the optimum rate of -1 °C per min. A slow cooling rate can be achieved using the following method or by using the recently available freezing containers (e.g., Nalge Com-

pany Product No. 5100-0001 or equivalent) as recommended by the manufacturers.

(a) Place the vials in a rack and place the rack in refrigerator at 4°C for 30 min, in a -20°C freezer for 30 min, and then in a -70°C freezer overnight. The transfers should be made as rapidly as possible.

To allow for more uniform cooling, wells adjoining each vial should remain empty

(b) Rapidly transfer vials into boxes or other containers for long-term storage.

To prevent substantial loss of cells during storage, temperature of cells should be kept constant after -70°C has been achieved.

6.8.3. Procedure for Thawing Cells

Cells must be thawed rapidly to decrease loss in cell viability.

(a) Place vials containing frozen cells into a 36°C water bath and agitate vigorously by hand until all ice has melted. Sterilize the outside surface of the vials with 0.5% I, in 70% ethanol.

(b) Add BGM cells to either 6 oz tissue culture bottles or 25 cm^2 tissue culture flasks containing an appropriate volume of growth medium (see Table 3). Use two vials of cells for 6 oz bottles and one vial for 25 cm^2 flasks.

(c) Incubate BGM cells at 36.5 ± 1 °C. After 18 to 24 h replace the growth medium with fresh growth medium and then continue the incubation for an additional five days. Pass and maintain the new cultures as directed in section 6.6.

7. Bibliography and Suggested Reading

ASTM. 1998. Standard Methods for the Examination of Water and Wastewater (L.S. Clesceri, A.E. Greenberg and A.D. Eaton, ed), 20th Edition. United Book Press, Baltimore, MD.

Barron, A.L., C. Olshevsky, and M.M. Cohen. 1970. Characteristics of the BGM line of cells from African green monkey kidney. Archiv. Gesam. Virusforsch. *32*:389-392.

Berg, G., D. Berman, and R.S. Safferman. 1982. A Method for concentrating viruses recovered from sewage sludges. Can. J. Microbiol. *28*:553-556.

Berg, G., R.S. Safferman, D.R. Dahling, D. Berman, and C.J. Hurst. 1984. USEPA Manual of Methods for Virology. U.S. Environmental Protection Agency Publication No. EPA/600/4-84-013, Cincinnati, OH.

Berman, D., G. Berg, and R.S. Safferman. 1981. A method for recovering viruses from sludges. J. Virol. Methods. *3*:283-291.

Brashear, D.A., and R.L. Ward. 1982. Comparison of methods for recovering indigenous viruses from raw wastewater sludge. Appl. Environ. Microbiol. 43:1413-l 418.

Dahling, D.R., and B.A. Wright. 1986. Optimization of the BGM cell line culture and viral assay procedures for monitoring viruses in the environment. Appl. Environ. Microbiol. *51*:790-812.

Dahling, D.R., G. Berg, and D. Berman. 1974. BGM, a continuous cell line more sensitive than primary rhesus and African green kidney cells for the recovery of viruses from water. Health Lab. Sci. *11*:275-282.

Dahling, D. R., G. Sullivan, R. W. Freyberg and R. S. Safferman. 1989. Factors affecting virus plaque confirmation procedures. J. Virol. Meth. *24*:111-122.

Dahling, D. R., R. S. Safferman, and B. A. Wright. 1984. Results of a survey of BGM cell culture practices. Environ. Internat. *10*:309-313.

Dulbecco, R. 1952. Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc. Natl. Acad. Sci. U.S.A. *38*:747-752.

Eagle, H. 1959. Amino acid metabolism in mammalian cell cultures. Science. *130*:432-437.

Hay, R. J. 1985. ATCC Quality Control Methods for Cell Lines. American Type Culture Collection, Rockville, MD.

Hurst, C. J. 1987. Recovering viruses from sewage sludges and from solids in water, pp. 25-51. *In G.* Berg (ed), Methods for Recovering Viruses from the Environment. CRC Press, Boca Raton, FL.

Katzenelson, E., B. Fattal, and T. Hostovesky. 1976. Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water. Appl. Environ. Microbiol. *32*:638-639.

Lennette, E. H. and N. J. Schmidt (ed.). 1979. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th ed. American Public Health Association, Inc., Washington, D.C.

Safferman, R. S., M. E. Rohr and T. Goyke. 1988. Assessment of recovery efficiency of beef extract reagents for concentrating viruses from municipal wastewater sludge solids by the organic flocculation procedure. Appl. Environ. Microbiol. *54*:309-316.

Stetler, R. E., M. E. Morris and R. S. Safferman. 1992. Processing procedures for recovering enteric viruses from wastewater sludges. J. Virol. Meth. 4067-76.

Ward, R. L., and C. S. Ashley. 1976. Inactivation of poliovirus in digested sludge. Appl. Environ. Microbiol. *31*:921-930.

Appendix I
Test Method for Detecting, Enumerating, and Determining the Viability of Ascaris Ova in Sludge.

1. Scope

1.1 This test method describes the detection, enumeration, and determination of viability of *Ascaris* ova in water, wastewater, sludge, and compost. These pathogenic intestinal helminths occur in domestic animals and humans. The environment may become contaminated through direct deposit of human or animal feces or through sewage and wastewater discharges to receiving waters. Ingestion of water containing infective *Ascaris* ova may cause disease[1].

1.2 This test method is for wastewater, sludge, and compost. It is the user's responsibility to ensure the validity of this test method for untested matrices.

1.3 This standard does not purport to address all of the safety problems, if any, associated with it use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. For specific hazard statements, see section 9.

2.0 Referenced Documents

2.1 ASTM Standards: (Use words "Specification for," "Practice for," "Test Method for," etc.)

(Include standards listed below and others that are referred to in the test method.)

