FINAL REPORT—SULFATE-REDUCING BACTERIA REACTIVE WALL DEMONSTRATION

MINE WASTE TECHNOLOGY PROGRAM
ACTIVITY III, PROJECT 12

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IAG No. DW89938870-01-1

and

U.S. Department of Energy
National Energy Technology Laboratory
Pittsburgh, Pennsylvania 15236
Contract No. DE-AC22-96EW96405

July 2002

REVIEWS AND APPROVALS:

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Foreword

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E. Timothy Oppelt, Director
National Risk Management Research Laboratory

Executive Summary

This document is a final report on the performance of sulfate-reducing bacteria (SRB) bioreactors that were constructed and operated for Mine Waste Technology Program (MWTP) Activity III, Project 12, Sulfate-Reducing Bacteria Reactive Wall Demonstration. The MWTP is funded by the U.S. Environmental Protection Agency (EPA) and jointly administered by the EPA and the U.S. Department of Energy (DOE) through an Interagency Agreement (IAG) and under DOE contract number DE-AC22-96EW96405.

Efforts reported in this document focused on the demonstration of a passive technology that could be used for remediation of thousands of abandoned mine sites existing in the Western United States that emanate acid mine drainage (AMD). This passive remedial technology takes advantage of the ability of SRB to increase pH and alkalinity of the water and to immobilize dissolved metals by precipitating them as metal sulfides or hydroxides.

The SRB technology was demonstrated by constructing three bioreactors at an abandoned mine site (Calliope Mine) in the vicinity of Butte, Montana. The bioreactors were fed by AMD emanating from a large waste rock pile. The quality of this AMD and its pH are related to the amount of atmospheric water that infiltrates into the waste rock pile and leaches metals. With the exception of the first 8 months of operation, atmospheric precipitation was well below normal. Consequently, the pH of the AMD increased, and the load of metals in the AMD significantly decreased, bringing concentrations of iron, aluminum, and manganese in the influent AMD below the target treatment levels for the project. The bioreactors operated from December 1998 to July 2001 when they were then decommissioned.

Two bioreactors were placed below ground (Bioreactors II and III), and one was placed above ground (Bioreactor IV). The aboveground bioreactor was built to evaluate the effect of cold weather and freezing on an SRB system. In addition, Bioreactors II and IV were built with a pretreatment section to evaluate the effect on the efficiency of the SRB of inducing an improved pH and oxidation-reduction potential (E_H).

Each bioreactor was filled with a combination of organic matter, crushed limestone, and cobbles placed in two or four discrete chambers. The first two chambers of Bioreactors II and IV constituted the pretreatment section and included a chamber filled with organic matter and a chamber filled with crushed limestone. Following the pretreatment section, there was another chamber with organic matter and a chamber filled with cobbles. A pretreatment section was not included in Bioreactor III in order to evaluate its contribution to overall bioreactor efficiency by comparison to Bioreactor II.

Bioreactors II and III, 71.5 feet and 61 feet in length respectively, were constructed below ground in 14-foot-wide trapezoidal (4-foot-wide bottom) trenches. Bioreactor IV, 72.5 feet in length, was constructed in a 12-foot-wide metal half-culvert elevated above ground. The chambers filled with organic matter or limestone were each 5 feet in length, whereas the chambers filled with cobbles were 50 feet in length.

The organic matter, an electron donor and carbon source for the SRB, was provided as an 80% to 20% by volume mixture of cow manure and cut straw. The cut straw was added to provide secondary porosity to the mix and to prevent settling of the medium. TerraCell™ material, commonly used in landscaping for slope stabilization and made of high density polyethylene, was used to form a cellular containment system

(CCS)[1] to house the organic matter. The CCS prevented the organic matter from settling to the bottom of the bioreactor, thus fostering the flow of AMD through the entire cross-sectional area without channeling. Each layer (lift) of TerraCell™ was positioned at 60 degrees off the horizontal plane so that the cells of each lift would be partially offset with respect to the cells of adjacent lifts. Each lift was 6 inches thick (as measured along the horizontal direction of flow) and contained 11-inch by 8.5-inch rhombohedral-shaped cells.

The two belowground bioreactors (II and III) were designed to flow year-round. The aboveground bioreactor (IV) was designed to be shut down for winter to let it freeze while full of AMD. The reactors flowed at a rate of 1 gallon per minute for the majority of time. This flow rate corresponded to a calculated 5-1/2 day residence time for the AMD in Bioreactors II and IV and a 4-1/2 day residence time in Bioreactor III. The residence time of the AMD in a single organic matter chamber was approximately 10 hours.

Bioreactor performance was monitored monthly by taking pH, E_H, dissolved oxygen, and temperature measurements and collecting samples of influent and effluent for chemical analysis. The analytes included SRB population; alkalinity; and concentrations of sulfate, sulfide, dissolved metals, aluminum, arsenic, cadmium, copper, iron, manganese, and zinc.

At the end of the project, the bioreactors were decommissioned, and the site was restored to nearly original conditions. The decommissioning activity also included an autopsy of the solid matrix material that was not accessible during the operational time. Autopsy sampling included collection of solid matrix samples for chemical analyses to determine concentrations of total metals [aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn], sulfate, sulfide, nitrogen, phosphorous, and total organic carbon (TOC) in the chambers of organic matter and limestone. Bacteriological analyses were also conducted to determine SRB population in the organic substrate and in the limestone. Because the cobbles did not have a visually discernible film of bacteria or chemical precipitate, no solid matrix samples were collected from these chambers.

Aqueous samples were also collected from the previously inaccessible bottom of the crushed limestone and cobble chambers and analyzed for total and dissolved metals.

The autopsy on the bioreactors revealed a convoluted biochemical environment that was probably caused by the dramatic change in the AMD chemistry after the first 10 months of operation. The material examined during the autopsy showed the mixed results of processes that were occurring at low pH and a reasonably high load of metals with the subsequent reactions that were characteristic for water of neutral pH laden with much less of the dissolved metals.

Interpretation of monthly monitoring results combined with the autopsy findings allowed for the formulation of a number of conclusions and recommendations, the most essential of which are listed below.

[1] U.S. Patent No. 6,325,923

- The CCS worked very well in preventing settling of the organic matter and ensuring uniform flow of AMD throughout the entire cross section of the organic carbon with no preferential flow paths (channeling).

- Configuring the bioreactors to accommodate flow in a horizontal plane (rather than in the vertical direction) was successful. Problems that were experienced with reductions in flow rate turned out to be associated with the AMD distribution system that was plugged by chemical precipitates. This hindrance, however, is common to both configurations.

- It takes some time for SRB population to be established in the bioreactors. Once established and supplied with organic matter, they maintained a population of E+4 most probable number (MPN)/milliliter or higher in the aqueous phase at temperatures ranging from 2 EC to 16EC.

- The SRB average population of 2.06E+6 MPN/cubic centimeter in the solid matrix of organic matter was two orders of magnitude greater than the SRB population present in the aqueous phase.

- The AMD in the bioreactors was notably stratified with respect to oxidation-reduction potential that was up to 400 millivolts lower at the bottom of the bioreactors than at the top. Because maintaining reduced conditions is required for SRB, the bioreactors should have been more carefully isolated from atmospheric air. A plastic liner placed on the top of bioreactors is preferred over the straw bails used for this project.

- Only Zn, Cu, and Cd were being removed as sulfides due to SRB activities. Changes in concentrations of other metals (Fe, Mn, Al, As), which do not necessarily precipitate as sulfide, seemed to be affected by SRB only in an indirect manner by responding to increased pH caused by SRB activity.

- For the Calliope site climatic and hydrochemical conditions, the thresholds for the removal of Zn, Cd, and Cu were approximately 500 micrograms per liter (µg/L), 5 µg/L, and 80 µg/L, respectively. These thresholds were slightly lower for Bioreactors II and IV than for Bioreactor III, which did not include a pretreatment cell. This indicates that the removal thresholds were dependent on the configuration of the bioreactor but were not affected by the shutdown and freezing of a bioreactor during winter.

- Most of the metal sulfides that were formed due to the SRB activity precipitated within the organic matter. The same seems to be true for the rest of the metals that must have formed hydroxides and carbonate compounds. The role of the cobble chamber was limited to a collection sump for a small mass of precipitates that escaped the organic matter chambers. This demonstrated that there was no need for the large cobble chamber, which could have been substituted with a smaller "trap" sump.

- The abundance of TOC present (20% by weight) in the organic matter chamber at the end of the project demonstrated that the bioreactors would have worked equally efficiently with a much smaller supply of organic carbon, provided the same residence time of AMD was maintained. Since the organic matter mass inhibits permeability, it is prudent to reduce the ratio of organic carbon to the permeability enhancing component (e.g., gravel, shell, etc.) and have more permeable medium.
- Since most of the material that caused plugging was found within and adjacent to the outlets of the AMD distribution system, there was a need to devise a system that would allow for occasional breakdown and removal of that material. Such a system might involve only a few outlets rather than the three dozen used in this design. It may include ports extended to the ground surface that would facilitate blowing in combustion engine exhaust to destroy plugging material that would then be removed by bailing.

Overall, the project documented that SRB technology, as applied in this demonstration, is effective in removing Zn, Cu, and Cd by precipitating them as sulfides. Removal mechanisms for Fe, Al, Mn, and As were overshadowed by a dramatic change of the quality of the influent AMD. The results of the project have also allowed the formulation of an important recommendation regarding the design and construction of SRB bioreactors.

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Acronyms and Abbreviations

ABA	acid-base accounting (analytical method for sulfide)
Al	aluminum
AMD	acid mine drainage
As	arsenic
Ca	calcium (also used as the designation for limestone chamber in figures)
$CaCO_3$	calcium carbonate
CCS	cellular containment system
Cd	cadmium
CO	cobble chamber (used only in tables or figures)
Cu	copper
DM	dissolved metals
DO	dissolved oxygen
DOE	U.S. Department of Energy
E_H	oxidation-reduction potential expressed with reference to standard hydrogen electrode
EPA	U.S. Environmental Protection Agency
Fe	iron
Fe^{2+}	ferrous iron
Fe^{3+}	ferric iron
$Fe(OH)_3$	ferrihydrite
FeS_2	iron disulfide (pyrite)
H_2S	hydrogen sulfide
HCO_3	bicarbonate
HDPE	high density polyethylene
IAG	Interagency Agreement
ICP	inductively coupled argon plasma
Mg	magnesium
mil	1/1,000 of an inch
Mn	manganese
MCL	maximum contaminant level
MPN	most probable number
MSE-TA	MSE Technology Applications, Inc.
MWTP	Mine Waste Technology Program
Or1	organic matter chamber No. 1
Or2	organic matter chamber No. 2
ORP	oxidation-reduction potential measured using silver/silver chloride reference electrode
pH	measure of hydrogen ion activity
PVC	polyvinyl chloride
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
SHE	standard hydrogen electrode
SMCL	suggested maximum contaminant level
SRB	sulfate-reducing bacteria
TCLP	Toxicity Characteristic Leaching Procedure
TOC	total organic carbon
TM	total metals
VFA	volatile fatty acids

Zn	zinc

Acknowledgment

Work was conducted through the DOE National Energy Technology Laboratory at the Western Environmental Technology Office under DOE Contract Number DE-AC22-96EW96405.

1. Introduction

This document is a final report on the performance of sulfate-reducing bacteria (SRB) bioreactors that were constructed and operated for Mine Waste Technology Program (MWTP) Activity III, Project 12, Sulfate-Reducing Bacteria Reactive Wall Demonstration. The MWTP is funded by the U.S. Environmental Protection Agency (EPA) and jointly administered by the EPA and the U.S. Department of Energy (DOE) through Interagency Agreement (IAG) Number DW89938870-01-1 and under DOE contract number DE-AC22-96EW96405.

1.1 Problem Definition

Acid mine drainage (AMD) emanates from many abandoned mines in the Western United States, causing significant environmental problems by contaminating surface waters and groundwater with dissolved metals and raising their acidity. Conventional active treatment of AMD is often not feasible due to the remoteness of the site, the lack of power, and limited site accessibility. Thus, for such sites, there is a need for a passive remedial technology to immobilize metals and increase the pH of the AMD.

Sulfate-reducing bacteria have the ability to increase pH and alkalinity of the water and to immobilize dissolved metals by precipitating them as metal sulfides. Some metals [e.g., aluminum (Al)] are removed as hydroxides due to the increase in pH.

1.2 Principles of the Sulfate-Reducing Bacteria Technology and its Application

Acid mine drainage is a typical result of mining sulfide-rich ore bodies. Acid mine water is formed when sulfide-bearing minerals, particularly pyrite [iron disulfide (FeS_2)], are exposed to oxygen and water as described by the following overall reaction (1-1).

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O \text{ ---> } Fe(OH)_3 + 2SO_4^{2-} + 4H^+ \quad (1\text{-}1)$$

This reaction results in increased acidity of the water (lowered pH), increased metal mobility, and the formation of dissolved sulfate.

When provided with an organic carbon source, SRB are capable of reducing the sulfate to soluble sulfide by using sulfate as a terminal electron acceptor; bicarbonate ions are also produced. The soluble sulfide reacts with some metals in the AMD to form insoluble metal sulfides (Reactions 1-2 and 1-3). The bicarbonate ions increase the pH and alkalinity of the water.

$$SO_4^{2-} + 2CH_2O \text{ -------> } H_2S + 2HCO_3^- \quad (1\text{-}2)$$

$$H_2S + M^{2+} \text{ --------> } MS + 2H^+, \text{ where M = metal} \quad (1\text{-}3)$$

The SRB technology was demonstrated in the field by engineering the SRB favorable conditions within three bioreactors that were fed by AMD emanating from an abandoned mine site (Calliope Mine) in the vicinity of Butte, Montana. The bioreactors, on which construction was completed in November 1998, operated from December 1998 to July 2001 (32 months). Performance of the bioreactors was monitored monthly by sampling and conducting chemical and bacteriological analyses of the influent and effluent of the bioreactors. The bioreactors were decommissioned in July 2001, and samples of solid matrix from the bioreactors were collected and analyzed for chemical, physical, and bacteriological parameters. This report includes a description of the site, reactor design and construction, details of the 32 months of monitoring data, data acquired during the decommissioning of the bioreactor, and data interpretation.

2. Site Description

2.1 Location

This project was conducted at the Calliope mine (Calliope/Mary Emma Mining Claims) located (Figure 2-1) in Silver Bow County, Montana, in NW¼ of SE¼ and SW¼ of NE¼ both of SW¼ of Section 10, T3N, R7W. The majority of the site where the bioreactors were installed is located on the Calliope mining claim, Mineral Survey No. 2972. The Mary Emma mining claim, Mineral Survey No. 5478, which is adjacent to the north of the Calliope, was also used for some installations.

2.2 Acid Mine Drainage Source

The abandoned Calliope mine site includes a collapsed adit discharging water into a large waste rock pile. The exposed volume of the waste rock pile is estimated to be 66,000 cubic yards; however, the approximately 50-foot-tall pile that is visible at the present time may not constitute the entire volume of mine waste disposed at the mine. The bottom part of the pile has probably been covered with fill material that was placed there during the construction of Interstate Highway 15 (I-15) and forms a distinct morphological shelf extending westward from the lower pond and the toe of the present waste rock pile.

The AMD discharging from the collapsed adit is of relatively good quality with the pH ranging from 6.5 to 7. This AMD flows over the top of the waste rock and accumulates in a small, approximately 50-foot-diameter flow-through pond (Upper Pond). Overflowing the Upper Pond, the AMD forms a surface drainage that, flowing on the surface of the waste rock pile, reaches another flow-through pond (the Lower Pond, which is 35 feet in diameter). In the Lower Pond, the AMD mixes with low pH subsurface seepage that enters the pond along its banks and through its bottom. This subsurface seepage is fed by atmospheric precipitation that infiltrates the waste rock pile and reappears on the surface at the toe of the pile. This seep is enriched in metals with a pH ranging from 2.6 to 3.6. Under natural conditions, the Lower Pond overflows and drains to an adjacent gulch. For this project, approximately 20% of the AMD that flows through the Lower Pond was diverted for treatment in three engineered bioreactors that were built at the site to demonstrate the SRB technology.

The quality of the Lower Pond water and its pH are related to the amount of atmospheric precipitation that infiltrates into the waste rock pile and leaches metals. With the exception of the first 8 months of bioreactor operation, atmospheric precipitation was well below normal. Consequently, the quality of the Lower Pond water significantly improved after the first 10 months. Table 2-1 includes analytical information on the water quality of the influent to the bioreactor system.

2.3 Bioreactor Layout and Configuration

All three bioreactors (denoted II, III, and IV) were designed and constructed in parallel downstream from the Lower Pond (Ref. 1) (Figure 2-2). This allowed the AMD to be piped to and treated by the bioreactors using gravity flow. The bioreactors, constructed in the fall of 1998, were designed to evaluate the SRB technology applied in slightly different environmental conditions. Bioreactors II and III were placed below ground, and Bioreactor IV was placed above ground. The belowground bioreactors were built to minimize temperature changes and to prevent freezing. The aboveground bioreactor was built to evaluate the effect of cold weather and freezing on the system. In addition, Bioreactors II and IV were built with a pretreatment section to evaluate the effect on the efficiency of the SRB to improve pH and oxidation-reduction potential (E_H). Due to budget constraints, Bioreactor I was not constructed.

Each bioreactor was filled with a combination of organic matter, crushed limestone, and cobbles placed in two or four discrete chambers (Figure 2-2). The first two chambers of

Bioreactors II and IV constituted the pretreatment section and included a chamber filled with organic matter and a chamber filled with crushed limestone. Following the pretreatment section was a primary treatment section that included a chamber with organic matter and a chamber filled with cobbles. A pretreatment section was not included in Bioreactor III in order to evaluate its contribution to overall bioreactor efficiency by comparison with Bioreactor II.

Each media component was expected to play an important role in the treatment train:

- organic matter was the nutrient (the electron donor) for the SRB;
- for the pretreatment section, a chamber with organic matter was included to lower the E_H of AMD;
- crushed limestone provided buffering capacity to increase the alkalinity of AMD in the pretreatment section; and
- cobbles placed in the last chamber of each bioreactor were to provide a stable surface for bacterial attachment.

Bioreactors II and III, 71.5 feet and 61 feet in length respectively, were constructed below ground in 14-foot-wide trapezoidal (4-foot-wide bottom) trenches. Bioreactor IV, 72.5 feet in length, was constructed in a 12-foot-wide metal half-culvert elevated above ground. The chambers filled with organic matter or limestone were each 5 feet in length, whereas the chambers filled with cobbles were 50 feet in length.

Table 2-1. Acid Mine Drainage Analytical Data and Target Concentrations for Bioreactors

Analyte	Concentration (µg/L)			Comments
	AMD (maximum)	AMD (minimum)	Target Effluent	
Aluminum	14,100	11.0	1,000	50 to 200 µg/L*
Cadmium	41.9	3.1	5	5 µg/L**
Copper	3,050	2.8	100	1,300 µg/L**
Iron	8,670	8.0	1,000	300 µg/L***
Manganese	3,770	690	2,000	50 µg/L***
Zinc	11,100	990	4,000	5,000 µg/L***
Arsenic	10.9	1.1	NA	50 µg/L**
Sulfate	229,000	69,800	NA	250,000 µg/L***
pH	7.52	3.29	6 to 8	

*Suggested maximum contaminant level (SMCL)
** Maximum contaminant level (MCL)
***Secondary maximum contaminant level
NA = not applicable

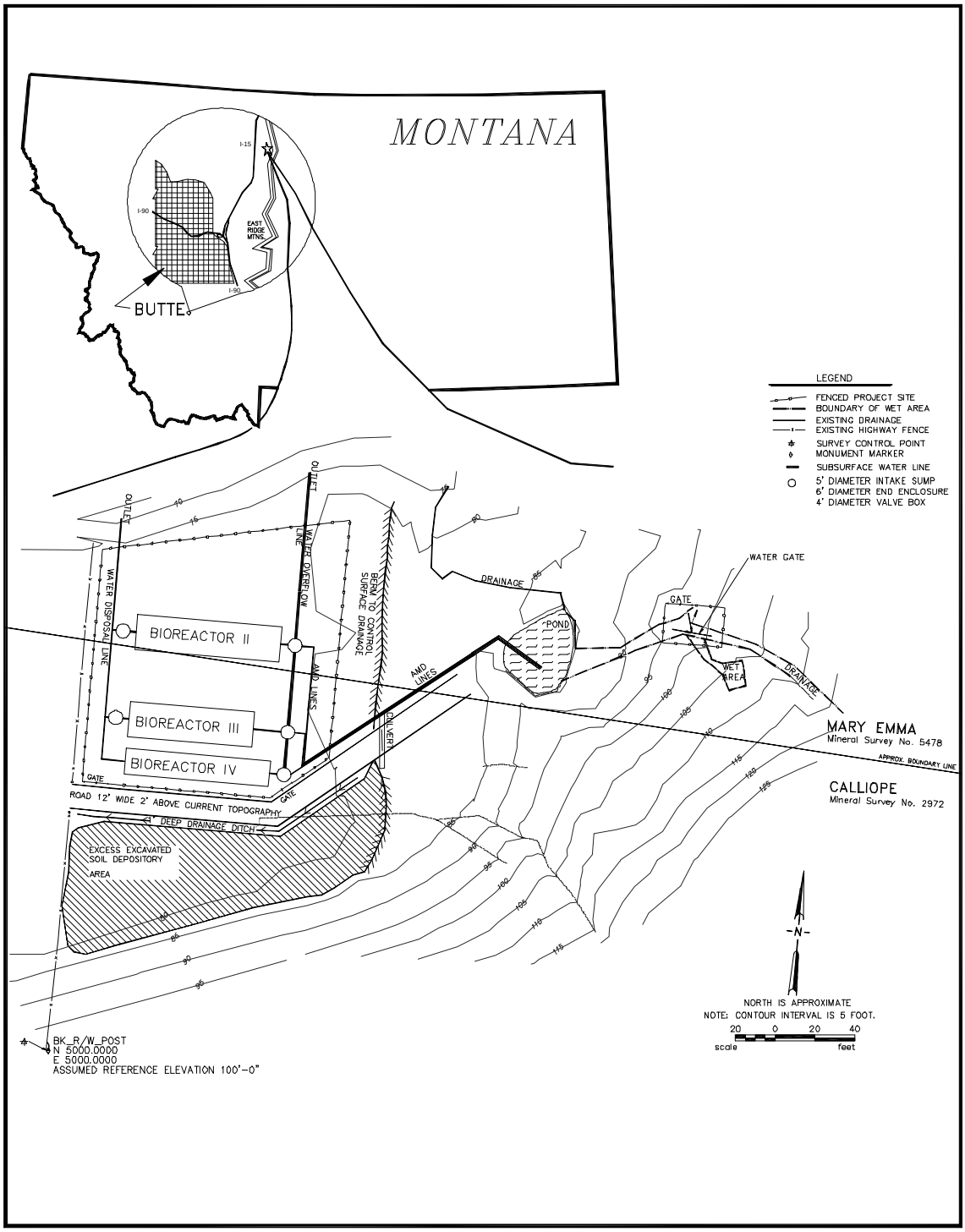

Figure 2-1. Calliope site location map.

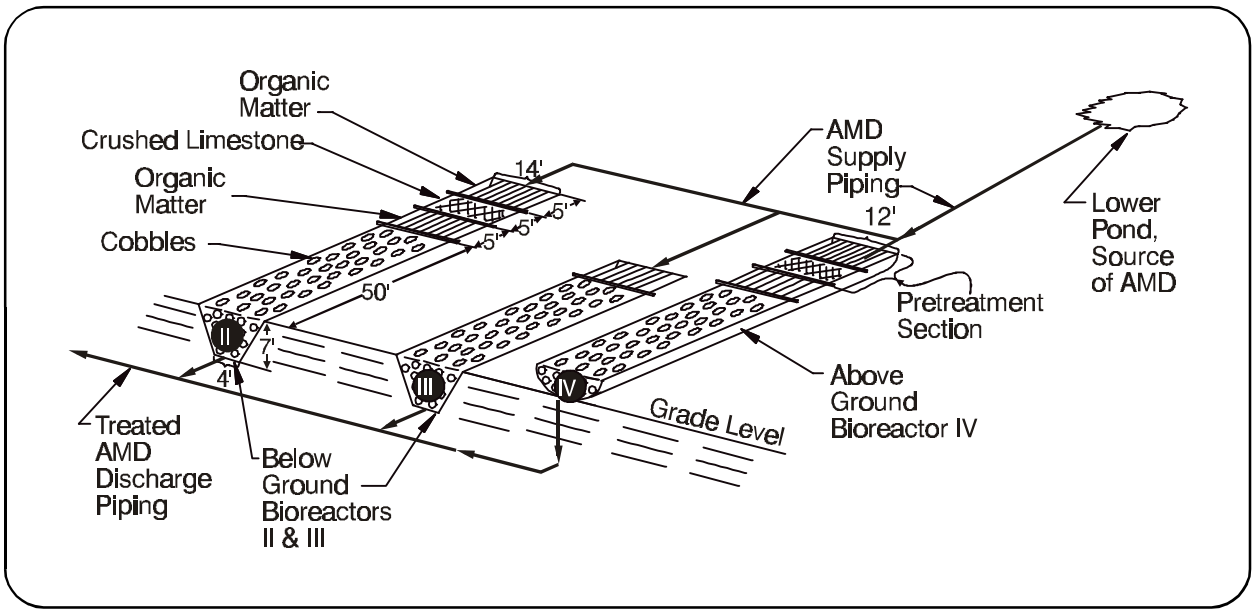

Figure 2-2. Layout of the bioreactors.

3. Design and Construction

3.1 Design Requirements

Several functional and operational constraints were identified before the design of the bioreactors began. The most important constraints are listed below with their design solutions.

- The entire system needed to be passive. This condition was satisfied allowing for gravity flow by incorporating the site topography and flow control instrumentation into the design.

- Construction of the bioreactors had to allow for investigation of the impact of subfreezing temperature on SRB activity. This requirement was satisfied by designing an aboveground bioreactor with features similar to one of the belowground bioreactors.

- Construction of the bioreactors had to allow for control of the water level to simulate seasonal droughts, if deemed appropriate. This requirement was achieved by constructing intake sumps where the hydraulic head could be controlled through a system of valves and overflow piping.

- The chambers with organic matter had to be designed so they fostered permeation of the AMD through the entire cross-sectional area (without channeling) and prevented settling of the medium. A cellular containment system[1] (CCS) was built into the organic carbon chambers to satisfy these requirements.

3.2 Construction

Construction activities at the Calliope began in August 1998 and were completed in October 1998. Excavation and grading for construction of all bioreactors and associated piping was completed while maintaining the existing slope grade of 2.5%. This grade was maintained to provide for natural runoff in the SRB construction area and, more importantly, to avoid the influx of surface runoff into the belowground bioreactors. Figure 3-1 is a simplified longitudinal cross section through the bioreactors. Bioreactor III differs from the other two bioreactors by having no pretreatment section (i.e., it consists only of one organic matter chamber and a chamber with cobbles).

3.2.1 Materials Used

All bioreactors were constructed with similar materials. Whenever possible, off-the-shelf, acid-resistant building materials were used. These materials and their use are described below.

- Schedule 40 polyvinyl chloride (PVC) piping, nontreated finished lumber, 40-mil woven geotextile, and 40-mil PVC liner. The PVC liner, sandwiched between woven geotextile, was used for lining the bottom and the sides of each bioreactor.

- A heavy gauge, multiple section, galvanized steel half culvert with fabricated steel end-walls was assembled to form an elevated trough to house the aboveground bioreactor.

- Precast reinforced concrete was used for the inlet and outlet walls for the belowground bioreactors. Precast reinforced concrete was also used for the intake sumps, and the end enclosures required for construction of the belowground bioreactors.

- TerraCell™ material, commonly used in landscaping for slope stabilization, made of high density polyethylene (HDPE), was used to form a CCS to house and support the organic matter. Each layer (lift) of TerraCell™ was 6 inches high and contained 11-inch by 8.5-inch rhombohedral-shaped cells.

- The organic matter was provided as an 80% to 20% by volume mix of cow manure and cut straw. The cut straw was added to provide "secondary" porosity to the mix and prevent settling of the medium.

[1] U.S. Patent No. 6,325,923

- The mixture of cow manure and straw was installed in the CCS, which consisted of 10 lifts of TerraCell™ material (Figure 3-2) and would limit settling (if it occurred) of the organic matter to each individual cell. The TerraCell™ lifts were positioned at 60 degrees off the horizontal plane so the cells of each lift would be partially offset (only partially overlapping) with respect to the cells of adjacent lifts. Such a configuration promoted migration of AMD along the organic matter chamber in a wavy-shaped flow line and facilitated the packing of each individual cell with the organic matter. The TerraCell™ material (lifts) was individually fastened to a grid of 2-inch by 4-inch lumber positioned at the top of the organic matter chamber. The grid was supported by 6-inch by 6-inch wood beams positioned across the reactor above its top.

- The limestone chambers of Bioreactors II and IV contained crushed limestone that was 3 to 5 inches in size. This crushed limestone was placed directly on the last CCS lift of the first organic matter chamber. The front face of the limestone chamber also sloped 60 degrees off the horizontal plane to provide a support surface for the second organic matter chamber.

- Cobbles (mostly granodiorite), which were 3 to 5 inches in diameter, filled the remaining portion of the bioreactors.

- Two lifts of straw bales, sized 16 inches by 18 inches by 48 inches and placed on top of the belowground bioreactors, were used to create a 32-inch-thick layer to provide thermal insulation. Only one lift of straw bales was used for the aboveground bioreactor.

3.2.2 Belowground Bioreactors

The belowground bioreactors, II and III, were constructed in lined trapezoidal cross-section trenches. The liner system, which was the same for the below- and aboveground bioreactors, consisted of a 40-mil PVC liner sandwiched between two layers of a 40-mil woven geotextile. The latter was used to provide additional abrasion resistance for the PVC liner for all subsequent construction activities.

Steel-reinforced cement end-walls, with the appropriate piping penetrations, were precast at the site and installed on top and behind the liner system for the AMD inlet and outlet, respectively. Unique to the construction of the belowground reactors was the embedding of a 2-inch inlet manifold for the AMD distribution system (Figure 3-3) into the 60-degree precast inlet end-wall. This distribution system allows the AMD to enter the bioreactors and flow evenly throughout the CCS.

A precast reinforced concrete intake sump to control water levels within the bioreactor was installed directly upgradient of each bioreactor (Figure 3-4). Water levels within the bioreactor were controlled via the intake sump. A hose, connected to an overflow drain line, was installed in all intake sumps to control (raising or lowering) the water level in each bioreactor. Water was piped from these intake sumps to the bioreactor manifold distribution systems.

Precast reinforced concrete end enclosures (i.e., large manholes) were installed at the end of the belowground bioreactors to control flow rate and to house equipment to monitor bioreactor performance. Effluent from the bioreactors was piped from the end enclosures and applied to the land surface in the gulch (see Figure 3-5).

3.2.3 Aboveground Bioreactor

The aboveground bioreactor (IV) was constructed using multiple sections of a heavy gauge, galvanized steel culvert with fabricated steel end-walls. Individual sections were joined together using carriage bolts. The aboveground bioreactor was lined in a manner similar to the belowground bioreactors.

Embedding the manifold in this reactor was not possible because the end-wall was fabricated from steel; therefore, the inlet manifold was placed on top of the liner system and supported with wooden blocking material placed on the 60-degree end-wall under the liner system. An end enclosure was not

required for this reactor as it was constructed above ground. Other features including flow control and monitoring equipment and the intake sump were like those used for the belowground bioreactors.

3.2.4 Sampling Locations and Ports

The bioreactors were equipped with a number of sampling ports to monitor performance of each bioreactor. The ports were either installed at the selected locations of the piping system that was assembled to supply the bioreactors with AMD and to discharge the treated effluent (see Figure 3-5) or were constituted by piezometers that were installed within the body of the bioreactors (Figure 3-1).

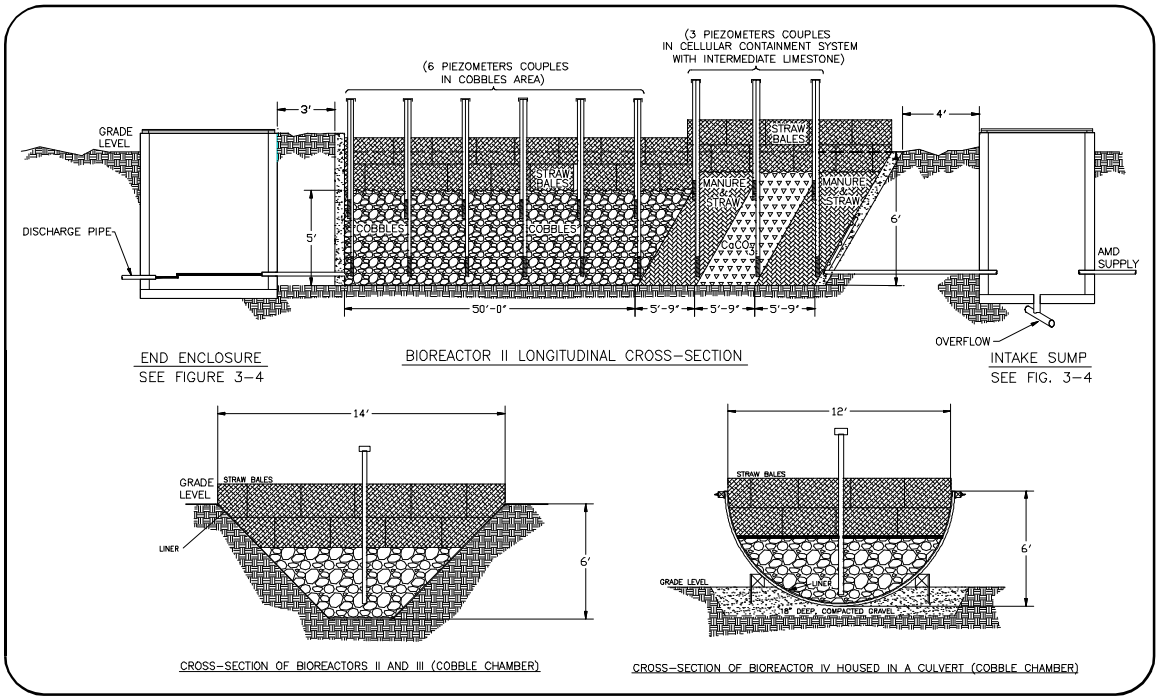

Figure 3-1. Simplified cross-sections of Bioreactors II and IV.

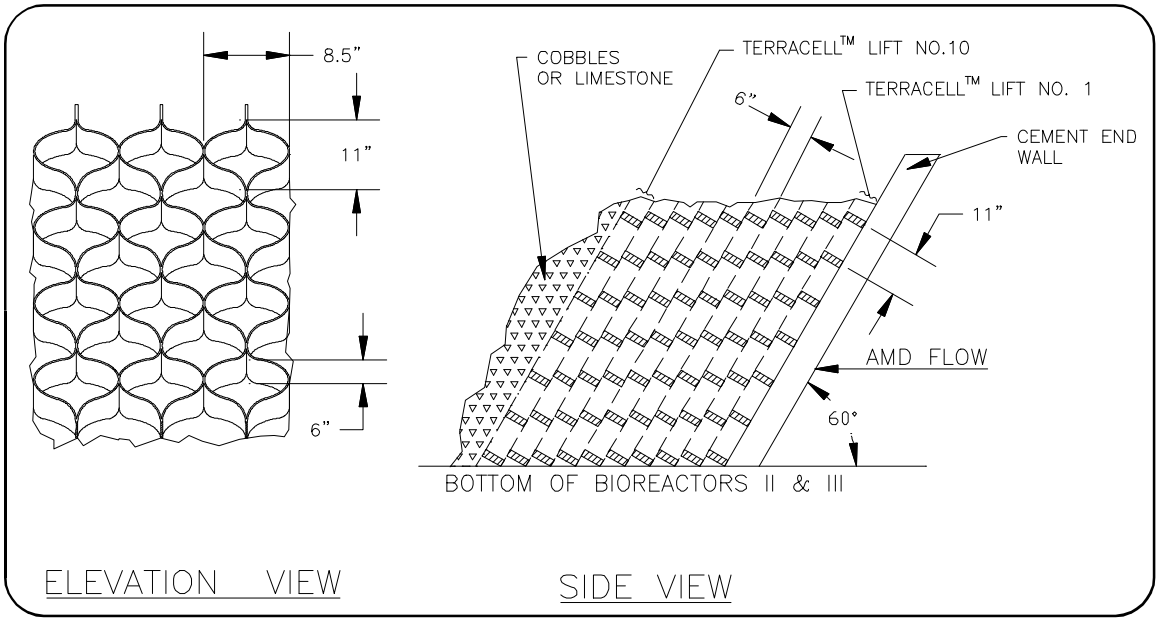

Figure 3-2. Cellular containment system for organic substrate.