D 1129 Terminology Relating to Water[2]

D 1193 Specification for Reagent Water[3]

D 2777 Practice for Determination of Precision and Bias of Applicable Methods of committee D-l 9 on Water

2.2 Other Documents: (Include any standards or codes from other organizations that are required to conduct test; if all from same organizations, use that as the title.)

3.0 Terminology

(Definitions and Descriptions of Terms must be approved by the Definitions Advisor.)

3.1 Definitions - For definitions of terms used in this test method, refer to Terminology D 1129.

3.2 Descriptions of Terms Specific to This Standard:

3.2.1 The normal nematode life cycle consists of the egg, 4 larval stages and an adult. The larvae are similar in appearance to the adults; that is, they are typically worm-like in appearance.

3.2.2 Molting *(ecdysis)* of the outer layer *(cuticle)* takes place after each larval stage. Molting consists of 2 distinct processes, the deposition ofthe new cuticle and the shedding of the old one or exsheathment. The cuticle appears to be produced continuously, even throughout adult life.

3.2.3 A molted cuticle that still encapsulates a larva is called a *sheath.*

3.2.4 Ascarid egg shells are commonly comprised of layers. The outer tanned, bumpy layer is referred to as the mammillated layer and is useful in identifying Ascaris eggs. The mammillated layer is sometimes absent. Eggs that do not possess the mammillated layer are referred to as *decorticated eggs.*

3.2.5 A potentially infective Ascaris egg contains a third stage larva[4] encased in a the sheath of the first larval stage.

4. Summary of Test Method

4.1 This method is used to concentrate pathogenic *Ascaris* ova from wastewater, sludge, and compost. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particulates. The solids in the screened portion are allowed to settle out and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate (specific gravity 1.20). This flotation proce-

[1]The boldface numbers in parentheses refer to the list of references at the end of this test method.
[2]Annual Book of ASTM Standards, Vol 11 .01.
[3]Annual Book of ASTM Standards, Vol 11 .01.

[4]P.L. Geenen. J. Bresciani, Jeap Boes, A. Pedersen. Lis Eriksen, H.P. Fagerholm, and P. Nansen (1999) The Morphogenesis of *Ascaris suum* to the infective third-stage larvae within the egg, J. Parasitology, 85(4):616-622

dure yields a layer likely to contain *Ascaris* and some other parasitic ova if present in the sample. Small particulates are removed by a second screening on a small mesh size screen.[5] Proteinaceous material is removed using an acid-alcohol/ethyl acetate extraction step. The resulting concentrate is incubated at 26EC until control *Ascaris eggs* are fully embryonated. The concentrate is then microscopically examined for the categories of *Ascaris* ova on a Sedgwick-Rafter counting chamber.[6]

5. Significance and Use

5.1 This test method is useful for providing a quantitative indication of the level of *Ascaris* ova contamination of wastewater, sludge, and compost.

5.2 This test method will not identify the species of *Ascaris* detected nor the host of origin.

5.3 This method may be useful in evaluating the effectiveness of treatment.

6. interferences

6.1 Freezing of samples will interfere with the buoyant density of *Ascaris* ova and decrease the recovery of ova.

7. Apparatus

7.1 A good light microscope equipped with brightfield, and preferably with phase contrast and/or differential contrast optics including objectives ranging in power from 1 OX to 45x.

7.2 Sedgwick-Rafter cell.

7.3 Pyrex beakers, 2 L. Coat with organosilane.

7.4 Erlenmeyer flask, 500 mL. Coat with organosilane.

7.4 A centrifuge that can sustain forces of at least 660 X G with the rotors listed below.

7.4.1 A swinging bucket rotor to hold 100 or 250 ml centrifuge glass or plastic conical bottles.

7.4.2 A swinging bucket rotor to hold 15 ml conical glass or plastic centrifuge tubes.

7.5 Tyler sieves.

7.5.1 20 or 50 mesh.

7.5.2 400 mesh, stainless steel, 5 inch in diameter.

7.5.3 A large plastic funnel to support the sieve. Coat with organosilane.

7.6 Teflon spatula.

7.7 Incubator set at 26EC.

7.8 Large test tube rack to accommodate 100 or 250 mL centrifuge tubes.

7.9 Small test tube rack to accommodate 15 mL conical centrifuge tubes.

7.10 Centrifuge tubes, 100 or 250 mL. Coat with organosilane.

7.11 Conical centrifuge tubes, 15 mL. Coat with organosilane.

7.12 Number "0" stoppers.

7.13 Wooden applicator sticks.

7.14 Pasteur pipettes. Coat with organosilane.

7.15 Vacuum aspiration apparatus.

7.15.1 Vacuum source.

7.15.2 Vacuum flask, 2 L or larger.

7.15.3 Stopper to fit vacuum flask, fitted with a glass or metal tubing as a connector for 1/4 inch tygon tubing.

7.16 Wash bottles (500 mL), label "Water".

7.17 Spray bottles (16 fl oz.) (2).

7.17.1 Label one "Water".

7.17.2 Label one "1% 7X".

8. Reagents and Materials

(This section must be approved by Reagents Advisor.)

8.1 Purity of Reagents — Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society[7]. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8.2 Purity of Water — Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I.

---

[5] The preceeding portion of the procedure is adapted from: Reimers, R.S., M.D. Little, T.G. Akers, W.D. Henriques, R.C. Badeaux, D.B. McDonnel, and K.K. Mbela. 1989. Persistance of pathogens in lagoon-stored sludge. Cooperative Agreement N. 810289. U.S. Environmental Protection Agency. EPA/600/2-89/015.

[6] This portion of the procedure was adapted from: Task Method for Detecting, Enumerating, and Determining the Viability of *Ascaris* Ova in Wastewater and Sludge. Draft No. 01.

[7] Reagent Chem Speci American Chemical Society fications, American Chemical Society, Washington, DC. For suggestions on the testing of Reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulary, US. Pharmaceutical Convention, Inc. (USPC),

8.3 Preparation of Reagents — Prepare reagents in accordance with Practice E200. (List other reagents in alphabetical order by the critical word, from highest to lowest concentration, with concentrations in parentheses. Example: Copper Solution, Standard (1 mL = 1 mg Cu).)