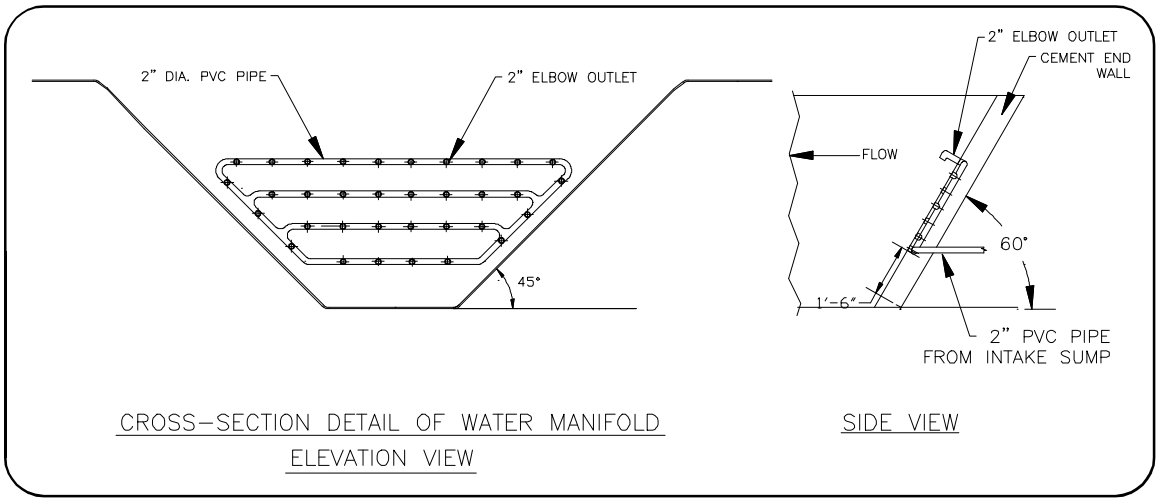

Figure 3-3. AMD distribution system manifold.

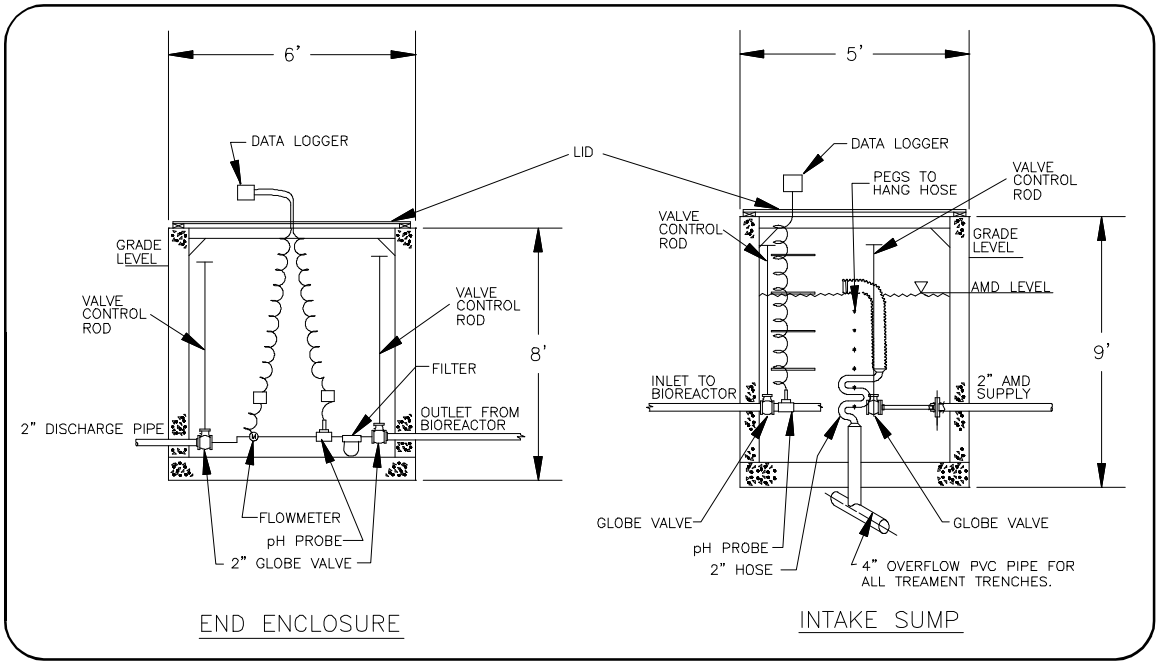

Figure 3-4. End enclosure and intake sump for Bioreactors II and IV.

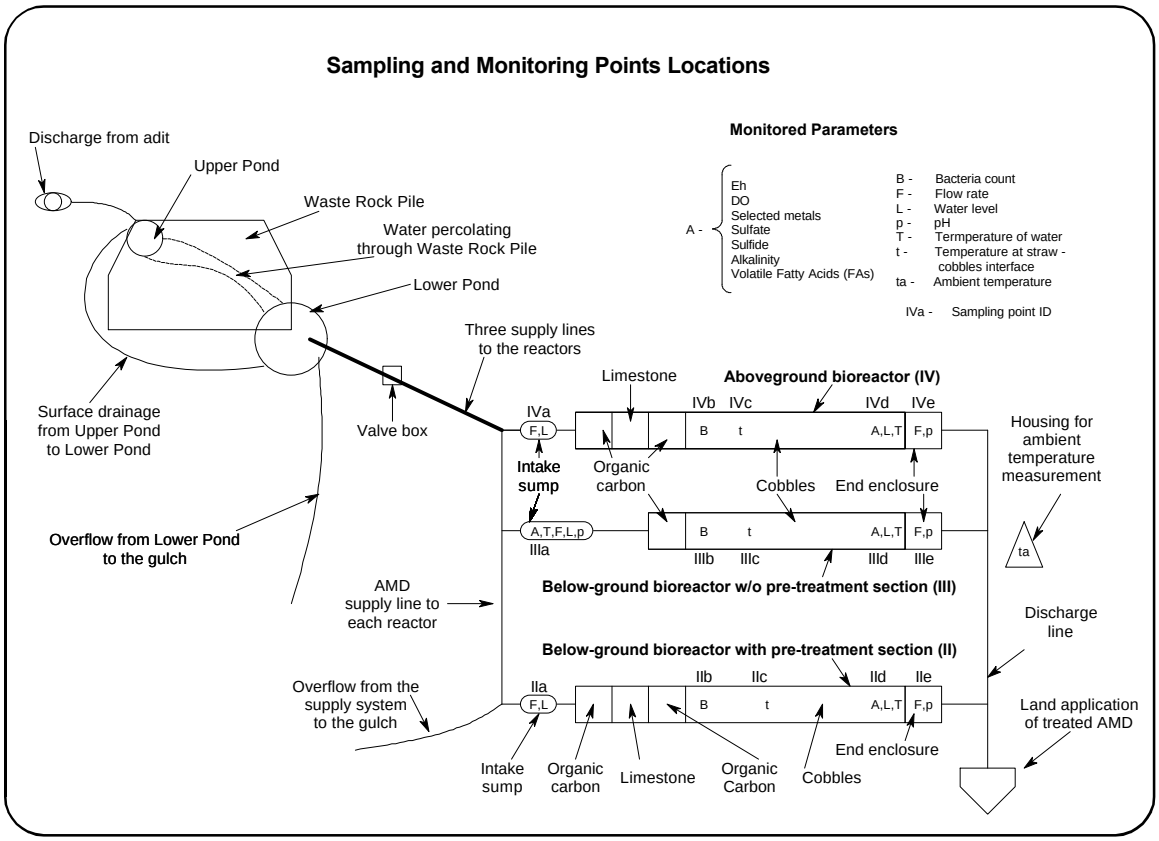

Figure 3-5. Sampling and monitoring point locations.

4. Operation

4.1 Flow Rates

The bioreactors operated from December 1998 to July 2001 (32 months). The two belowground bioreactors (II and III) were designed to flow year-round. The aboveground bioreactor (IV) was designed to be shut down for winter to let it freeze while full of AMD. The reactors flowed at a rate of 1 gallon per minute (gpm) for the majority of time (Figure 4-1 and Table 4-1a). For 4 months in the summer of 2000, the flow rate was doubled to nearly 2 gpm. Although the flow for Bioreactor IV was shut down for the winter, a center portion of this bioreactor did not freeze due to a small (0.05-gpm) leak through the liner that must have been inadvertently perforated during construction. Although the location of the leak was not defined, the rate of the leak was determined by measuring the water level changes with the valve on the influent closed.

A flow rate of 1 gpm corresponds to a 5-1/2 day calculated residence time for the AMD in Bioreactors II and IV and a 4-1/2 day residence time in Bioreactor III. The residence time of the AMD in a single organic matter chamber was approximately 10 hours for the flow rate of 1 gpm.

Flow through Bioreactors III and IV was maintained as desired for most of the demonstration. However, the flow rate through Bioreactor II started to decrease in May 1999 and ceased at the beginning of June 1999. The flow rate was restored in July 1999 after the upgradient cell with organic matter was chemically treated and blown out with air using an air compressor to remove biofouling and associated plugging. Similar behavior in Bioreactor II was observed again in May 2000. In this case, the permeability of the upgradient chamber was restored by sparging it with combustion engine exhaust. Cessation of flow in Bioreactor II (indicated in Figure 4-1) in March 2001 was actually caused by sediment that accumulated within the inlet valve. The flow was restored by cleaning the valve.

4.2 Acid Mine Drainage Levels

Acid mine drainage levels (or water levels, as it is also referred to in this document) in the bioreactors were controlled by setting their levels in the intake sumps. In general, water level was maintained to just below the top surface of the 5-foot-thick layer of cobbles. A diagram of water level changes in the bioreactors is presented in Figure 4-2, where water level elevations are plotted with reference to the bottom of each bioreactor at its outlet. For Bioreactor II, which experienced two plugging episodes, the water level dropped close to the outlet level, as shown in Figure 4-3, for the episode on June 1999. In each case, most of the water level drop took place between the inlet sump and the first organic matter chamber, indicating plugging within or immediately adjacent to the AMD distribution system. Numerical values of flow and water level measurement for the diagrams in Figures 4-2 and 4-3 are compiled in Table 4-1a and 4-1b, respectively.

4.3 Sampling and Performance Monitoring

Performance of the bioreactors was monitored monthly by taking measurements manually and collecting samples of influent and effluent for chemical analysis. All aqueous samples were collected and then analyzed by the HKM Analytical Laboratory following the quality assurance project plan (QAPP) (Ref. 2). In general, samples were submitted to the laboratory as raw water with the exception of samples for dissolved metals. All samples were preserved as required by the QAPP.

In addition, an attempt was made to monitor the pH, temperature, flow rate, and water level of the influent and effluent using stationary transducers or sensors and recording the measurements using data loggers. The recoding interval was set for 30 minutes during the first 8 months of operation and for every 4 hours thereafter. However, the reliability of the transducer-generated measurements was unacceptable due to either

deterioration of the signal because of organic and/ or chemical coating or repetitive failures of the data loggers. Therefore, the performance reported in this document is based on the records derived through monthly sampling events. The list of measured or analyzed parameters is include in Table 4-2, which also includes references to the sampling locations and monitoring ports shown in Figure 3-5.

4.4 Decommissioning

The site was decommissioned beginning in July 2001 in accordance with regulatory guidelines and requirements imposed by the Montana Department of Environmental Quality. The majority of components of the system were either removed from the site or abandoned in place (if they were located in the subsurface), and the site was restored to its predemonstration condition. The only infrastructure remaining at the site are two subsurface inlet sumps and the subsurface piping system to feed the inlet sumps with AMD from the Lower Pond. This infrastructure is currently used for investigations conducted for MWTP Project 24, Improved SRB.

The decommissioning process also included autopsy sampling of the interior of each reactor to evaluate how the SRB material was used and if undesired preferred flow paths were developed. The autopsy sampling was neither included in the project work plan (Ref. 3) nor in the project QAPP (Ref. 2); it was conducted as a value-added investigation to the project to substantiate recommendations for technology improvements and enhance lessons learned conclusions.

Autopsy sampling focused on the collection of solid matrix material that was inaccessible during the operational phase of the project. The majority of this visual inspection and sample collection took place within 30 feet of the respective AMD intake to the reactors (i.e., in chambers containing organic matter and limestone). Cobble chambers were inspected adjacent to the respective organic matter chambers.

Consistent with the above sampling needs, the cobble chambers for Bioreactors II and III were abandoned in place. Organic matter and limestone was excavated, examined, and reburied in place. All material of aboveground Bioreactor IV was removed from the half-culvert and examined in the same manner as the material from Bioreactor II. After examination and sampling, the material from Bioreactor IV (organic matter, limestone, cobbles) was spread on or near the existing waste rock pile. Other materials (e.g., lumber, TerraCell™, aboveground portions of inlet and outlet enclosures, PVC monitoring pipes, and metal culvert) were salvaged or disposed in a local landfill. Underground pipes that were left in place were plugged. The straw bales covering the bioreactors were used for mulch associated with the final restoration of the site.

4.4.1 Sampling

Autopsy sampling included collecting solid matrix samples for chemical analyses to determine concentrations of total metals [aluminum (Al), arsenic (As), cadmium (Cd), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), zinc (Zn)], sulfate, sulfide, nitrogen, phosphorous, and total organic carbon (TOC) in the organic matter and limestone chambers. The EPA Toxicity Characteristic Leaching Procedure (TCLP), Method 1311 (EPA SW-846) was used for samples of the organic matter retrieved from the upstream organic matter chamber. Bacteriological analyses were conducted to determine the SRB population in the organic matter and in the limestone. Because the cobbles did not have a visually discernible film of bacteria or chemical precipitate, no solid matrix samples were collected from this chamber.

Aqueous samples were collected from the bottom of the limestone and cobble chambers and analyzed for the same total metals as those listed for the samples of solid matrix retrieved from the organic matter chamber. The aqueous samples from the cobble chambers were also analyzed for dissolved metals that included Al, As, Cd, Cu, Ca, Fe, Mg, Mn, and Zn. The analytical work performed for the autopsy phase is summarized in Table 4-3.

Table 4-3 indicates that the analytical work performed for Bioreactors II and IV was identical. Each upstream organic matter chamber (Or1) of Reactors II and IV had 5 samples of organic matter recovered for chemical analyses from the 10 CCS lifts that made up each chamber. The samples were collected from the center portion of the lifts, starting with lift No. 9 and then every other lift. Two additional organic matter samples (one from lift No. 9 and one from lift No. 2) were recovered from each Or1 and analyzed for SRB population. A single organic matter sample was collected from Or1 and analyzed by TCLP. Thus, samples from eight locations were collected from each upstream organic matter chamber.

A single organic matter sample (lift No.5) was recovered from each downstream organic matter chamber (Or2) and analyzed for the identical constituents as the samples collected from the Or1 chamber, including the SRB population count but excluding the TCLP.

Each limestone chamber was sampled for a nonaqueous sample (a precipitate coating on the crushed limestone) and an aqueous sample (stagnant water from the base of the chamber). The suite of analyses for the nonaqueous sample was the same as for the samples collected from the Or2 chamber. An aqueous sample collected from each limestone chamber was analyzed for total metals, sulfide, and sulfate only.

For the cobble chambers, stagnant water that accumulated near the base of the bioreactor was stirred, and one aqueous sample for each bioreactor was collected and analyzed for total and dissolved metals, sulfide, and sulfate.

Analytical work performed for Bioreactor II was identical as that for the upstream chamber of organic matter (Or1) and the cobble chamber of Bioreactors II and IV.

Table 4-1a. Flow and Water Level Measurements

	Bioreactor II		Bioreactor III		Bioreactor IV	
Date	**Flow (gpm)**	**Water level (ft from bottom of Bioreactor)**	**Flow**	**Water level (ft from bottom of Bioreactor)**	**Flow**	**Water level (ft from bottom of Bioreactor)**
12/11/98		4.15		4.5		4.58
12/14/98	0.974		0.557		0.798	
12/22/98	0.822		0.633		0	
1/4/99	0.998		1.008		0	
2/3/99	0.827		0.859		0	
2/4/99		4.03		4.41		4.87
3/3/99	0.996		1.04		0	
4/5/99	0.99		0.696		0	
5/5/99	0.611		0.537		0	
5/7/99		1.63		3.57		4.62
5/20/99		1.42		4.09		4.66
6/3/99	0		0.578		0	
6/25/99		3.81		4.38		4.53
6/27/99		3.55		4.28		4.48
7/6/99		3.15		4.06		
7/12/99	0.191		0.954		0.861	
7/14/99		2.8		3.88		3.7
7/15/99		2.66		3.8		
7/19/99		6.43		5.46		
7/21/99		5.36		5.54		
7/30/99		4.62		5.43		4.49
8/11/99	1.064	4.13	0.802	5.33	0.487	4.31
8/20/99		3.4		5.38		4.1
8/29/99		2.63		3.66		3.92

Table 4-1a. Flow and Water Level Measurements

	Bioreactor II		Bioreactor III		Bioreactor IV	
Date	Flow (gpm)	Water level (ft from bottom of Bioreactor)	Flow	Water level (ft from bottom of Bioreactor)	Flow	Water level (ft from bottom of Bioreactor)
9/1/99		5.41		4.08		4.06
9/8/99	1.002	5.43	1.063	2.92	1.012	4.69
9/21/99		4.89		4.51		4.2
9/24/99		4.92		4.47		4.13
9/27/99		5.34				
10/7/99	0.947		0.962		1.273	
10/26/99		5.06		3.96		4.37
11/9/99	0.862	4.97	1.004	3.81	0.537	4.48
12/8/99	0.868	4.99	0.921	3.74	0	4.12
1/6/00	0.877		0.763		0	
1/10/00		4.6		3.66		4.45
2/3/00	0.976		0.91		0	
2/8/00		4.03		3.59		4.49
3/6/00	0.789	2.66	0.809	3.27	0	4.61
3/16/00		6				
4/5/00	1.009	5.67	0.602	3.58	0	4.43
5/8/00	0.863	3.98	1.019	5.57	0	4.68
5/25/00		2.49				
5/28/00		4.28				
6/7/00	1.071	3.29	1.89	4.05	0	4.61
6/14/00		2.89				4.51
6/16/00		1.93				4.35
6/18/00		0.47				
6/30/00		4.95				
7/5/00		4.63				
7/6/00	1.868	4.46	1.793	4.3	1.964	4.32
8/10/00	1.324	4.12	1.104	4.21	1.693	4.18
9/7/00	1.969	2.57	2.116	4.58	2.528	3.28
9/25/00	1.247	4.38	1.823	4.55	2.048	3.71
10/31/00	1.428	4.62	1.105	4.59	1.278	4.44
12/4/00	1.029	5.21	0.715	4.53	0	3.96
1/8/01	0.891	5.19	0.897	4.63	0	4.26
2/12/01	0.815	4.3	0.916	4.58	0	3.83
3/14/01	0.883	0.84	0.754	4.35	0	4.28
3/27/01	0				0	
4/4/01	0				0	
4/9/01	0.983		0.886		0	
5/9/01	0.854	4.81	0.752	4.06	0	4.41
6/6/01	0.812	4.61	0.706	3.9	0.917	4.54
7/9/01	0.919	4.24	0.786	3.75	0.988	4.49

Table 4-1b. Water Level Profile in Bioreactor II

Point	Water level - feet above bottom of Bioreactor II 12/11/1998	05/20/1999
influent sump	5.01	4.96
II fd	4.94	2.93
II gd	4.66	2.62
II hd	4.66	2.01
II bd	4.66	1.92
II cd	4.66	1.93
II id	4.65	1.92
II jd	4.66	1.93
II kd	4.64	1.92
II ld	4.65	1.92
II dd	4.65	1.91

Table 4-2. Monitored Parameters and Analytes

Measurement	Planed Sample Frequency[1]	Sample Location[2]	Reported frequency and/or comments
Dissolved metals (Al, As, Cd, Cu, Fe, Zn, Mn)	days 10, 20, 30, every month thereafter	IIIa, IId, IIId, IVd	As planned
Alkalinity, SO_4, E_H, DO, Soluble sulfide $(HS)^-$	days 10, 20, 30, every month thereafter	IIIa, IId, IIId, IVd	As planned
Volatile fatty acids (VFA)	days 10, 30, every 3rd month thereafter	IIIa, IId, IIId, IVd	Increased to every month
pH	every half-hour	IIIa, IIe, IIIe, I'VE	Monthly; probe got coated
SRB count	days 10, 30; every 3rd month thereafter	IIb, IIIb, IVb	Increased to every month
Water temperature	every half-hour	IIIa, IId, IIId, IVd	monthly; data logger malfunctioned
Flow rate	every half-hour	IIe, IIIe, I'VE	monthly; data logger malfunctioned
Water level	every half-hour	IId, IIId, IVd	monthly; data logger malfunctioned
Air temperature at straw/rock interface	days 10, 20, 30; every month thereafter	IIc, IIIc, IVc	Incomplete data; probes corroded
Ambient air temperature	every half-hour	Ambient temperature housing	No records; instrument broke[3]

[1] Samples for Bioreactor IV were not taken during the months in which freezing occurs
[2] See Figure 3-5 for locations
[3]Addressed in Section 8, Quality Assurance/Quality Control

Table 4-3. Analysis for the Autopsy of the Bioreactors

Total No. of analyses	Analyzed parameter	Reactors												
		IV (Aboveground) Chamber				Total	III Chamber		Total	II Chamber				Total
		Or1[4]	Ca[5]	Or2[4]	CO[6]		Or1	CO		Or1	Ca	Or2	CO	
3	TCLP[1]	1[10]				1	1[10]		1	1[10]				1
24	TM[2]	5[10]	2[11,12]	1[10]	1[11]	9	5[10]	1[11]	6	5[10]	2[11,12]	1[10]	1[11]	9
3	DM[3]				1[11]	1		1[11]	1				1[11]	1
24	Fe Spc[7]	5[10]	2[11,12]	1[10]	1[11]	9	5[10]	1[11]	6	5[10]	2[11,12]	1[10]	1[11]	9
24	Sulfide[8]	5[10]	2[11,12]	1[10]	1[11]	9	5[10]	1[11]	6	5[10]	2[11,12]	1[10]	1[11]	9
24	Sulfate[9]	5[10]	2[11,12]	1[10]	1[11]	9	5[10]	1[11]	6	5[10]	2[11,12]	1[10]	1[11]	9
19	N[13]	5[10]	1[12]	1[10]		7	5[10]		5	5[10]	1[12]	1[10]		7
19	P[14]	5[10]	1[12]	1[10]		7	5[10]		5	5[10]	1[12]	1[10]		7
19	TOC[15]	5[10]	1[12]	1[10]		7	5[10]		5	5[10]	1[12]	1[10]		7
10	SRB	2[10]	1[12]	1[10]		4	2[10]		2	2[10]	1[12]	1[10]		4
169						**63**			**43**					**63**
Number of Sample Locations		8	3	2	1	14	8	1	9	8	3	2	1	14

1) Toxicity Characteristic Leaching Procedure, EPA Method 1311 (samples of organic matter)
2) Total metals, EPA Method 6010A/Method 3005
3) Dissolved metals, EPA Method 6010A/Method 3005
4) Or1[4] and Or2[4] the upstream and downstream organic matter chambers, respectively (see Figure 2-3)
5) Limestone chambers (see Figure 2-3)
6) Cobble chambers (see Figure 2-3)
7) Fe Spc (Iron Speciation) (Standard Method 3500-Fe);
8) Sulfide, EPA Method 376.1
9) Sulfate, EPA Method 375.2
10) Organic matter (nonaqueous)
11)Aqueous sample (2[11,12] -limestone/cobble chambers - one of two samples is aqueous)
12) Nonaqueous sample (film, slime, or precipitate coating),
(2[11,12] - limestone/cobble chambers-one of two samples is nonaqueous)
13) Total nitrogen
14) Phosphorous
15) Total organic carbon

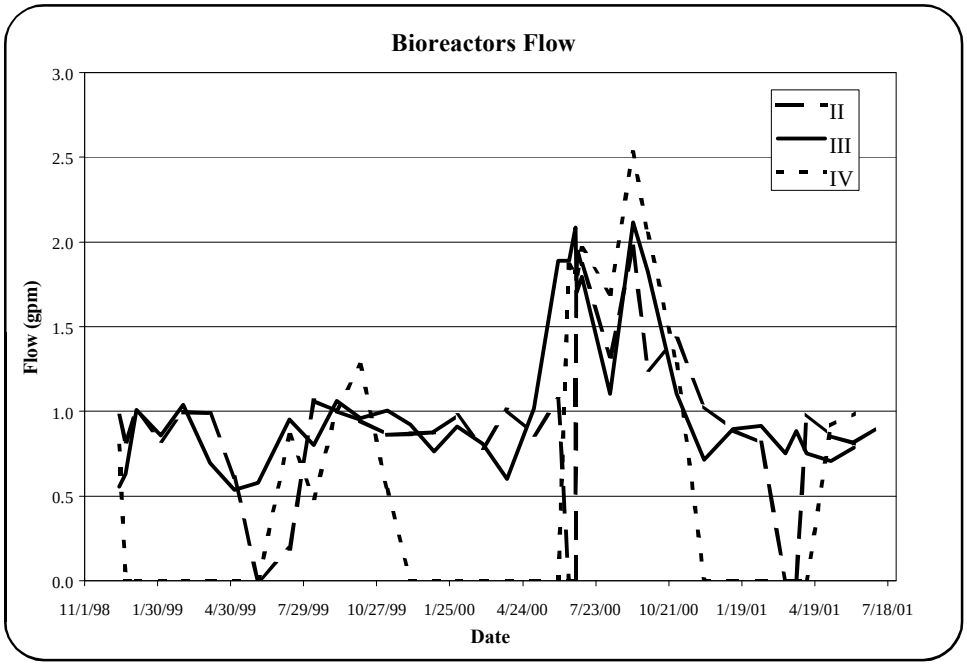

Figure 4-1. Bioreactor flow.

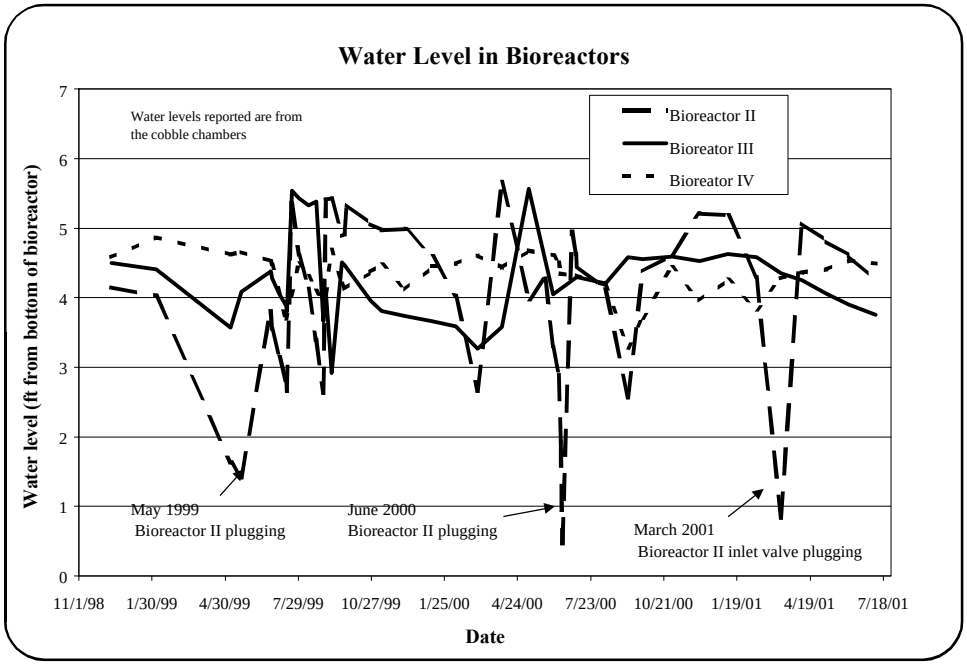

Figure 4-2. Water level in the bioreactors.

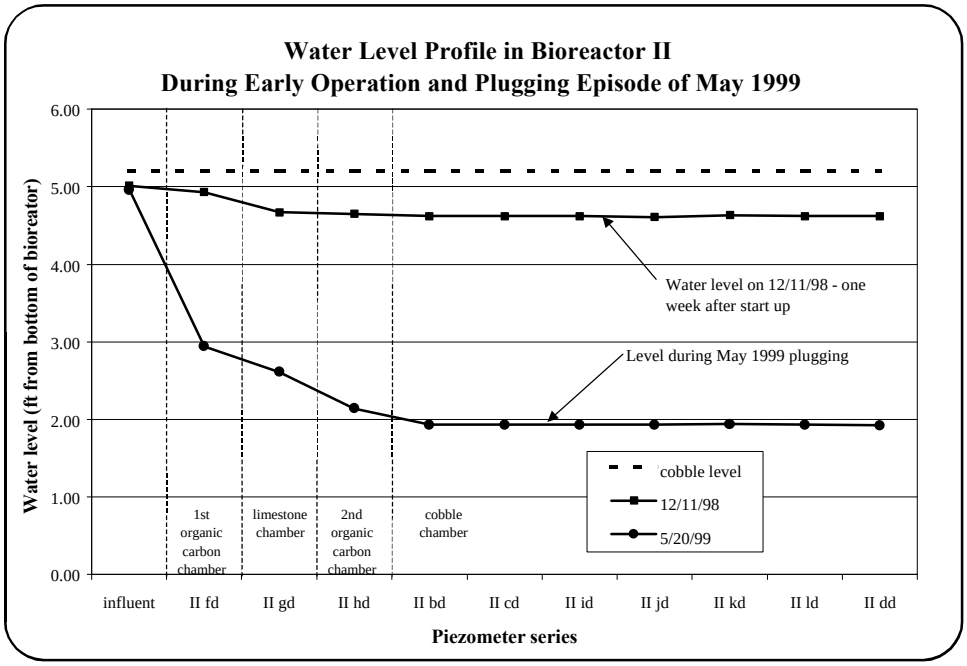

Figure 4-3. Water level profile in Bioreactor II during early operation and the plugging episode of May 1999.

5. Operation Phase Results

Results reported in this section include measurements and analyses performed on the aqueous samples. They include parameters measured in the field, bacteriological analyses of the SRB population, and chemical analyses for sulfate, sulfide, alkalinity, and selected dissolved metals.

5.1 Field Parameters

Field measurements are compiled in Tables 5-1 and 5-2 and are presented in a graphical form in the figures that are individually referred to in this section of the report. Other field parameters (i.e., the flow rate and water levels in the bioreactors) are presented in Section 4 together with other information regarding operation of the bioreactors.

5.1.1 pH, E_H, Dissolved Oxygen, and Temperature

Diagrams 5-1, 5-2, 5-3, and 5-4 present pH, E_H, dissolved oxygen (DO), and temperature, respectively, for the influent and effluent from each bioreactor during the 32-month operation period. These results are also tabulated in Table 5-1.

As mentioned in Section 2.2, the quality of water in the Lower Pond improved significantly after the first 10 months of operation. This is also evidenced in the pH trends diagram (Figure 5-1) where pH of the intake water (Lower Pond AMD) increased from a minimum value of 3.29 in May 1999 to 7.14 in January 2000 and then stayed at this or a slightly lower level for the duration of the project, with the exception of the spring months.

The initial increase in effluent pH (including June 1999) can largely be attributed to alkalinity present within the organic matter rather than to the presence of limestone. This is indicated by an insignificant pH difference in the effluent from Bioreactors II and III, the latter having no limestone pretreatment chamber. As the SRB became established and the effluent pH from each bioreactor dropped to 8, 7.5, and 7 for Bioreactors IV, II and III respectively, the pH differential between the influent and effluents could be attributed to SRB activity. The slightly lower pH of the effluent from Bioreactors II and IV during this period may have been due to the limestone chambers in these bioreactors.

Values of pH of the effluent from Bioreactor III fell below the pH values of its influent during the period from August 2000 to April 2001. However, the subsequent decrease of the influent pH in May and June 2001 to a value of 5 had no effect on the effluent pH, indicating that the bioreactor was still capable of improving the quality of the AMD. This decrease in the influent pH did not impact the pH of the other two reactors.

Oxidation reduction potential (ORP) measurements in the field were taken using an ORP electrode with a silver/silver-chloride reference electrode. Since the E_H is defined (Ref. 4) as a voltage reading with a reference to the standard hydrogen electrode (SHE), these field values were converted to the E_H values by adding 200 millivolts (mV) and corrected for temperature to report it for 25 EC. The corrected E_H values are presented in Figure 5-2.

The E_H diagram shows that with the exception of a few time periods, the most noticeable being from late summer 1999 through winter 1999/2000 for Bioreactors II and IV and late summer through fall of 1999 for Bioreactor III, the E_H values were positive, indicating an apparent oxidizing environment. There was, however, a significant difference in E_H of the influent and the effluent AMD; the former being in the 300-mV to 400-mV range for most of the operating time. This difference indicates that the bioreactors significantly lowered the E_H. Moreover, the bioreactors fostered conditions favorable for the SRB population growth, as indicated further in this document. Therefore, it is postulated that the E_H measurements of aqueous samples collected at the outlets of the bioreactors (location A in Figure 3-5) may not reflect the E_H present in the "pockets" of organic matter where the SRB lived. Additionally,

the E_H and pH profiling of the bioreactor (see Section 5.1.2) showed that the E_H measured at the outlet of the bioreactors was higher than that measured for aqueous samples collected from the organic matter chambers where most of the sulfate reduction took place.

The DO diagram (Figure 5-3) indicates that the oxygen level ranging from 4 milligrams per liter (mg/L) to 14 mg/L in the influent decreased in the bioreactors to less than 1 mg/L for the majority of the operation time. However, the 14 mg/L peak in the influent DO concentration in December 2000 was also reflected as a maximum DO concentration in Bioreactor III, which had no limestone chamber and only one organic matter chamber. Moreover, both peak values correlate well with very high E_H values recorded for Bioreactor III at the same time (Figure 5-2).

The temperature of the influent and effluent are presented in Figure 5-4. The temperatures reflect seasonal variation and change from 0.5 EC to 15.5 EC for the influent and 1 EC to 16 EC for the effluent from both belowground bioreactors. Temperature of the effluent from these two bioreactors was very similar throughout the operating period with the exception of the first 4 months when the effluent from Bioreactor II was up to 2 EC higher. This difference is attributed to the fermentation processes of the organic matter that took place at the beginning of the operation and were more intensive in Bioreactor II, which contained two organic matter chambers.

The above-freezing temperatures inside the aboveground bioreactor (IV) during winter were caused by a small (approximately 0.05-gpm) leak from the bioreactor that prevented the bioreactor from freezing solid, as anticipated by the design.

The 2 EC higher temperature of the effluent from Bioreactor IV during summer is attributed to its exposure to higher ambient air temperature because of its aboveground location.

5.1.2 pH, E_H, and Temperature Profiles

Although profiling the bioreactors for pH and E_H values was not in the original sampling plan, it was conducted to assist in data interpretation. The pH and E_H measurements of the AMD at all deep and shallow piezometers for each bioreactor were taken four times per month beginning in November 1999. The results obtained were similar for all measurement events (Table 5-2). Figures 5-5 and 5-6 present the results of measurements taken in December 1999 for Bioreactors II and III, respectively. Measurements taken in Bioreactor IV are not plotted because they depict nonflowing conditions (the reactor was shut down for winter).

The pH profiles document very little change along the flow in both bioreactors with the exception of the first organic matter chamber where pH values dropped by approximately 0.2 units upon the influent entry. This slight decrease in the pH values was probably caused by the release of protons when hydrogen sulfide (H_2S) reacted with dissolved metals and precipitated them as metal sulfides as shown in Reaction 1-3.

The pH increased approximately 0.5 units in the limestone chamber in Bioreactor II and in the cobble chamber in Bioreactor III. Worth noting is a small difference in the pH measurements taken in the shallow and deep piezometers in the cobble section. The slightly lower values of pH at shallow depth correlates well with the higher E_H measurements taken at the same locations.

The E_H values were more differentiated than the pH readings for both bioreactors. The AMD flow in the bioreactors were notably stratified with respect to E_H, which in Bioreactor II was up to 400 mV higher in the shallow piezometers than in the deep piezometers. This difference, thus also stratification, diminished downgradient and close to the outlet of the bioreactors due to mixing of the water flowing through the cobble section. This mixing process was increased by the placement of the outlet pipe located 6 inches above the bottom of the bioreactors.

The water mixing process seems to also be responsible for higher values of E_H at the monitoring location at the outlet of the bioreactors in comparison to measurements taken at the bottom of the first organic matter chambers. This difference was approximately 50 mV and 150 mV for Bioreactors II and III, respectively.

5.2 Sulfate-Reducing Bacteria Populations

The first 8 months of operation can be described as a period in which the microbial populations were established within the bioreactors. It should be noted that the bioreactors were started in the winter when temperatures were not ideal for microbial growth. As the bioreactor temperatures (Figure 5-4) began to increase in April and May 1999, an increase in SRB populations (Figure 5-7 and Table 5-3) was also seen. During the second winter of operation, the well-established SRB population was not affected by the low temperatures.

The correlation of the SRB population with E_H can be seen in Figure 5-2. The E_H decreased to -80 mV for Reactor III in September 1999 and -200 mV for Reactor IV in October 1999. During the same time, the SRB populations grew to a level of 2E+5 most probable number (MPN) for Reactor III. There was a small decrease in the SRB population during the winter of 1999-2000, with a subsequent increase in the spring of 2000. This increasing trend ended at the same time as the flow rates were doubled, indicating that SRB might have been flushed out at that flow velocity. The subsequent decrease in the SRB population through the winter of 2000/2001 is considered a delayed effect of doubling the flow rate in the summer of 2000. When the temperature increased in the spring of 2001, the SRB population returned to above the E+4 MPN level.

Based on the metals-removal results explained further in this document (Section 5.5), the population of SRB at the E+4 MPN level worked well for the geochemical and climatic conditions present at the Calliope site. It is worth noting, however, that this SRB population should not be considered the optimum and/or recommended population for a SRB bioreactor in general. This is because the activity of the SRB is probably more important than the population size. In other words, a small population of SRB that are very active may be more efficient than a large population of less active cells. Reliable methods for the direct measurement of SRB activity are not currently available for routine sample analysis. Methods have been developed based on the uptake of radioactively labeled sulfate; however, these require specialized equipment and have an inherent safety hazard that makes routine use difficult to justify. Such methods were not included in the monitoring program for the Calliope site.