8.3.1 Phosphatebuffered water (1 L = 34.0 g KH_2PO_4, pH adjusted to 7.2 ± 0.5 with 1 N NaOH).

8.3.2 1% (v/v) 7X ("Limbro" laboratory detergent) (1 L = 999 mL phosphate-buffered water, 1 mL 7X "Limbro", Adjust pH to 7.2 ± 0.1 with 1 N NaOH).

8.3.3 Magnesium sulfate, sp. gr. 1.20. (1 L = 215.2 g $MgSO_4$, check specific gravity with a hydrometer; adjust as necessary to reach 1.20).

83.4 Acid alcohol. 0.1. N H_2SO, made in 35% ethyl alcohol. (100 mL = 35 mL EtOH, 0.9807 g H_2SO_4)

8.3.5 Ethyl Acetate, reagent grade.

8.4 Organosilane. For coating glassware. Coat all glassware according to manufacturer's instructions.

8.5 Fresh *Ascaris* ova for positive control, either dissected from *Ascaris suum* gravid adult female worms or purified from *Ascaris* infected pig fecal material.

9. Precautions

(To be reviewed by the Reagents Advisor)

9.1 When harvesting *Ascaris* ova from gravid female worms, the analyst must wear latex gloves, a surgical mask and protective goggles or full face mask, and laboratory coat before dissecting the worms. Moreover, it is recommended that the *Ascaris* ova harvest be carried out either in a biological safety cabinet or minimally a chemical hood. These precautions are designed to prevent the development of an allergy to *Ascaris* pseudocoelomic fluid. If infective *Ascaris* ova are ingested they may cause disease.

10. Sampling

10.1 Collect 1 liter of compost, wastewater, or sludge in accordance with Practice D 1066, Specification D 1192, and Practices D 3370, as applicable.

10.2 Place the sample container(s) on wet ice or around chemical ice and ship back to the laboratory for analysis within 24 hours of collection.

10.3 Store the samples in the laboratory refrigerated at 2 to 5°C. Do not freeze the samples during transport or storage.

11. Preparation of Apparatus

(Give instructions in imperative mood.)

12. Calibration and Standardization

(To be reviewed by the Reagents Advisor) (Give instructions in imperative mood.)

13. Procedure

(To be reviewed by the Reagents Advisor) (Give instructions in imperative mood.)

13.1 The percentage moisture of the sample is determined by analyzing a separate portion of the sample, so the final calculation of ova per gram dry weight can be determined. The concentration of ova in liquid sludge samples may be expressed as ova per unit volume.

13.2 Initial preparation:

13.1.1 Dry or thick samples: Weigh about 300 g (estimated dry weight) and place in about 500 ml water in a beaker and let soak overnight at 4 - 10EC. Transfer to blender and blend at high for one minute. Divide sample into four beakers.

13.1.2 Liquid samples: Measure 1,000 ml or more (estimated to contain at least 50 g dry solids) of liquid sample. Place one half of sample in blender. Add about 200 mL water. Blend at high speed for one minute transfer to a beaker. Repeat for other half of sample.

13.3 Pour the homogenized sample into a 1000 mL tall form beaker and using a wash bottle, thoroughly rinse blender container into beaker. Add 1% 7X to reach 900 ml final volume.

13.4 Allow sample to settle four hours or overnight at 4 - 10EC. Stir occasionally with a wooden applicator, as needed to ensure that material floating on the surface settles. Additional 1% 7X may be added, and the mixture stirred if necessary.

13.5 After settling, vacuum aspirate supernatant to just above the layer of solids. Transfer sediment to blender and add water to 500 ml, blend again for one minute at high speed.

13.6 Transfer to beaker, rinsing blender and add 1% 7X to reach 900 ml. Allow to settle for two hours at 4 - 1 OEC, vacuum aspirate supernatant to just above the layer of solids.

13.7 Add 300 ml 1% 7X and stir for five minutes on a magnetic stirrer.

13.8 Strain homogenized sample through a 20 or 50 mesh sieve placed in a funnel over a tall beaker. Wash sample through sieve with a spray of 1% 7X from a spray bottle.

13.9 Add 1% 7X to 900 mL final volume and allow to settle for two hours at 4 - 1 OEC.

13.10 Vacuum aspirate supernatant to just above layer of solids. Mix sediment and distribute equally to 50 mL graduated conical centrifuge tubes. Thoroughly wash any sediment from beaker into tubes using water from a wash bottle. Bring volume in tubes up to 50 ml with water.

13.11 Centrifuge for 10 minutes at 1000 X G. Vacuum aspirate supernatant from each tube down to just above the level of sediment. (The packed sediment in each tube should not exceed 5 mL. If it exceeds this volume, add water and distribute the sediment evenly among additional tubes, repeat centrifugation, and vacuum aspirate supernatant.)

13.12 Add 10 to 15 mL of $MgSO_4$ solution (specific gravity 1.20) to each tube and mix for 15 to 20 seconds on a vortex mixer. (Use capped tubes to avoid splashing of mixture from the tube.)

13.13 Add additional $MgSO_4$ solution (specific gravity 1.20) to each tube to bring volume to 50 mL. Centrifuge for five to ten minutes at 800 to 1000 X g. DO NOT USE BRAKE.

13.14 Allow the centrifuge to coast to a stop without the brake. Pour the top 25 to 35 mL of supernatant from each tube through a 400 mesh sieve supported in a funnel over a tall beaker.

13.15 Using a water spray bottle, wash excessive flotation fluid and fine particles through sieve.

13.16 Rinse sediment collected on the sieve into a 100 mL beaker by directing the stream of water from the wash bottle onto the upper surface of the sieve.

13.17 After thoroughly washing the sediment from the sieve, transfer the suspension to the required number of 15 mL centrifuge tubes, taking care to rinse the beaker into the tubes. Usually one beaker makes one tube.

13.18 Centrifuge the tubes for three minutes at 800 X G, then discard the supernatant.

13.19 If more than one tube has been used for the sample, transfer the sediment to a single tube, fill with water, and repeat centrifugation.

13.20 Resuspend the pellet in 7 mL acid alcohol solution and add 3 mL ethyl acetate.

13.21 Cap the tube with a rubber stopper and invert several times, venting after each inversion.

13.22 Centrifuge the tube at 660 x G for 3 minutes.

13.23 Aspirate the supernatant above the solids.

13.24 Resuspend the solids in 4 mL 0.1 N H_2SO_4 and pour into a 220-mL polyethylene scintillation vial or equivalent with loose caps.

13.25 Before incubating the vials, mark the liquid level in each vail with a felt tip pen. Incubate the vials, along with control vials containing *Ascaris* ova mixed with 4 mL 0.1 N H_2SO_4, at 26°C for three to four weeks. Every day or so, check the liquid level in each vial. Add reagent grade water up to the initial liquid level line as needed to compensate for evaporation. After 18 days, suspend, by inversion and sample small aliquots of the control cultures once every 2 - 3 days. When the majority of the control *Ascaris* ova are fully embryonated, samples are ready to be examined.

13.26 Examine the concentrates microscopically using a Sedgwick-Rafter cell to enumerate the detected ova. Classify the ova as either unembryonated, embryonated to the first, second or third larval stage. In some embryonated *Ascaris* ova the larva may be observed to move. See Figure 1 for examples of various Ascaris egg categories.

14. Calculation

(To be reviewed by the Results Advisor.) (Provide directions in the imperative mood and include equations using appropriate quantity symbols and key.)

14.1 Calculate % total solids using the % moisture result:

$$\% \text{ Total solids} = 100\% - \% \text{ moisture}$$

14.2 Calculate catagories of ova/g dry weight in the following manner:

$$\text{Ova/g dry wt} = \frac{(NO) \times (CV) \times (FV)}{(SP) \times (TS)}$$

Where:

NO = no. ova

CV = chamber volume(= 1 mL)

FV = final volume in mL

SP = sample processed in mL or g

TS = % total solids

15. Report

(To be reviewed by the Results Advisor.) (State detailed information required in reporting results, as particular procedure used; can include report forms or worksheets as Figures.)

15.1 Report the results as the total number of *Ascaris* ova, number of unembryonated *Ascaris* ova, number of 1 st, 2nd or 3rd stage larva; reported as number of *Ascaris* ova and number of various larval *Ascaris* ova per g dry weight. Representative reporting forms are shown in Figure 2.

16. Precision and Bias

(Approval of the Results Advisor is required for the proposed program both before testing is initiated and for the calculations and final precision statement that appears in the test method. See Practice D 2777-86 for precision and bias requirements and formats.)

Figure 1. ***Ascaris*** ovum: potentially non-fertile, note bumpy mammilated outer layer.

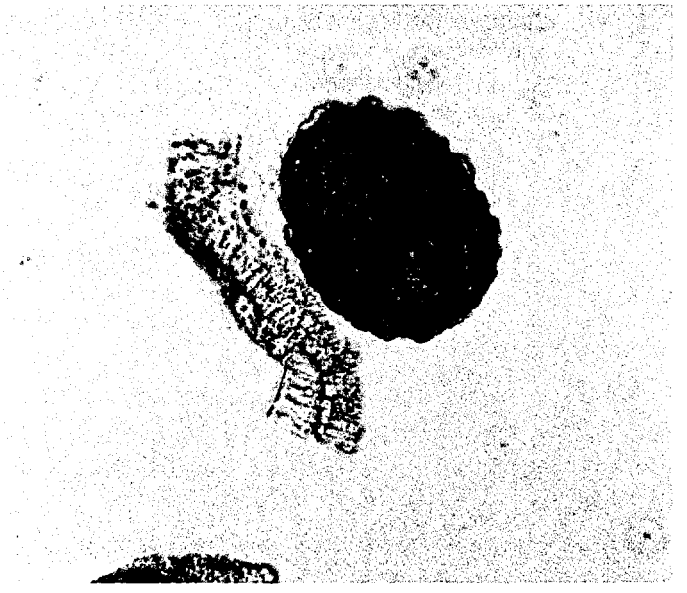


FIgure 2. ***Ascaris*** ovum: fertile, note the bumpy outer mammilated layer.

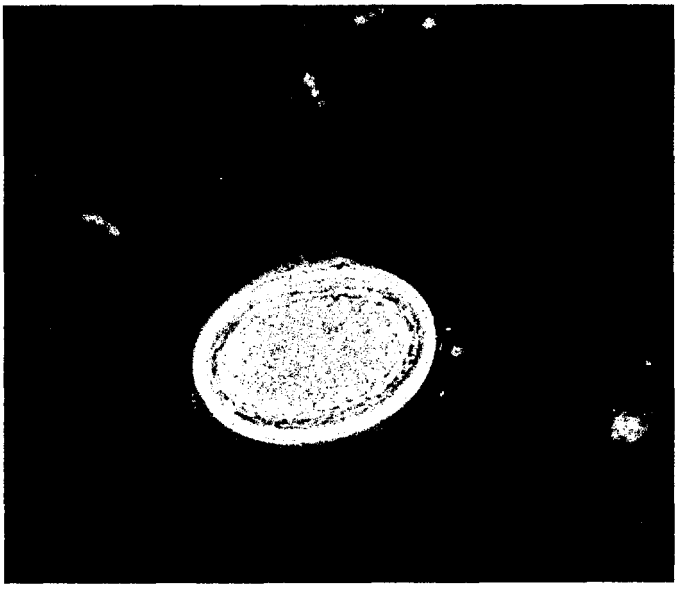


Figure *3.* ***Ascaris*** ovum: decorticated, unembryonated. Note the outer mammilated layer is gone.