5.3 Sulfate and Sulfide

Sulfate and sulfide concentrations in the influent and effluents of the bioreactors (Figure 5-8 and 5-9, respectively, and Table 5-4) do not give conclusive results regarding sulfate reduction rates. These diagrams are included in this report mainly for documentary purposes. The main reason for the inconclusiveness of the sulfate records could be a high concentration of sulfates in the organic matter built in the reactors, as indicated by often higher concentrations of sulfate in the effluent than in the influent. The use of analytical results for sulfide is limited due to the analytical procedure selected for the project. As this procedure did not require filtering of the sampled material, some of the sulfide detected might have come from suspended metal sulfides. This is especially true for sulfide analyses of the influent AMD that show up to 4 mg/L of sulfide, which should not exist in dissolved form under E_H and pH conditions of influent and in the presence of dissolved Cu. Moreover, there was a noticeable H_2S odor coming from the influent AMD.

5.4 Alkalinity

Analytical data for alkalinity, expressed as milligrams of calcium carbonate ($CaCO_3$), are presented in Figure 5-10 and Table 5-5. Although this diagram shows the total alkalinity, it is actually a bicarbonate (HCO_3^-) alkalinity for most of the operating time. Only during the first 3 months of operation (2 months for Bioreactor II) did

carbonate alkalinity (CO_3^{2-}) contribute to the total alkalinity, with the hydroxide alkalinity never detected.

The alkalinity of a typical AMD is zero, as indicated on Figure 5-10, where, until October 1999, alkalinity of the influent AMD at the intake location was zero. As the AMD at the site improved (due to the change of climatic conditions) and its pH increased to 6.5, the alkalinity of the influent AMD increased to the range of 20 mg/L to 30 mg/L, with the exception of March 2001 when it peaked to 189 mg/L.

The alkalinity of the treated AMD is a product of sulfate reduction by SRB that use organic carbon as the electron donor, as described by Reaction 1-2. Therefore, the alkalinity buildup in the effluent from all bioreactors at the Calliope site is a good indication of biochemical reactions taking place in the bioreactors.

5.5 Metals

The primary objective of this project was to assess various configurations of the bioreactors and monitor their ability to produce a high-quality effluent (Ref. 3). It was the goal of the field demonstration to achieve the effluent characteristics given in Table 2-1.

These target concentrations were set arbitrarily at the beginning of the project when the quality of AMD in the Lower Pond was at its worst or close to it. The quality of the AMD improved with time due to climatic conditions, and some of the metals (Al, Zn, and Mn) became irrelevant because their concentrations in the influent were already close to or even below the target concentrations.

Analytical results for concentrations of seven dissolved metals (Zn, Cu, Cd, Al, As, Fe, and Mn) in the influent and effluent of the bioreactors are compiled in Table 5-6. These monthly results are also presented in a graphical form in the figures that are individually referred to in this section.

Changes in Zn concentrations are presented in Figure 5-11. During the first 7 months of the demonstration, Zn concentrations were rising as the sorptive capacity of the organic matter was being filled. During this period, the percent of Zn removal was as high as 99%. Once the sorptive capacity was filled, Zn concentration stabilized at a threshold of approximately 500 µg/L for Reactors II and IV and 800 µg/L for Reactor III. The slightly lower percent of removal in Reactor III is attributed to a smaller total supply of organic carbon, as this reactor has only one chamber with organic matter.

Concentrations of Cu are presented in Figure 5-12. Much of the Cu removal observed during the first 7 months of operation can be attributed to adsorption. Once sorption sites fill and the SRB populations become established, Cu was removed through SRB activity to a threshold level of 50 µg/L on average for Bioreactors II and IV and, again, a slightly higher threshold level of approximately 80 µg/L for Bioreactor III.

Cadmium concentrations are presented in Figure 5-13 (in linear scale). Similarly to Zn and Cu, the removal of Cd observed during the first 7 months of operation can be attributed to adsorption. Once sorption sites fill and the SRB populations become established, Cd was removed through SRB activity to a threshold level ranging between 4 µg/L to 5 µg/L, until March 2001 when the Cd concentration in the influent dropped to approximately 3 µg/L for a 2-month period. Cadmium concentration in the effluent in the same time period decreased to less than 1 µg/L.

Figure 5-14, which presents the concentration of Al, also shows a reduction of concentration in the effluents, but only for the first 10 months of operation. During this time, Al was removed due to adsorption on the organic matter, similarly to Zn, Cu, and Cd. After September 1999 when the Al concentration in the influent decreased to 100 µg/L or below level, there is no indication of further removal of Al.

Figure 5-15, which presents As concentrations, indicates that As content did not decrease in the bioreactor effluents but rather the effluents were

enriched with As for most of the operating time. The reason behind such behavior is two-fold. First, it was found by Canty (Ref. 5) that the manure obtained from a similar source but used for another demonstration site, contained elevated (in comparison to the AMD) levels of As. Thus, it is prudent to assume that some As was flushed from the organic matter chambers down to the sampling points. Second, as Robins and Huang (Ref. 6) explain, under oxidized conditions, as present for the source of AMD, Fe^{3+} (ferric iron) precipitates as ferrihydrite $Fe(OH)_3$, which effectively adsorbs As. However, under reducing conditions, such as in the bioreactors, ferrous iron (Fe^{2+}) becomes the predominant iron species. Because Fe^{2+} is much more soluble than Fe^{3+}, it is released into solution along with the previously adsorbed As. Because the analytical work for the operation phase of the project did not include speciation of Fe, it is impossible to determine which of the above-described mechanisms was predominantly responsible for the high concentrations of As in the effluent.

Iron concentrations, presented in Figure 5-16, show that with exception of the initial 8 months, Fe concentration in the influent was significantly lower than that in the effluents. During the initial 8-month period, Fe seemed to be removed by the bioreactors due to initially high sorptive capacity of the organic matter.

Higher dissolved Fe concentration in the effluent than in the influent (beginning in July 1999) can be explained by a very possible, but never measured, difference in dissolved versus total Fe concentrations in the influent. As stated earlier in this document, under oxidizing conditions and nearly neutral pH, such as those present in the Lower Pond, the prevailing form of iron (Fe^{3+}) precipitates as $Fe(OH)_3$. Because the AMD that was piped from the Lower Pond was not filtered, it certainly brought suspended $Fe(OH)_3$ to the bioreactors. It is possible that under reduced conditions in the bioreactors, Fe^{3+} in $Fe(OH)_3$ was reduced to Fe^{2+} and released to the aqueous phase as dissolved Fe, increasing its concentration in the effluent.

The Mn diagram (Figure 5-17) indicates that the bioreactors were not efficient in lowering concentrations of Mn for most of the demonstration. In fact, reduction of Mn concentration took place only at the beginning of the project and close to its end (for Bioreactor II). At the beginning of the demonstration, Mn was sorbed (like other metals) to the organic matter. At another date in late winter through early spring 2001, Mn was efficiently removed in Bioreactor III. In this case, the efficient removal of Mn coincides with an unexplained increase in DO concentration (Figure 5-3) that was subsequent to the also unexplained peak of E_H in the winter of 2000/2001.

Table 5-1. Field Parameters

Location	Date	pH	E_H (mV)	DO (mg/L)	Temperature (EC)
Influent	12/14/98	3.87	700	6.8	1.6
	12/22/98	6.01	426	8.85	1.1
	1/4/99	4.51	571	10.19	0.9
	2/3/99	5.70	466	11.19	0.6
	3/3/99	5.62	480	8.29	0.5
	5/5/99	3.29	726	6.06	4.6
	6/3/99	3.64	611.4	3.93	9.9
	7/12/99	4.19	315.4	6.39	15.3
	8/11/99	5.14	probe malfunction		14.4
	9/8/99	6.08	403	8.61	9.7
	10/7/99	6.68	309	8.94	7.3
	11/9/99	6.58	357	9.76	4.3
	12/8/99	6.87	294.4	9.94	1.3
	1/6/00	7.14	247.5	10.5	0.7
	2/3/00	6.81	302	8.81	1.2
	3/6/00	6.61	334	8.89	4.7

Table 5-1. Field Parameters

Location	Date	pH	E_H (mV)	DO (mg/L)	Temperature (EC)
	4/5/00	6.07	368	8.42	5.2
	5/8/00	6.83	363	8.59	7.5
	6/7/00	5.80	409	7.66	15.4
	7/6/00	6.24	366	7.53	13.3
	8/10/00	6.48	342	7.43	15.5
	9/7/00	6.83	310	7.89	10.5
	9/25/00	6.92	267	8.89	6.6
	10/31/00	7.08	267	9.03	4.3
	12/4/00	7.40	422	13.84	1.6
	1/8/01	6.94	probe malfunction	11.49	1.2
	2/12/01	7.52	230.9	10.49	1.5
	3/14/01	7.10	365	9.67	2.7
	4/9/01	7.33	251	9.93	3.8
	5/9/01	6.48	32	probe malfunction	8.3
	6/6/01	4.85	394		8.5
	7/9/01	5.01	401	7.1	16.5
Bioreactor II	12/14/98	9.95	76	0.51	4.9
	12/22/98	9.51	252	0.63	5.9
	1/4/99	8.75	342	1.09	4.1
	2/3/99	8.42	378	0.93	2.4
	3/3/99	8.37	353	0.11	2.1
	5/5/99	8.29	87	0.04	6.1
	6/3/99	7.87	62.8	0.95	8.9
	7/12/99	7.61	106.5	0.46	13.2
	8/11/99	7.68	probe malfunction		15.4
	9/8/99	7.09	-76	0.2	14.1
	10/7/99	7.14	-125	0.24	9.2
	11/9/99	7.20	-36	0.53	6.4
	12/8/99	7.69	-62	0.43	4.4
	1/6/00	7.29	-35	0.39	2.4
	2/3/00	7.46	29	0.48	1.9
	3/6/00	7.37	42	0.30	2.9
	4/5/00	6.98	-68	0.51	2.9
	5/8/00	6.79	-74	0.21	5.3
	6/7/00	7.25	78	0.29	10.6
	7/6/00	7.10	16	0.27	13.5
	8/10/00	6.76	151	0.31	15.8
	9/7/00	7.31	-14	0.61	12.9
	9/25/00	6.73	-34	0.27	12.0
	10/31/00	7.06	133	0.46	6.3
	12/4/00	7.17	330	2.58	3.7
	1/8/01	6.98	probe malfunction	0.82	2.9
	2/12/01	7.21	296	0.56	2.2
	3/14/01	7.74	-83	0.59	2.7
	4/9/01	6.83	289	0.48	2.9
	5/9/01	6.61	94	probe malfunction	4.8
	6/6/01	6.76	98		9.1
	7/9/01	6.76	15	0.47	12.4
Bioreactor III	12/14/98	9.12	219	0.05	4.2
	12/22/98	9.32	279	0.18	3.4
	1/4/99	9.34	403	0.44	2.2
	2/3/99	8.43	444	0.76	1.7
	3/3/99	7.78	328	0.17	1.3
	5/5/99	7.56	273	0.04	5.4
	6/3/99	7.62	187.2	0.08	9.6
	7/12/99	7.54	72.9	0.15	13.8
	8/11/99	7.03	probe malfunction		15.0
	9/8/99	7.27	-82	0.21	14.8
	10/7/99	7.02	-81	0.24	10.9
	11/9/99	6.75	37	0.27	6.3
	12/8/99	7.21	59	1.18	3.2
	1/6/00	6.98	68	1.43	2.2

Table 5-1. Field Parameters

Location	Date	pH	E_H (mV)	DO (mg/L)	Temperature (EC)
	2/3/00	6.92	259	0.66	1.6
	3/6/00	7.00	145	2.23	3.6
	4/5/00	6.82	76	0.34	4.1
	5/8/00	6.84	-49	0.12	6.7
	6/7/00	6.63	41	0.27	11.8
	7/6/00	6.39	114	0.91	14.1
	8/10/00	6.35	196	0.67	15.7
	9/7/00	6.45	164	2.53	12.9
	9/25/00	6.45	241	1.79	10.0
	10/31/00	6.77	187	0.91	5.8
	12/4/00	6.73	569	5.69	2.8
	1/8/01	6.65	probe malfunction	5.28	2.5
	2/12/01	6.79	379.4	3.91	2.1
	3/14/01	6.64	113	3.80	2.0
	4/9/01	6.82	334	3.22	3.1
	5/9/01	6.7	-48	probe malfunction	4.4
	6/6/01	6.8	161		6.8
	7/9/01	6.26	34	0.43	13.7
Bioreactor IV	12/14/98	9.98	69	0.15	1.0
	12/22/98	9.87	339	0.40	0.5
	1/4/99	9.68	337	0.24	0.3
	2/3/99	9.19	346	0.46	0.1
	3/3/99	8.37	135	0.06	0.1
	5/5/99	8.63	17	0.28	2.0
	6/3/99	8.27	53.6	0.09	10.3
	7/12/99	7.59	77.3	0.15	16.2
	8/11/99	8.12	probe malfunction		17.0
	9/8/99	7.80	-82	0.2	13.8
	10/7/99	7.90	-100	0.25	7.6
	11/9/99	7.91	-191	0.28	5.6
	12/8/99	8.25	-60	0.3	2.1
	1/6/00	7.96	-31	0.31	0.7
	2/3/00	8.17	95	0.77	0.0
	3/6/00	7.88	53	0.28	1.6
	4/5/00	7.92	-87	0.37	3.1
	5/8/00	8.06	-33	0.08	5.0
	6/7/00	7.49	37	0.27	14.1
	7/6/00	7.23	37	0.3	14.5
	8/10/00	6.99	30	0.30	17.3
	9/7/00	7.81	-53	0.45	12.4
	9/25/00	7.54	-31	0.31	7.5
	10/31/00	7.57	98	0.24	5.5
	12/4/00	7.84	289	0.60	0.8
	1/8/01	7.70	probe malfunction	1.06	1.2
	2/12/01	8.47	242	0.73	0.5
	3/14/01	7.68	-16	0.50	0.4
	4/9/01	7.75	254	0.48	0.8
	5/9/01	7.79	-87	probe malfunction	2.6
	6/6/01	7.18	39		8.2
	7/9/01	6.97	-40	0.42	18.1

Table 5-2. E_H and pH Profiles for Bioreactors II and III

Location	Piezometer	E_H				pH		
		11/2/99	12/6/99	1/11/00	2/9/00	12/6/99	1/11/00	2/9/00
Influent		418.20	438.8	301.9	302	6.79	6.78	6.67
Bioreactor II	II fd	131.9	53.2	174	243	6.2	5.76	5.79
	II gd	-100.7	-135	-38	83	7.01	7.03	7.1
	II hd	-63.3	-101.2	-11	22	7.19	7.06	7.36
	II bd	19.5	-91.6	-21	-25	7.46	7.38	7.59
	II cd	-10.3	-111.7	-15	0	7.55	7.4	7.43
	II id	-56.2	-110.5	-18	-10	7.55	7.45	7.6
	II jd	-56.5	-108	-13	-13	7.55	7.45	7.7
	II kd	-61.8	-99.9	-1	4	7.6	7.48	7.65
	II ld	-57.8	-87.4	12	29	7.53	7.45	7.78
	II fs	328.5	331.7	dry		7.01	dry	
	II gs	312.1	284.3	150	214	7.31	6.76	dry
	II hs	380.4	114.7	70	192	7.59	7.43	7.29
	II bs	33.9	105.3	84	106	7.3	7.19	7.13
	II cs	-35.2	49.7	52	81	7.08	7.14	7.27
	II is	-76.5	33.5	32	24	7.13	7.23	7.34
	II js	-80.5	-26.8	32	11	7.13	7.19	7.72
	II ks	-82.3	-77.9	1	23	7.25	7.19	7.74
	II ls	-76.8	-95.4	-14	42	7.25	7.19	7.6
Bioreactor III	III fd	-13.5	-46.5	88	150	6.36	6.19	6.37
	III bd	41.5	-153.2	-23	60	7.61	7.27	7.19
	III gd	81.2	-110.8	-16	71	7.58	7.3	7.12
	III cd	108.2	-72.8	1	98	7.47	7.15	6.74
	III hd	87.6	-55.5	22	114	7.41	7.05	6.81
	III Id	50.4	-36.9	47	141	7.15	6.93	6.71
	III jd	34.9	16	115	259	6.99	6.73	6.55
	III fs				dry			
	III bs	116.7	97.3	127	202	7	6.85	6.52
	III gs	78.3	47.1	95	227	7	6.82	6.32
	III cs	62.9	56.2	97	229	6.99	6.73	6.14
	III hs	55.3	65.7	110	231	7.04	6.69	6.44
	III Is	41.8	50	96	219	6.65	6.62	6.51
	III js	23.9	53.5	126	245	6.83	6.56	6.59

Table 5-3. SRB Populations

	SRB populations (SRB/mL)		
Date	**Bioreactor II**	**Bioreactor III**	**Bioreactor IV**
12/14/98	1.40E+03	1.70E+01	7.80E+01
12/22/98	2.00E+02	1.70E+01	1.40E+02
1/4/99	9.20E+00	1.00E-01	1.00E-01
2/3/99	1.80E+00	1.80E+00	1.80E+00
3/3/99	1.10E+00	8.10E+00	8.10E+00
4/5/99	1.70E+01	2.40E+02	2.40E+02
5/5/99	1.70E+02	7.80E+03	2.00E+03
6/3/99	2.80E+03	2.00E+03	1.10E+04
7/12/99	2.80E+04	2.40E+03	1.40E+04
8/12/99	1.40E+06	2.80E+04	1.40E+04
9/8/99	4.50E+05	2.00E+05	2.40E+04
10/7/99	4.50E+04	4.50E+04	4.50E+04
11/9/99	1.70E+04	4.50E+03	1.40E+04
12/8/99	1.20E+04	1.10E+04	2.00E+03
1/6/00	1.10E+04	1.10E+04	2.40E+03
2/3/00	2.40E+04	1.70E+04	1.10E+04
3/6/00	1.70E+06	1.10E+05	1.40E+04
4/5/00	2.00E+03	1.10E+05	7.80E+04
5/8/00	1.70E+04	1.10E+05	7.80E+04
6/7/00	1.40E+05	2.00E+04	4.50E+04
7/6/00	1.10E+05	4.50E+04	6.80E+04
8/16/00	9.20E+04	1.10E+04	2.00E+04
9/7/00	4.00E+04	1.40E+04	2.00E+03
9/25/00	2.80E+02	2.00E+03	2.40E+02
10/31/00	2.10E+03	1.40E+03	2.40E+03
12/4/00	4.50E+03	2.40E+03	1.40E+04
1/8/01	7.80E+03	4.50E+03	1.40E+04
2/12/01	7.80E+03	2.00E+03	2.10E+04
3/14/01	2.40E+04	2.10E+03	2.40E+04
4/9/01	1.40E+05	4.50E+03	7.80E+04
5/9/01	9.30E+04	1.40E+04	2.00E+04
6/6/01	1.70E+04	2.80E+04	2.10E+04
7/9/01	1.10E+04	4.50E+04	4.50E+04