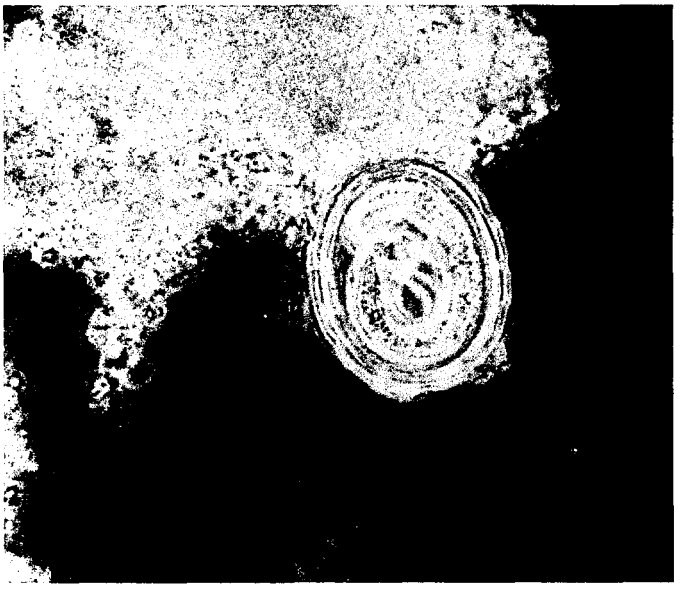


Figure *4.* ***Ascaris*** ovum: decorticated and embryonated.

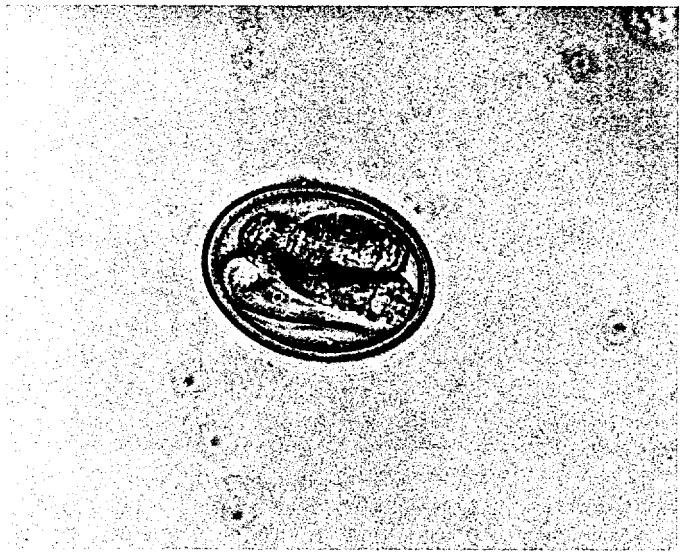


FIgure 5. ***Ascaris*** ovum: decorticated, embryonated.

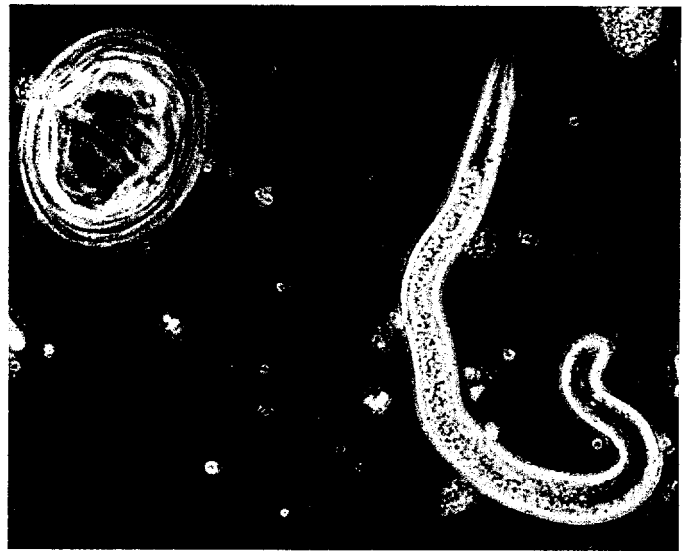


Figure 6. ***Ascaris*** ovum with second stage or potentially third stage larva; note the first stage larval sheath at the anterior end of the worm.

16.1 This test method was tested by laboratories, with each operator analyzing each sample on different days. These collaborative test data were obtained on reagent water and waters. For other matrices, these data may not apply.

16.1.1 Precision -The precision of this test method within its designated range may be expressed as follows: (This data may be included as a figure.)

16.1.2 Bias - Recoveries of known amounts of in a series of prepared standards were as follows: (This data may be included as a table.)

16.2 (The deficiency boilerplate should be added as applicable. It reads:)

, independent laboratories (and a total of operators) participated in the round robin study. Precision and bias for this test method conforms to Practice D 2777-77, which was in place at the time of collaborative testing. Under the allowances made in 1.5 of Practice D 2777-86, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D-l 9 test methods.

17. Keywords

17.1 *Ascaris*, ova, embryonation, viability assay, helminth.

Notice

The PEC was consulted in a recent (1998-1999) pilot study by Lyonnaise des Eaux concerning the use of a microscope in making helminth ova counts for different types of sludge. Solids and debris present in the sludge being viewed with the microscope were found to impair ones ability to count. Dilution of raw sludge and digested sludge, however, with phosphate-buffered water prior to analyzing them significantly improved the number of ova that could be counted. Raw sludges were diluted by a factor of 20 and digested sludges by a factor of 5. QA/QC procedures were followed to validate this procedure. The PEC should be consulted for more details.

Appendix J
The Biosolids Cornposting Process

Introduction

Composting is the biological decomposition of organic matter under controlled aerobic conditions. The objectives of composting are to reduce pathogens to below detectable levels, degrade volatile solids, and produce a usable product. Pathogen reduction is a function of time and temperature. Composted biosolids is one way to meet 40 CFR Part 503 pathogen (and vector attraction) reduction requirements. Composted biosolids can meet either a "Process to Significantly Reduce Pathogens" (PSRP/Class B) or a "Process to Further Reduce Pathogens" (PFRP/Class A) standard, depending upon the operating conditions maintained at the facility. Process and operational considerations must be taken into account when a facility desires to meet the pathogen and vector attraction requirements of 40 CFR 503. The 40 CFR Part 503 regulations require composted biosolids applied to the land to meet specific pollutant limits, site restrictions, management practices, and pathogen and vector attraction reduction processes, depending upon whether they: 1) are applied to agricultural land, forest, a public contact site, or a reclamation site; 2) are sold or given away in a bag or other container; or 3) are applied to a lawn or home garden. Discussions provided here are presented in summary form, it is recommended that the facility seek additional details in developing a compost operation.

Cornposting Process Discription

The addition of a bulking agent to sewage sludge provides optimum conditions for the composting process, which usually lasts 3 to 4 weeks. A bulking agent acts as a source of carbon for the biological process, increases porosity, and reduces the moisture level. The composting process has several phases, including the active phase, the curing phase, and the drying phase.

Active phase. During the active or stabilization phase, the sewage sludge/bulking agent mix is aerated and the sewage sludge is decomposed due to accelerated biological activity. The biological process involved in composting can raise the temperature up to 60°C or more. At these high temperatures, all of the disease-causing pathogens are destroyed. Windrow systems must meet this condition by achieving 55°C for a minimum of 15 consecutive days during which time the windrow is turned five times. The critical requirement is that the material in the core of the compost pile be maintained at the required temperatures (55°C) for the required time (3 days). Therefore, the first phase typically lasts 21 days. Aeration is accomplished in one of two ways: 1) by mechanically turning the mixture so that the sewage sludge is exposed to oxygen in the air; or 2) by using blowers to either force or pull air through the mixture.

Curing phase. After the active phase, the resulting material is cured for an additional 30 days to 180 days. At this time, additional decomposition, stabilization, pathogen destruction, and degassing takes place. Composting is considered complete when the temperature of the compost returns to ambient levels. Depending upon the extent of biodegradation during the active phase and the ultimate application of the finished product, the curing phase may not be carried out as a separate process.

Drying Phase. After curing, some operations add another step called the drying phase which can vary from days to months. This stage is necessary if the material is to be screened to either recover the unused bulking agent for recycling or for an additional finished product. If the product is to be marketable, the final compost should be 50% to 60% solids.

There are two main process configurations for the composting process:

Unconfined composting. This process is conducted in long piles (windrows) or in static piles. Operations using unconfined composting methods may provide oxygen to the compost by turning the piles by hand or machine or by using air blowers which may be operated in either a positive (blowing) or negative (suction) mode. For windrows without blower aeration, it is typical to turn the windrow two or three times a week, using a front-end loader. Properly operating aerated static piles do not require turning.

Confined (in-vessel) composting. This process is carried out within an enclosed container, which minimizes odors and process time by providing better control over the process variables. Although in-vessel composting has been effective for small operations, typically these operations are proprietary and therefore will not be described any further in this fact sheet.

Operational Considerations

The key process variables for successful composting are the moisture content and carbon to nitrogen (C:N) ratio of the biosolids/bulking agent mixture, and temperature and aeration of the compost pile. Other process parameters such as volatile solids content, pH, mixing and the materials used in the compost also affect the process.

Biosolids/Bulking Agent Mixture Moisture Content. Moisture control is an important factor for effective composting. Water content must be controlled for effective stabilization, pathogen inactivation, odor control and finished compost quality (Benedict, 1988). The optimum moisture content of the mix is between 40% and 60%. At less than 40% water, the material is too fluid, has reduced porosity and has the potential for producing septic conditions and odors; above 60% solids, the lack of moisture may slow down the rate of decomposition. Since typical dewatered sewage sludge or biosolids are often in the range of 15% to 20% solids for vacuum filtered sewage sludge or biosolids and 20% to 35% solids for belt press or filter pressed sewage sludge or biosolids, the addition of drier materials (bulking agents) is usually essential.

Biosolids/Bulking Agent Mixture Carbon to Nitrogen Ratio. Microorganisms need carbon for growth and nitrogen for protein synthesis. For efficient composting, the carbon to nitrogen (C:N) ratio of the biosolids/bulking agent mixture should be in the range of 25:1 to 35:1

Oxygen /eve/s. For optimum aerobic biological activity, air within the pile should have oxygen levels of between 5% and 15%. Lower levels of oxygen will create odors and reduce the efficiency of the composting. Excessive aeration will cool the pile, slow the composting process, and will not provide the desired pathogen and vector attraction reduction.

Conventional windrows obtain necessary oxygen through the natural draft and ventilation induced from the hot, moist air produced during active composting and from the periodic windrow turning. Where blowers are used for aeration, it is typical to provide at least one blower per pile.

Biosolids/Bulking Agent Mixture Volatile Solids Con tent. The volatile solids content of the biosolids/bulking agent mix should be greater than 50% for successful composting (EPA, 1985). This parameter is an indicator of the energy available for biological activity and therefore compostability.

Biosolids/Bulking Agent Mixture pH. The pH of the biosolids/bulking agent mix should be in the range of 6 to 9 for efficient composting (EPA, 1985). Higher pH mixtures may result if lime stabilized biosolids are used. They can be composted; however, it may take longer for the composting process to achieve the temperatures needed to reduce pathogens.

Biosolids and Bulking Agent Mixing. Uniform mixing is necessary in order to assure that moisture concentration is constant through the pile and that air can flow throughout *Type of Biosolids.* The type of biosolids used may have an effect on the composting process. Composting can be accomplished with unstabilized biosolids, as well as anaerobically and aerobically digested biosolids. Raw sludge has a greater potential to cause odors because they have more energy available and will, therefore, degrade more readily. This may cause the compost pile to achieve higher temperatures faster unless sufficient oxygen is provided and may also cause odors (EPA, 1985).