Table 5-4. Sulfate and Sulfide Concentrations

Date	Sulfate (mg/L)				Sulfide (mg/L)			
	Influent	II	III	IV	Influent	II	III	IV
12/14/98	102	250	172	148	3.1	1.2	2.3	2.1
12/22/98	115	80	111	114	2.8	2.2	2.9	3.3
1/4/99	113	145	123	143	3.7	6.4	2.4	1.9
2/3/99	104	111	115	137	0.0	1.2	1.6	1.2
3/3/99	143	122	152	186	0.0	1.3	2.4	0.0
4/5/99	135	193	178	187	0.0	0.0	0.0	0.0
5/5/99	229	281	223	300	0.0	0.0	0.0	1.2
6/3/99	200	304	197	457	0.0	15.0	2.2	1.3
7/12/99	122	136	126	326	0.0	0.0	0.0	0.0
8/12/99	118	92	80	74	2.7	2.8	4.1	2.0
9/8/99	109	113	76	92	1.2	3.2	3.0	1.3
10/7/99	99	73	88	106	0.0	0.0	0.0	1.2
11/9/99	93	87	100	99	1.0	9.6	0.6	0.9
12/8/99	88	83	89	98	1.1	1.4	3.2	0.8
1/6/00	81	82	85	125	3.1	4.8	3.9	4.2
2/3/00	84	82	87	152	1.6	2	1.2	1.3
3/6/00	83.2	73.8	81.4	114	1.37	1.13	1.53	1.45
4/5/00	103	100	95.6	69.2	1.5	1.5	2	1.2
5/8/00	88.3	81.2	69	127	1.7	1.3	3.7	0.85
6/8/00	91.6	82.4	84.8	91.9	2.6	2.3	2.9	2.8
7/6/00	92.5	72.7	93.5	64.7	5.1	4.8	4.7	4.7
8/10/00	78.5	95.4	78.5	80.3	4.3	4.9	7.2	6.0
9/7/00	81.4	88.5	83.8	93.1	3	3.2	2.3	3
9/25/00	82.9	59.2	81.5	86	3.1	5.2	5.6	3.2
10/31/00	69.8	73.4	86.2	84.7	3.6	3.4	2.3	2.6
12/4/00	73.3	65.9	79.7	163	3	3.2	3.3	3.2
1/8/01	79.7	68.1	74.9	208	3	2.6	2	2.9
2/12/01	72.4	55.4	56.4	263	4.5	3	4	4.6
3/14/01	60.6	8	59.6	251	5	3.7	4.5	5
4/9/01	1,400	196	73.7	204	2.6	2.2	1.7	2.5
5/9/01	81.6	131	113	241	2.8	1.7	1.9	2.6
6/6/01	118	128	113	121	2.2	1.3	1.6	2
7/9/01	101	88.7	102	79.7	2.8	3.9	4.1	2.4

Values enveloped by lines are considered outliers and were not grafted

Table 5-5. Alkalinity

Date	Total Alkalinity (mg/L as $CaCO_3$)			
	Intake	II	III	IV
12/14/98	0.0	215.0	277.0	294.0
12/22/98	0.0	111.0	192.0	238.0
1/4/99	0.0	105.0	93.8	223.0
2/3/99	0.0	95.0	95.8	203.0
3/3/99	0.0	95.8	94.2	275.0
4/5/99	0.0	102.0	86.0	251.0
5/5/99	0.0	126.0	85.4	241.0
6/3/99	0.0	474.0	99.0	262.0
7/12/99	0.0	128.0	78.2	214.0
8/12/99	0.0	247.0	359.0	290.0
9/8/99	0.0	238.0	248.0	446.0
10/7/99	0.0	124.0	75.8	87.8
11/9/99	14.4	54.0	26.4	90.4
12/8/99	12.4	53.6	35.0	68.2
1/6/00	24.8	70.4	37.0	125.0
2/3/00	19.6	79.0	41.0	129.0
3/6/00	16.2	90.4	35.2	146.0
4/5/00	0.0	100.0	47.0	220.0
5/8/00	14.0	87.2	126.0	426.0
6/8/00	11.0	74.2	38.4	77.2
7/6/00	18.0	152.0	27.2	124.0
8/10/00	19.4	54.6	29.8	81.8
9/7/00	18.2	45.8	23.6	59.6
9/25/00	22.2	122.0	28.0	56.4
10/31/00	20.8	62.2	39.6	65.2
12/4/00	28.0	63.8	26.2	102.0
1/8/01	21	54.0	29.2	120
2/12/01	25.4	70.2	26.8	154
3/14/01	189	95.8	24.2	25.8
4/9/01	25.2	90.4	40.2	286
5/9/01	16	70.0	46.6	347
6/6/01	0	83.4	75.0	202
7/9/01	0	83.8	33	97

Table 5-6. Metals Concentration

		Metals concentration (µg/L)						
Location	**Date**	**Al**	**As**	**Cd**	**Cu**	**Fe**	**Mn**	**Zn**
Influent	12/14/98	2400.0	2.8	15.2	884.0	524.0	1620.0	3740.0
	12/22/98	1670.0	2.5	18.1	737.0	377.0	2020.0	4420.0
	1/4/99	2080.0	4.4	14.9	689.0	603.0	1700.0	3610.0
	2/3/99	788.0	5.2	16.0	477.0	357.0	1830.0	3570.0
	3/3/99	1160.0	3.6	12.1	518.0	265.0	1620.0	3190.0
	4/5/99	3750.0	3.9	20.1	1020.0	713.0	2120.0	4870.0
	5/5/99	14100.0	1.8	41.9	3050.0	7220.0	3770.0	11100.0
	6/3/99	8770	3.1	31	2090	2960	2860	7890
	7/12/99	1580	1.3	16.5	451	272	1600	3370
	8/11/99	779	2.5	17.9	366	281	1950	3790
	9/8/99	140	6.5	16.4	200	372	1950	3520
	10/7/99	26.6	6.2	12.7	68.9	50.6	1580	2700
	11/9/99	35	6.7	7.3	78.8	154	1320	2130
	12/8/99	93.1	5.5	9.3	82.8	15.7	1170	2140
	1/6/00	18	10.9	10.9	7.2	115	1420	2080
	2/3/00	11	5.1	7	53	35.9	1130	1890
	3/6/00	44.5	6.1	8.2	114	20.1	1220	2050
	4/5/00	134	3.5	7.3	274	124	1360	2460
	5/8/00	32	7.6	8.4	145	27.2	1270	2020
	6/7/00	154	2.1	8.6	310	73.1	1230	2230
	7/6/00	35.3	1.5	6.3	174	20.5	1170	1840
	8/10/00	17.3	1.5	5.7	81.4	31.9	1070	1500
	9/7/00	36.1	2.5	3.7	92.7	15.5	1090	1580
	9/25/00	18.5	1.9	5.7	71.4	15.5	1140	1570
	10/31/00	18.9	2.1	5.1	44.2	20.1	1110	1420
	12/4/00	18.9	3.3	4.3	34.1	19.5	1000	1240
	1/8/01	73.4	5.7	4.3	28.1	19.5	882	1270
	2/12/01	45.5	4.7	4.3	2.8	19.5	787	1090
	3/14/01	18	3.4	2.8	26	8	690	990
	4/9/01	31	2	3.1	30	13	840	1000
	5/9/01	40.5	1.6	4.8	162	25.6	947	1570
	6/6/01	1650	1.1	8.8	635	70.2	1460	3180
	7/9/01	587	1.1	12.1	459	195	1190	2720
Bioreactor II	12/14/98	39.5	15.6	3.9	29.4	140.0	35.6	75.6
	12/22/98	24.6	10.0	3.9	11.5	77.6	31.1	18.6
	1/4/99	22.8	7.4	3.9	3.3	46.9	39.5	22.3
	2/3/99	30.8	5.1	4.8	4.8	93.7	117.0	22.3
	3/3/99	11.2	3.4	4.8	4.3	35.5	115.0	43.9
	4/5/99	10.7	4.3	4.8	47.5	54.2	357.0	128.0
	5/5/99	13.8	3.0	4.8	7.8	97.5	551.0	249.0
	6/3/99	111	26.5	2.5	75.2	1300	999	388
	7/12/99	49.3	5.4	2.5	25.6	686	964	717
	8/11/99	97	14.9	3.9	96.6	868	1040	704
	9/8/99	128	7.7	4.6	95.9	983	1340	1230
	10/7/99	35.0	5.0	4.9	36.8	545	885	1180
	11/9/99	15.8	5.6	4.7	19.4	284	774	750
	12/8/99	21.3	5	5.5	23.2	395	765	772
	1/6/00	30.6	5.4	4.7	15.8	352	785	453
	2/3/00	16.5	2.6	3.5	12.8	314	913	176
	3/6/00	24.6	1.6	3.5	21.4	305	982	210
	4/5/00	130	15.6	3.5	72.2	1810	1000	751
	5/8/00	56.5	10	3.5	49.7	1230	1140	467
	6/7/00	47.6	2.8	3.2	21	772	1000	331
	7/6/00	55.2	6.8	3.2	81.8	2110	1440	409
	8/10/00	17.3	3.6	3.4	17.7	261	984	372
	9/7/00	17.3	2.3	3.4	5.9	197	836	189
	9/25/00	98.2	3.6	3.9	89.1	926	1050	707
	10/31/00	49.6	3.5	4.3	57	578	564	517
	12/4/00	31.8	4.7	4.3	68.8	533	500	404
	1/8/01	18.9	6.8	4.3	31.5	234	296	419

Table 5-6. Metals Concentration

		Metals concentration (µg/L)						
Location	Date	Al	As	Cd	Cu	Fe	Mn	Zn
	2/12/01	46.5	3.3	4.3	23.7	460	871	648
	3/14/01	19	2.2	0.1	8	110	600	48
	4/9/01	140	4.7	0.3	100	600	2400	300
	5/9/01	130	11.4	4.8	142	454	721	1170
	6/6/01	47.8	5.5	4.8	50.6	638	849	518
	7/9/01	118	4	5	110	707	996	899
Bioreactor III	12/14/98	43.8	17.1	3.9	65.5	229.0	105.0	91.0
	12/22/98	40.0	11.3	3.9	20.3	92.4	54.5	34.8
	1/4/99	22.8	5.0	3.9	8.6	57.8	33.1	35.8
	2/3/99	43.4	6.2	4.8	18.1	69.8	124.0	35.9
	3/3/99	16.9	4.4	4.8	17.5	43.9	436.0	60.9
	4/5/99	10.7	4.6	4.8	6.4	49.8	768.0	241.0
	5/5/99	45.3	3.5	4.8	43.4	149.0	2100.0	459.0
	6/3/99	23.7	3.1	2.5	36.4	202	1940	1190
	7/12/99	59.6	2.9	3.1	28.3	337	1510	1030
	8/11/99	335	8.9	7	140	1380	3100	1100
	9/8/99	141	12.7	3.9	69.2	875	1360	847
	10/7/99	52.1	3.5	3.9	22.5	661	1260	753
	11/9/99	10.1	4.2	4.7	16.1	302	1230	364
	12/8/99	26.4	3.2	4.7	14	163	1150	659
	1/6/00	19.9	3.5	4.8	19.5	91.2	1170	934
	2/3/00	9.4	2.4	3.5	18.3	95.8	1200	707
	3/6/00	31.2	1.6	3.5	51.8	323	1270	682
	4/5/00	68.5	3.6	3.5	65.6	1240	1500	893
	5/8/00	240	11.7	4.7	114	3910	1450	1070
	6/7/00	50.9	3	4.4	49.3	1070	1130	723
	7/6/00	25.3	2.2	3.2	96.8	115	1120	956
	8/10/00	24.6	1.5	3.4	45.8	124	886	784
	9/7/00	49.6	2.8	4.4	68.8	116	631	1040
	9/25/00	48	2.3	3.5	69.5	115	631	1330
	10/31/00	83.3	3.3	5.6	128	161	493	1590
	12/4/00	18.9	1.9	4.3	24.3	43.9	310	836
	1/8/01	18.9	6.1	4.3	23	34.3	129	871
	2/12/01	18.9	3	4.3	20.4	25.2	73.5	937
	3/14/01	22	3.1	1	16	31	76	790
	4/9/01	85	2	2	65	150	280	850
	5/9/01	143	4.5	9.1	155	282	212	1720
	6/6/01	125	4.3	4.8	142	494	3040	1590
	7/9/01	80.3	2.7	4.8	122	638	2470	1010
Bioreactor IV	12/14/98	54.2	26.3	3.9	103.0	417.0	19.2	118.0
	12/22/98	27.0	21.6	3.9	79.4	155.0	20.4	53.3
	1/4/99	29.8	29.1	3.9	33.0	126.0	19.3	40.6
	2/3/99	45.2	19.7	4.8	39.5	95.7	27.5	119.0
	3/3/99	35.4	19.6	4.8	44.8	230.0	168.0	72.8
	4/5/99	18.8	14.6	4.8	32.3	180.0	215.0	51.3
	5/5/99	29.5	17.1	4.8	36.3	166.0	454.0	194.0
	6/3/99	9.7	26.3	2.5	36.2	301	983	421
	7/12/99	57.2	3.4	2.5	8.0	410	1310	306
	8/11/99	37.7	13.4	3.9	28.1	530	1070	672
	9/8/99	49.7	28.7	3.9	32.6	782	1480	595
	10/7/99	14.5	2.3	3.9	6.4	142	751	278
	11/9/99	25.3	3.4	4.7	23.5	304	883	491
	12/8/99	10.1	2.6	4.7	17.2	141	811	544
	1/6/00	13	7.8	4.7	16.9	188	934	438
	2/3/00	18.6	8.8	3.5	39.7	206	1060	466
	3/6/00	21.8	10.1	3.5	47.1	325	1070	471
	4/5/00	32.9	8.8	3.5	34.6	434	1150	410
	5/8/00	87.3	16.3	3.7	44.1	1900	2190	442
	6/7/00	43.4	9.6	3.2	47	761	1000	463
	7/6/00	23.4	1.6	3.2	20.1	448	1120	348

Table 5-6. Metals Concentration

		Metals concentration (µg/L)						
Location	Date	Al	As	Cd	Cu	Fe	Mn	Zn
	8/10/00	17.3	1.5	3.4	10.2	87.7	837	269
	9/7/00	17.3	1.8	3.4	9.8	67.1	629	222
	9/25/00	17.3	1.5	3.4	6.3	124	794	145
	10/31/00	45.4	2.5	4.3	52.9	506	942	383
	12/4/00	18.9	4.1	4.3	16.9	1090	1320	170
	1/8/01	51.1	7.2	4.3	13.6	1590	1810	203
	2/12/01	18.9	5.5	4.3	18.5	937	2000	315
	3/14/01	85	6	0.4	530	1100	2300	220
	4/9/01	22	8	0.7	35	2900	2300	250
	5/9/01	55.8	9.6	4.8	33.9	3540	2610	211
	6/6/01	81.7	4.1	4.8	132	1940	1400	505
	7/9/01	42.3	1.1	4.8	18.2	572	1270	292

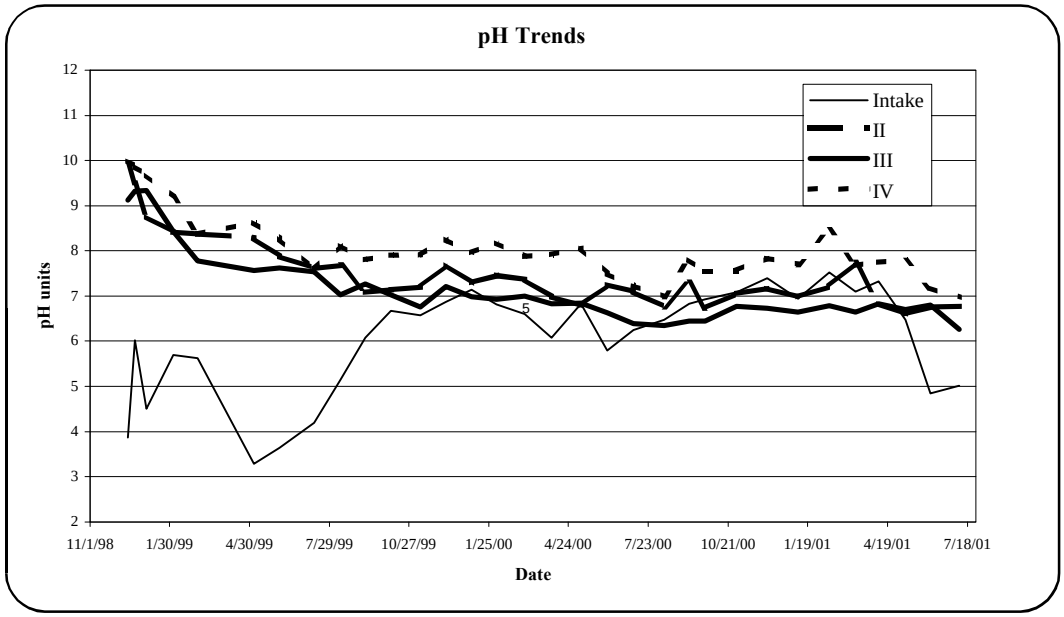

Figure 5-1. pH trends.

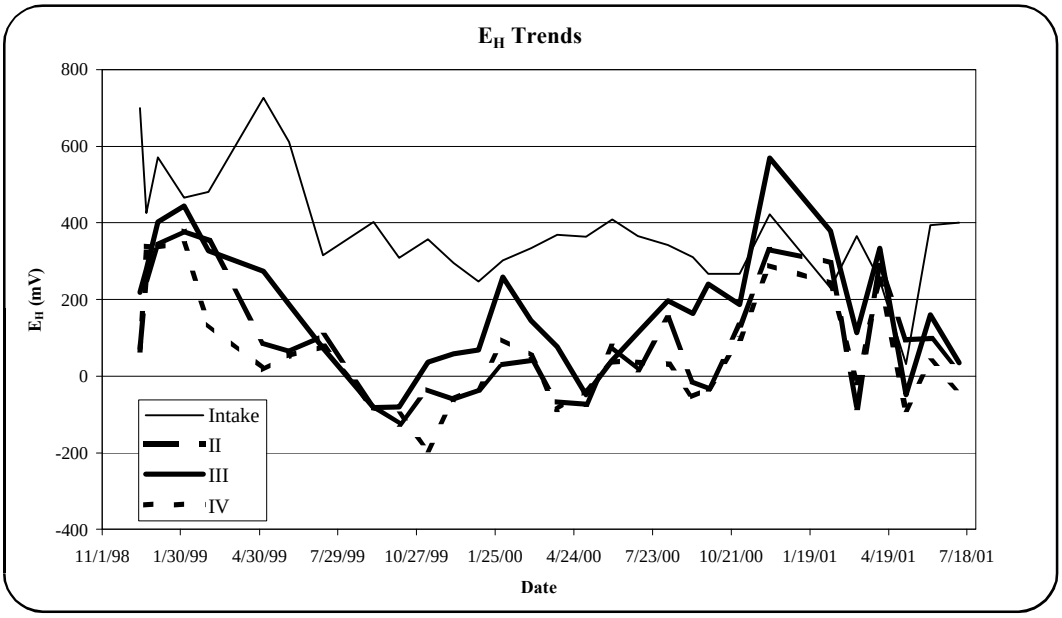

Figure 5-2. E_H trends.