Material for Bulking Agents. Materials such as wood chips, sawdust and recycled compost are usually added as "bulking agents" or "amendments" to the compost mixture to provide an additional source of carbon and to control the moisture content of the mixture. Other common bulking agents used by facilities around the country include wood waste, leaves, brush, manure, grass, straw, and paper (Goldstein, 1994). Because of their cost, wood chips are often screened out from the matured compost, for reuse. Although sawdust is frequently used for in-vessel composting, coarser materials such as wood chips, wood shavings, and ground-up wood are often preferred because they permit better air penetration and are easier to remove. Recycled compost is often used as a bulking agent in windrows, especially if bulking agents must be purchased. However, its use is limited because the porosity decreases as the recycle ages (EPA, 1989). The amount of biosolids and bulking agent which must be combined to make a successful compost is based on a mass balance process considering the moisture contents, C:N ratio, and volatile solids content.

Compost Pile Size. In general, assuming adequate aeration, the larger the pile the better. A larger pile has less surface area per cubic yard of contents and therefore retains more of the heat that is generated and is less influenced by ambient conditions. In addition, less cover and base material (recycled compost, wood chips, etc.) is needed as well as the overall land requirements for the compost operation. Larger piles tend to retain moisture longer. The surface area to volume ratio has an effect on the temperature of the pile. Assuming other factors are constant (e.g., moisture, composition, aeration), larger piles (with their lower surface area to volume ratio), retain more heat than smaller piles. Ambient temperatures have a significant impact on composting operations (Benedict, 1988).

A typical aerated static pile for a large operation would be triangularly shaped in cross section about 3 meters(m) high by 4.5 to 7.5 m wide (15 to 25 feet) at the base by 12 to 15 m long (39 to 50 feet) (Haug, 1980). One survey study indicates that extended aerated static pile (where piles are formed on the side of older piles) heights were typically 12 to 13 feet high. Minimum depths of base and cover materials (recycled compost, wood chips etc.) were 12 and 18 inches, respectively (Benedict, 1988).

In windrow composting, the compost mix is stacked in long parallel rows. In cross section, windrows may range from rectangular to trapezoidal to triangular, depending

upon the material and the turning equipment. Atypical trapezoidal windrow might be 1.2 m (4 feet) high by 4.0 m (13 feet) at its base and 1 .O m (3 feet) across the top (Haug, 1980).

Monitoring and Sampling of the Compost Pile

Unless the entire composting mass is subject to the pathogen reduction temperatures, organisms may survive and repopulate the mass once the piles or windrows are cooled. Therefore it is crucial that temperatures be attained throughout the entire pile. For aerated static piles or in-vessel systems using static procedures such as tunnels or silos, temperature monitoring should represent points throughout the pile, including areas which typically are the coolest. In aerated static piles this is usually the toes of the pile (Figure 1). Temperatures should be taken at many locations and at various depths to be assured that the core of the pile maintains the require temperature. Records of the temperature, date, and time should be maintained and reviewed on an ongoing basis. Microbial analysis should at a minimum be taken in a matter to represent the entire compost pile. Operational parameters such as moisture, oxygen as well as the others should be monitored at a frequency necessary to assure that the compost operation is operating within acceptable ranges.

For composting, vector attraction reduction (VAR) is achieved through the degradation of volatile solids. The extent to which the volatile solids are degraded is often referred to as compost stability. Stabilization requires sufficient time for the putrescible organic compounds and for other potential food sources for vectors to decompose. Under this vector attraction reduction option, the Part 503 requires that biosolids be maintained under aerobic conditions for at least 14 days, during which time temperatures are over 40°C (104°F), and the average temperature is over 45°C (113°F) (503.33(b)(5). These criteria are based on studies which have shown that most of the highly putrescible compounds are decomposed during the first 14 days of composting and that significant stability is achieved at mesophilic (<45 ° C) temperatures.

Recommendations for Specific Technologies

Aerated static pile – Aerated static piles should be covered with an insulation layer of sufficient thickness to ensure that temperatures throughout the pile, including the pile surface, reach 55° C. It is recommended that the insulation layer be at least 1 foot thick. Screened compost is a more effective insulation than unscreened compost or wood chips. Screened compost also provides more odor control than the other two materials.

Air flow rate and the configuration of an aeration system are other factors which affects temperature. Air flow must be sufficient to supply oxygen to the pile, but excessive aeration removes heat and moisture from the composting material. The configuration of an aeration system is also important. Aeration piping too close to pile edges may result in uneven temperatures in the pile and excessive cooling at the pile toes. If holes in the perforated piping are too large or not distributed properly, portions of the pile may receive too much air and be too cool as a result.

Windrows – Compliance with the pathogen reduction requirements for windrows depends on proper windrow size and configuration. If windrows are too small, the high surface area to volume ratio will result in excessive heat loss from the pile sides. Turning must ensure that all material