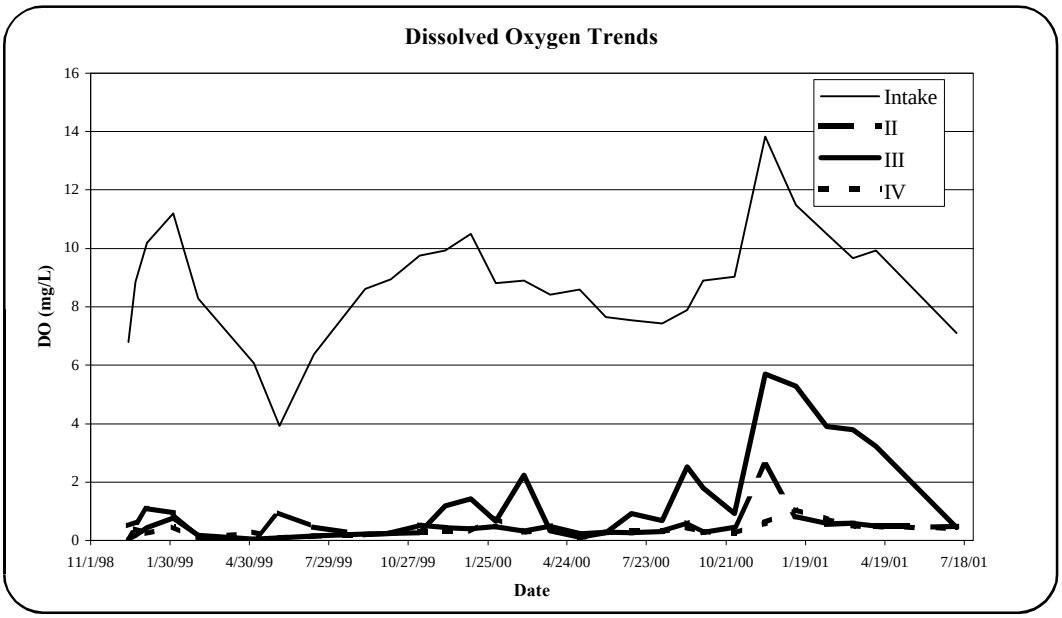

Figure 5-3. Dissolved oxygen trends.

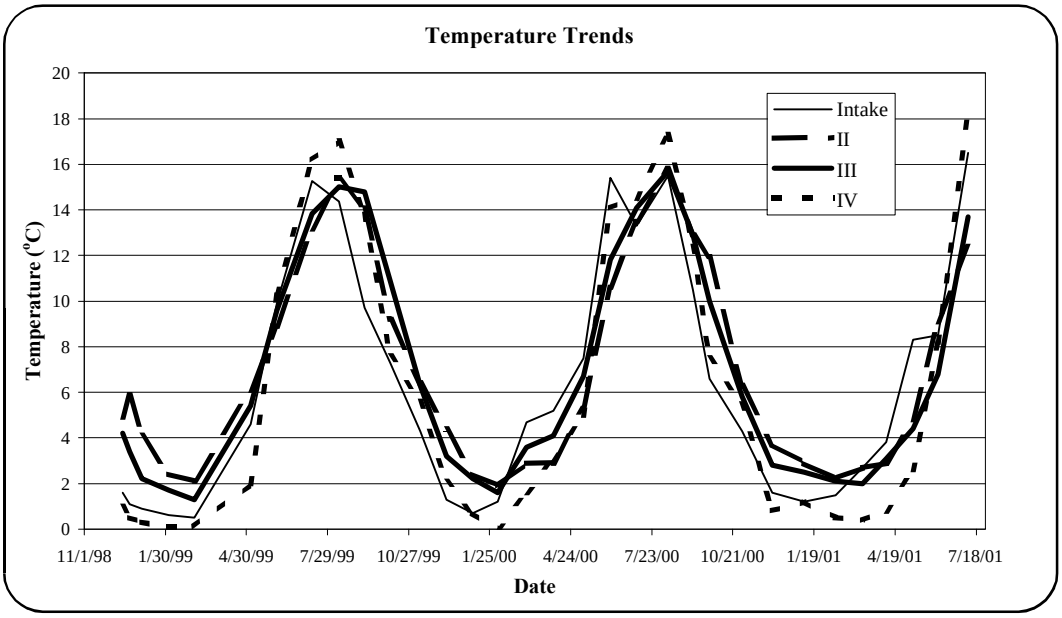

Figure 5-4. Temperature trends.

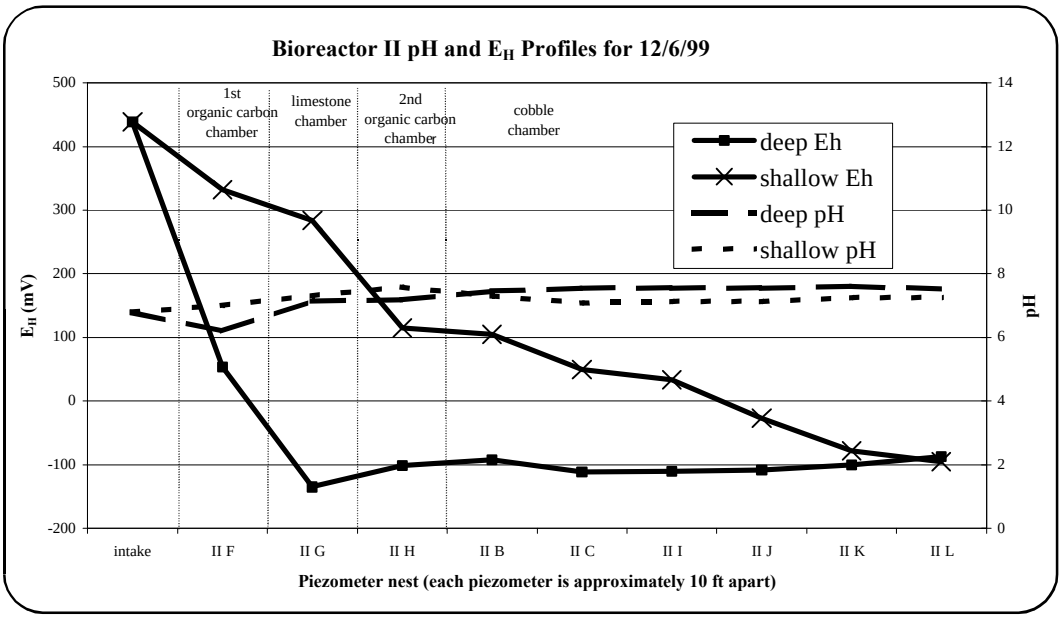

Figure 5-5. Bioreactor II pH and E_H profiles for 12/6/99.

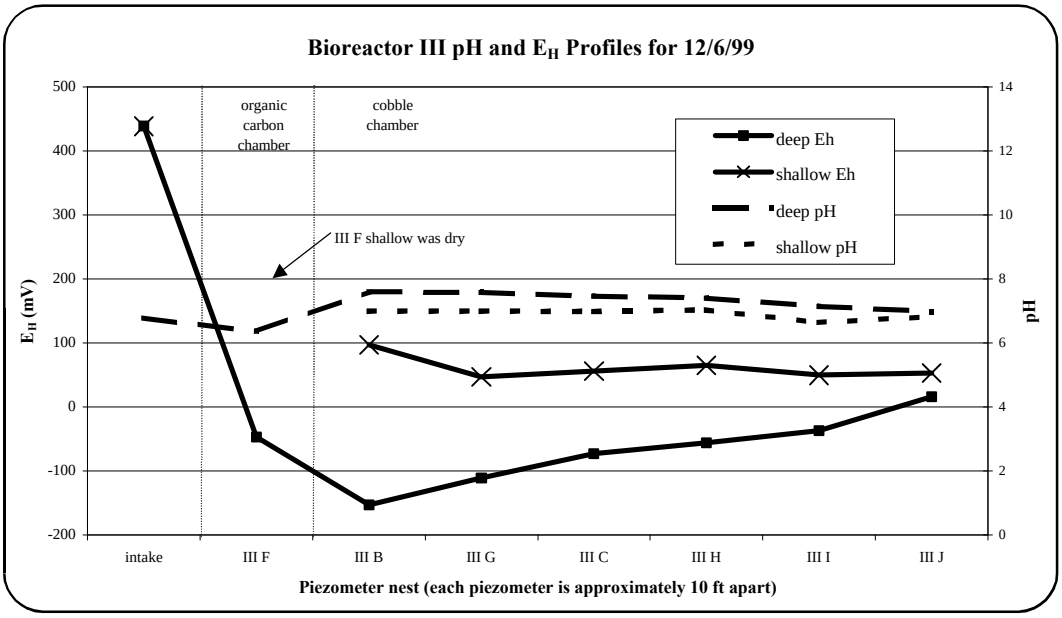

Figure 5-6. Bioreactor III pH and E_H profiles for 12/6/99.

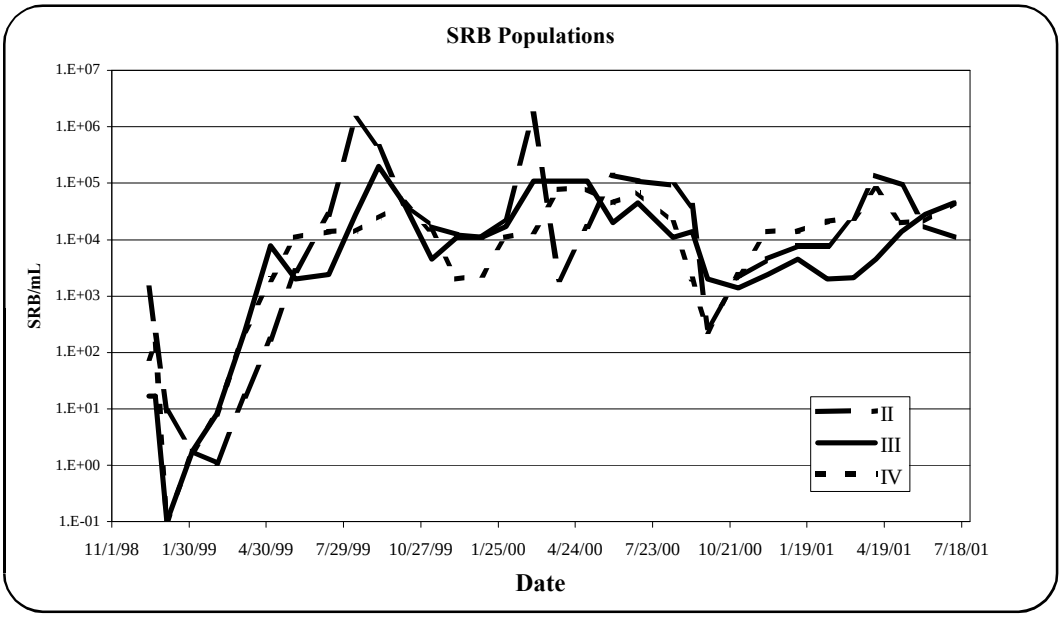

Figure 5-7. SRB populations.

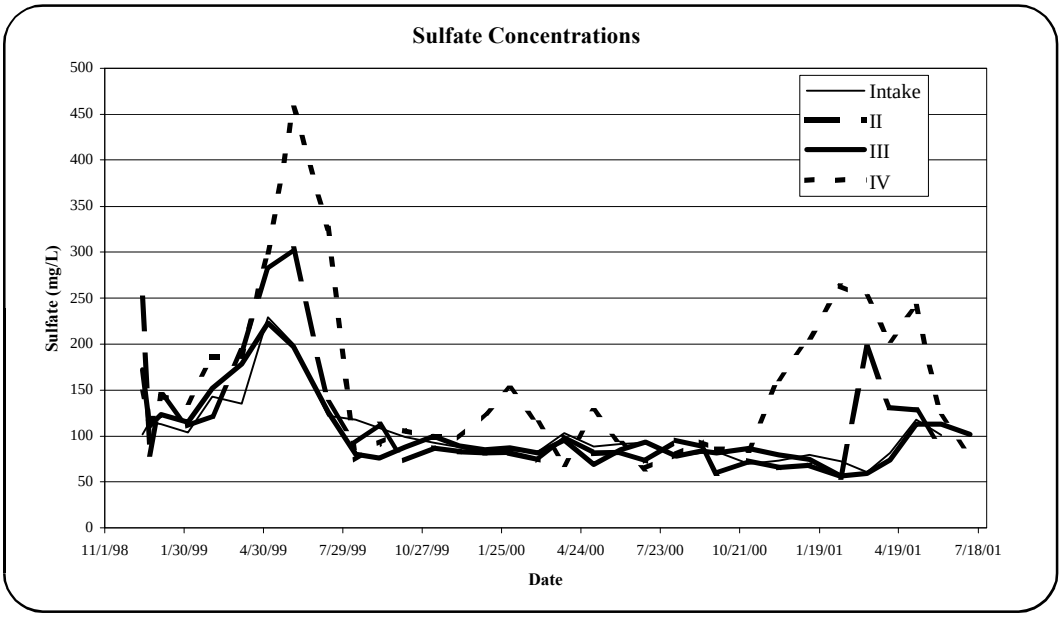

Figure 5-8. Sulfate concentrations.

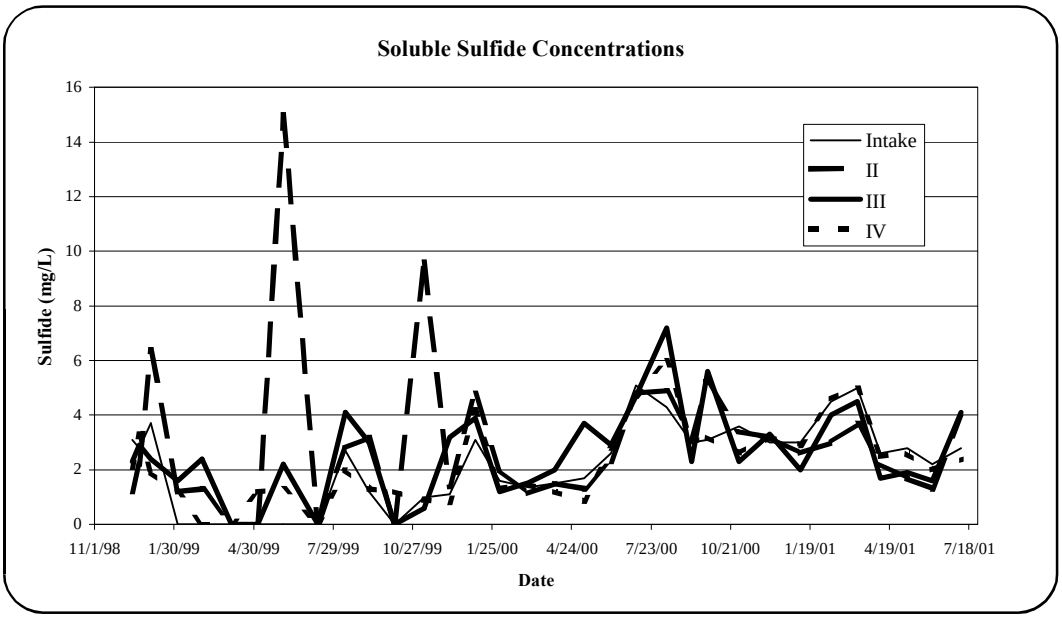

Figure 5-9. Soluble sulfide concentrations.

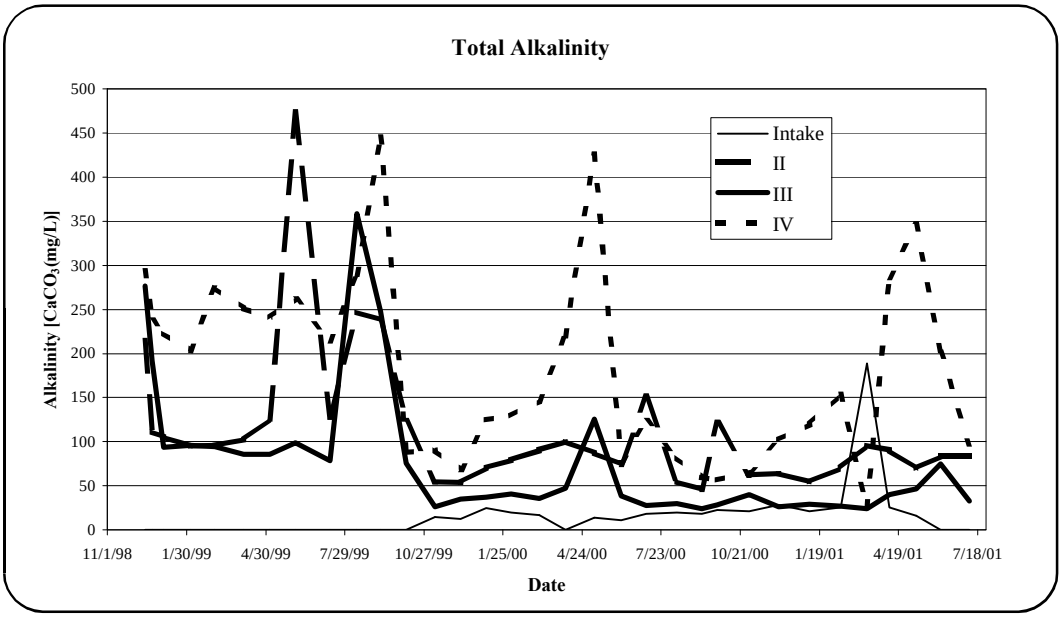

Figure 5-10. Total alkalinity.