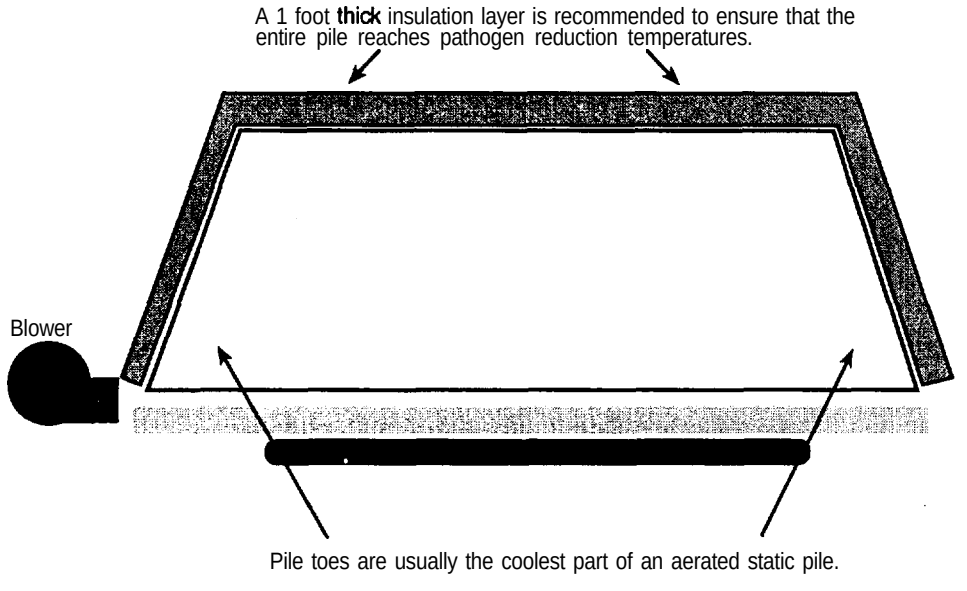
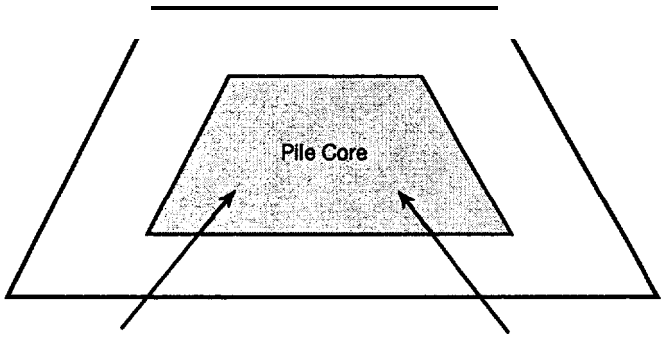


Figure **la.** Aerated static pile.



Material turned into the pile core reaches pathogen reduction temperatures. Operations must ensure that all material is turned into the core at some point during composting and that core temperatures rise to 55 degrees after turning.

Figure 1 b. Windrow.

in a windrow be introduced into the pile core and raised to pathogen reduction temperatures. This is most easily achieved with a windrow turning machine.

In-Vessel systems- It is difficult to provide guidance for these systems as there are numerous types with varying configurations. Two key factors that apply to all in-vessel systems are aeration and available carbon. As with aerated static piles, the air flow configuration and rate can affect the distribution of aeration to different parts of a composting mass and the temperature profile of a pile. Many in-vessel systems use sawdust as an amendment. This may not provide sufficient energy if the volatile solids in the biosolids are low.

Requirements for Class A/Class B Compost

For class A biosolids, aerated static pile, conventional windrow and in-vessel composting methods must meet the PFRP requirements, including the following temperature/ time requirements:

- Aerated static piles and in-vessel systems must be maintained at a minimum operating temperature of 55°C (131°F) for at least 3 days; and
- Windrow piles must be maintained at a minimum operating temperature of 55°C (131°F) for 15 days or longer. The piles must be turned five times during this period.

For class B biosolids, aerated static pile, conventional windrow and in-vessel composting methods must meet the PSRP requirements, including the following temperature/ time requirements:

- The compost pile must be maintained at a minimum of 40°C for at least five days; and
- During the five-day period, the temperature must rise above 55°C for at least four hours to ensure pathogen destruction. This is usually done near the end of the active composting phase in order to prevent inactivating the organic destroying bacteria.

To meet 40 CFR Part 503 vector attraction reduction requirements using the "aerobic process" alternative, composting operations must ensure that the process lasts for 14 days or longer at a temperature greater than 40°C. In addition, the average temperature must be higher than *45°C.*

Additional References

Benedict, Arthur et al., Composting Municipal Sludge: A Technology Evaluation, Pollution Technology Review No. 152, Noyes Data Corporation, Park Ridge, New Jersey, 1988.

BioCycle, Managing Sludge by Composting, JG Press Inc., Emmaus, PA, 1984.

Goldstein, N. et al., "1994 Biocycle Biosolids Survey." Biocycle: Journal of Composting and Recycling, December 1994.

Haug, Roger T., Compost Engineering, Principles and Practice, Ann Arbor Science Publishers, Ann Arbor, Michigan, 1980.

Information Transfer Inc., 1977 National Conference on Composting of Municipal Residues and Sludges, August 23-25, 1977, Information Transfer, Inc., Rockville, Maryland, 1978.

Jensen, Ric, Research Encourages Biosolids Re-use, Environmental Protection, December 1993.

The BioCycle Guide to In-Vessel Composting, JG Press Inc., Emmaus, PA 1986.

U.S. EPA, A Plain English Guide to the EPA Part 503 Biosolids Rule. Office of Wastewater Management. EPA/832/R-93/003. September 1994.

U.S. EPA Guidance for NPDES Compliance Inspectors, Evaluation of Sludge Treatment Processes. Office of Wastewater Enforcement and Compliance, Office of Water. November 1991.

U.S. EPA, Summary Report, In-Vessel Composting of Municipal Wastewater Sludge. EPA/625/8-89/016. September 1989.

U.S. EPA, Seminar Publication: Composting of Municipal Wastewater Sludges. Center for Environmental Research Information, Office of Research and Development. EPA 625/4-85-014. August, 1985.

U.S. EPA, Environmental Regulations and Technology, Use and Disposal of Municipal Wastewater Sludge. EPA 625/10-84/003. 1984.

United States
Environmental Protection Agency
Center for Environmental Research information
Cincinnati, OH 45268

Please make all necessary changes on the below label, detach or copy. and return to the address in the upper left-hand comer.

If you do not wish to receive these reports CHECK HERE ☐; detach, or copy this cover, and return to the address in the upper left-hand comer.

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

Official Business
Penalty for Private Use
$300

EPA/625/R-92/013