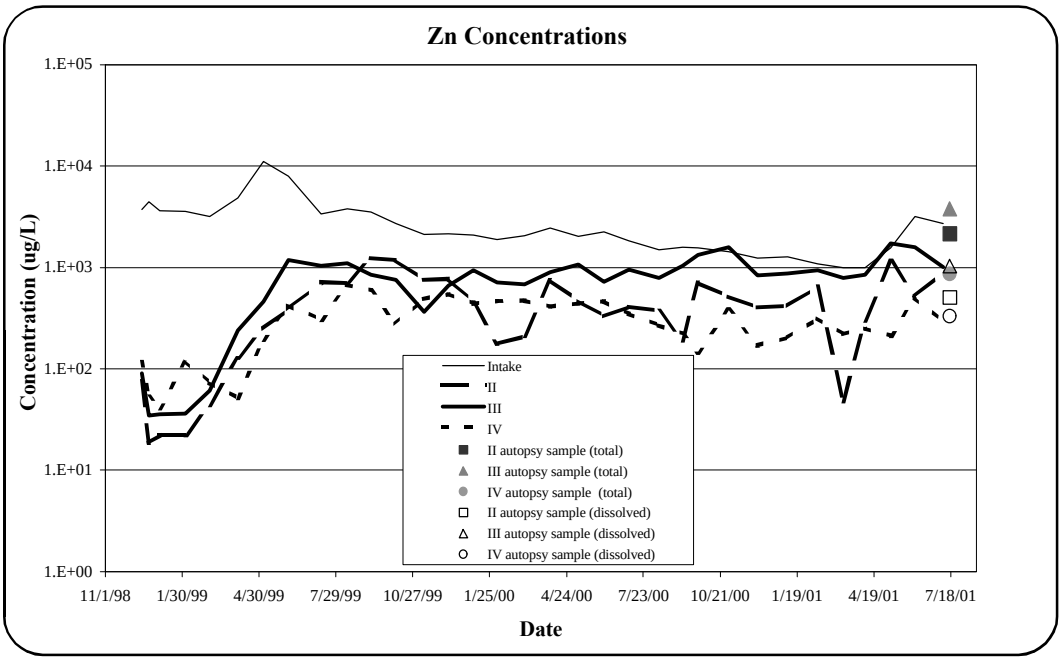

Figure 5-11. Zinc concentrations.

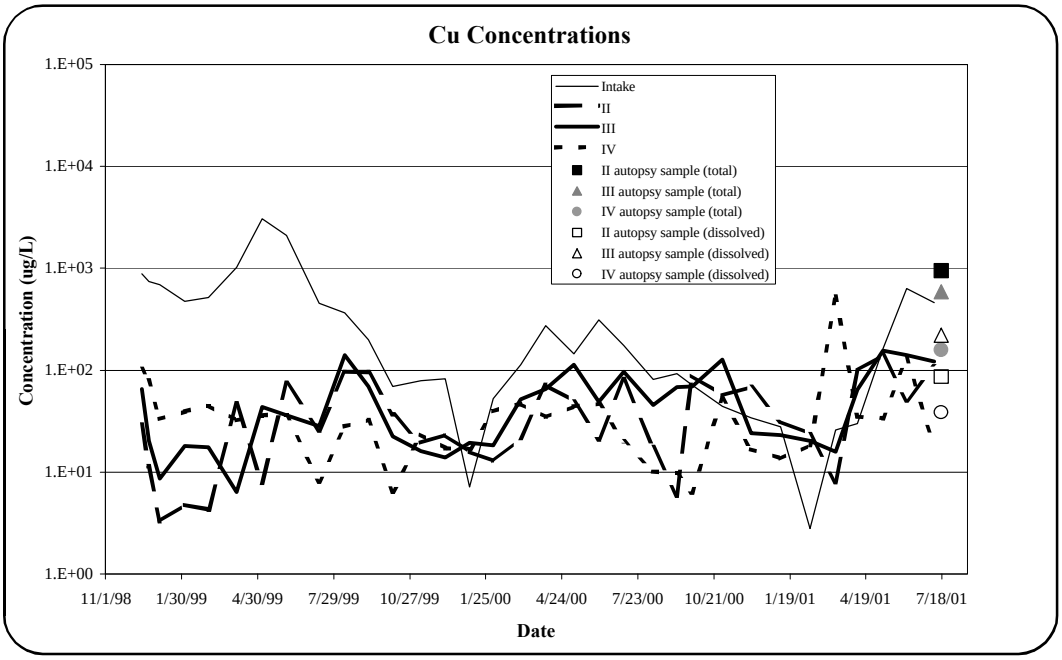

Figure 5-12. Copper concentrations.

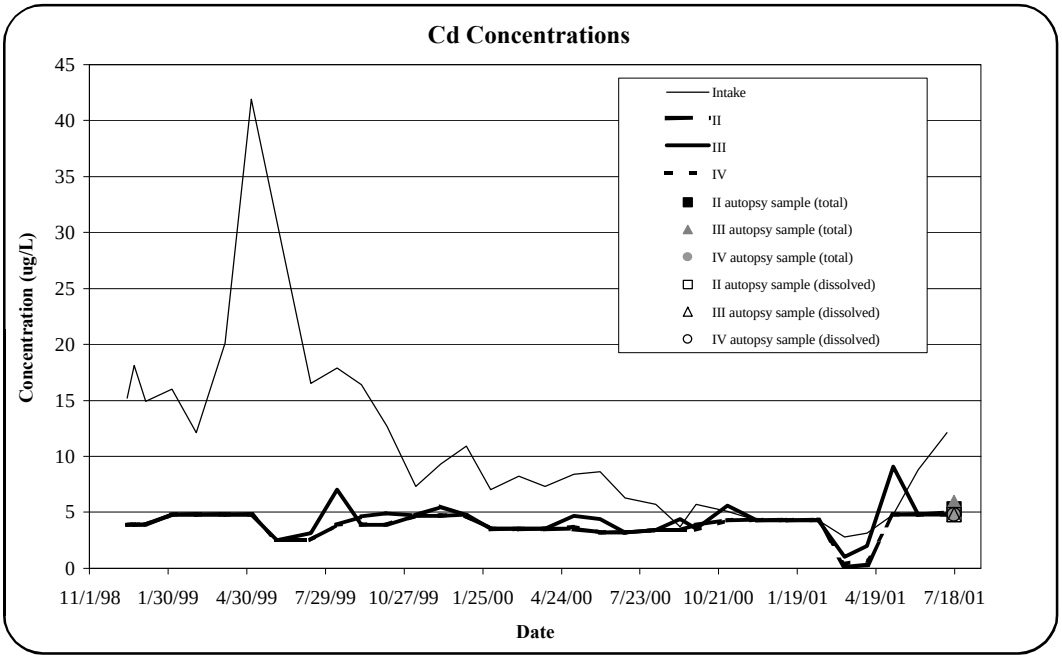

Figure 5-13. Cadmium concentrations.

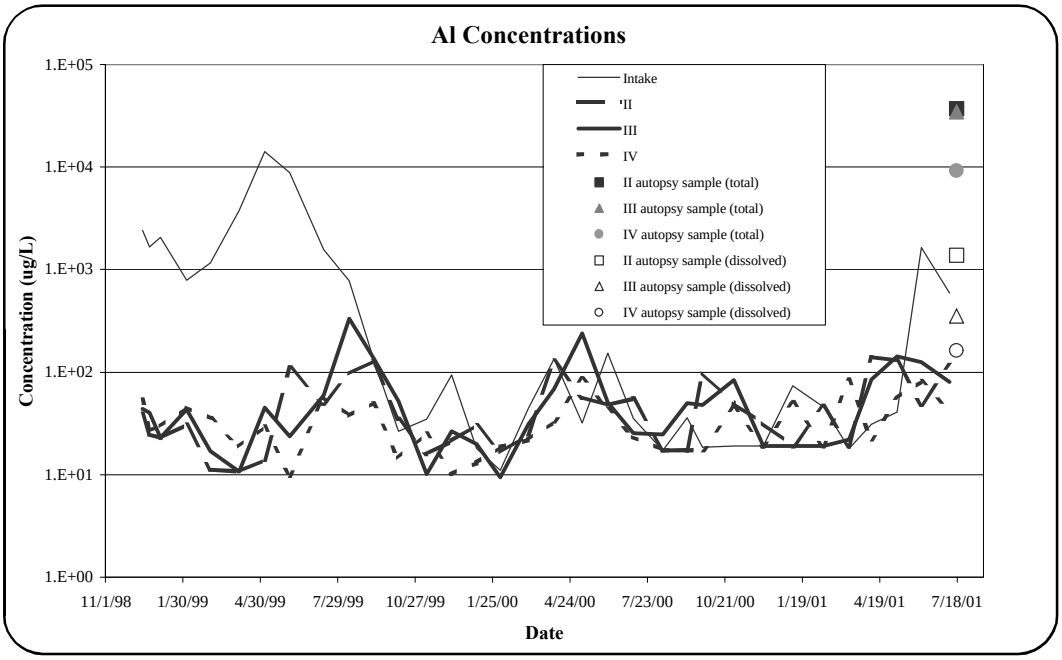

Figure 5-14. Aluminum concentrations.

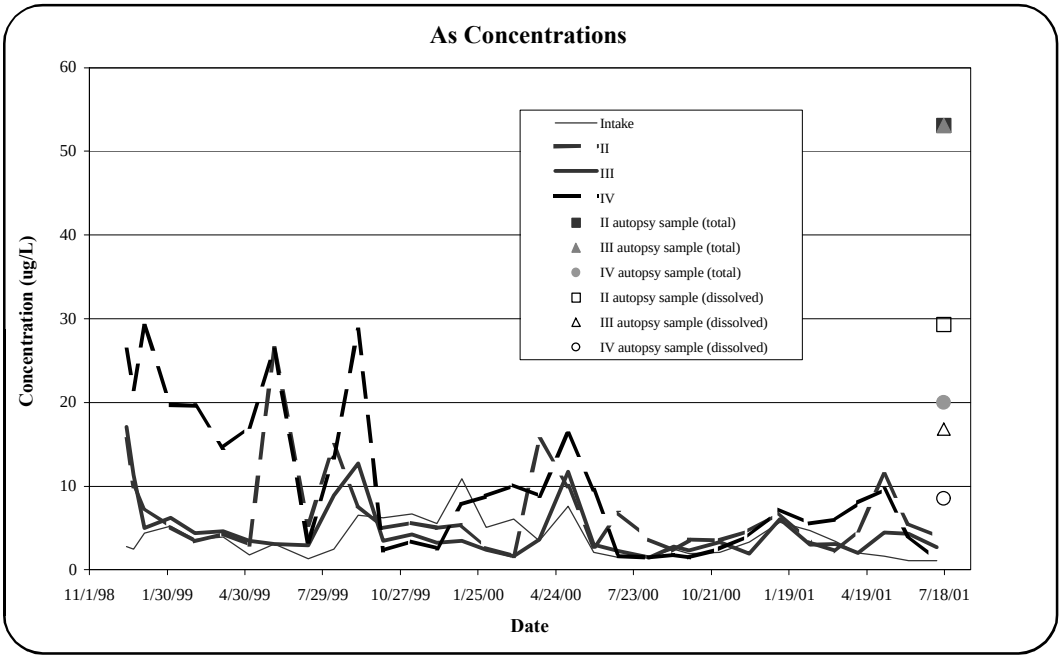

Figure 5-15. Arsenic concentrations.

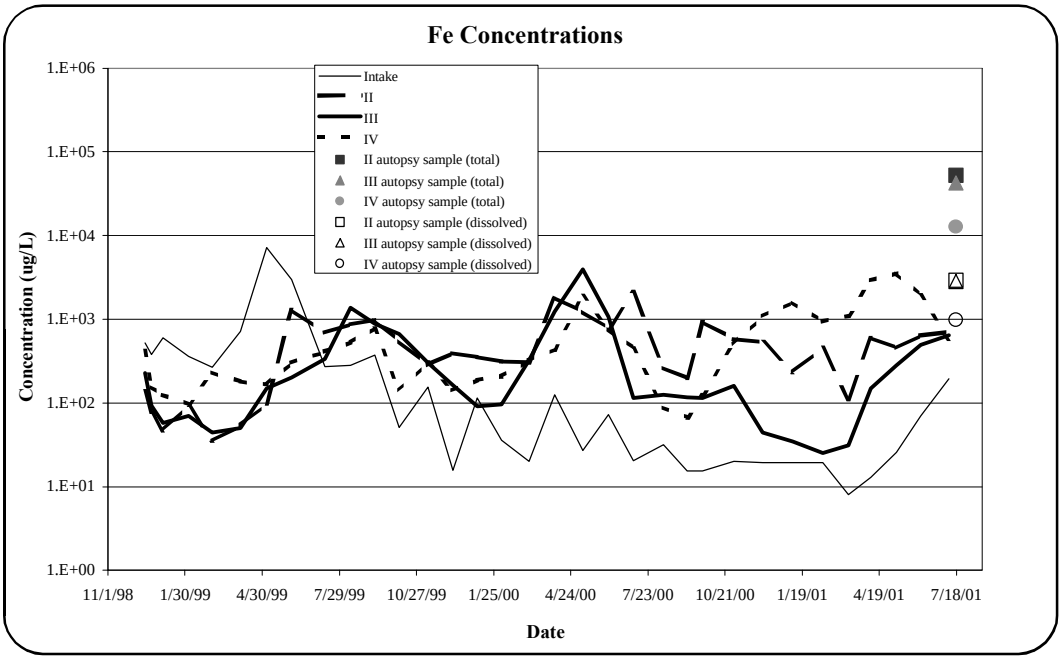

Figure 5-16. Iron concentrations.

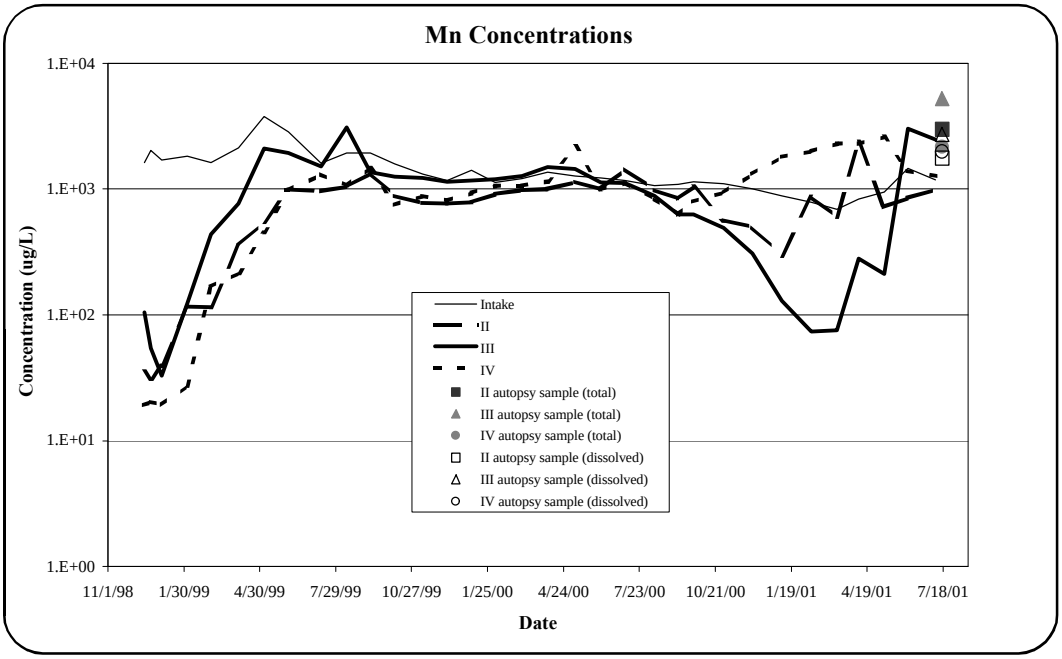

Figure 5-17. Manganese concentrations.

6. Autopsy Results

Reporting on autopsy sampling in this section is descriptive in its character; it documents the results obtained and includes basic qualitative interpretation. It is expected that a portion of the autopsy data will be used in a quantitative manner to improve and validate a computer program for bioreactor design begun in one of the tasks of another MWTP project (i.e., Project 24, Improved SRB).

6.1 Aqueous Samples

Although the autopsy sampling focused on collecting the solid matrix material that was inaccessible during the operational phase of the project, it also included aqueous samples collected from the bottom of the bioreactors within chambers filled with limestone cobbles. To minimize the impact of atmospheric oxygen on the aqueous samples, the samples were collected immediately after the limestone cobbles were removed to provide access to the treated AMD that remained at the bottom of the bioreactors. Aqueous samples collected at the bottom of the cobble chamber were analyzed for total and dissolved metals, iron speciation, sulfate, and sulfide. Samples collected at the bottom of the limestone chambers were analyzed for the same analytes with the exception of dissolved metals. Analytical results are compiled in Table 6-1 (a, b, c). The concentrations of selected analytes are also presented in graphical form in the figures that are individually referred to in this section.

6.1.1 Cobble Chambers

Figures 6-1a and b, 6-2a and b, and 6-3a and b include diagrams of dissolved versus total and suspended metals for Bioreactors II, III, and IV respectively. Values used for these diagrams are tabulated in Table 6-1a. Figures denoted with the letter "a" show total versus dissolved metals, and figures denoted with the letter "b" present suspended metals calculated as the difference between the total and dissolved metals.

The autopsy dissolved metal concentrations compare well to the corresponding metals concentrations of the final sampling event as documented in Table 6-2. The differences may be attributed to a 1-week gap between the sampling events and the different sampling locations. In general, the dissolved metal concentrations were higher than during the final monthly sampling event with the biggest difference for As, Al, and Fe. The autopsy dissolved and total metal concentrations are also marked in Figures 5-11 through 5-17.

Although not detected by a meter, the smell of H_2S was present during sampling of the "soupy" grey water that accumulated at the bottom of the bioreactors. This smell was indicative of reducing conditions and sulfate reduction at these locations. Such judgement was supported by results of speciation analyses for dissolved Fe (Figure 6-4 and Table 6-1b) in the aqueous samples collected from the cobble and limestone chambers. Ferric iron (Fe^{3+}) for those samples was found only in the sample taken from the limestone chamber of Bioreactor II and the limestone and cobble chambers of Bioreactor IV. The maximum concentration of Fe^{3+} for these locations was 0.17 Fg/L or 9% of Fe^{2+} in the limestone chamber of Bioreactor II. However, the analyses for sulfide and sulfate in the dissolved phase (Table 6-1c) show only 3.9 mg/L, 2.2. mg/L, and 4.2 mg/L concentrations of sulfide for Bioreactors II, III, and IV respectively. This indicates that only a small portion of dissolved metals in reduced form were balanced in the solution by sulfides. These were probably Zn, Cu, Cd, and a small portion of Fe.

The rest of the metals dissolved in solution, including high concentrations of Ca (Table 6-1a), are electronically balanced by a high concentration of sulfate (Table 6-1c), hydroxide, and bicarbonate. Concentrations of the latter two were not analyzed for the autopsy samples.

Series "b" diagrams that show a high load of suspended metal compounds in a "soupy" solution attested to the successful operation of the bioreactors. Provided no suspended metals drained from the bioreactors through their outlets, the mass of metal compounds found in the lower portion of the bioreactors together with metal compounds that accumulated within the organic matter and on the limestone should be related to the mass of metals removed from the influent AMD.

The analytical data available do not allow for determination of chemical compounds that contained these metals. However, considering the observed (during the autopsy on the bioreactors) orange-color precipitate present on the PVC liner and the HDPE of the CCS, it is possible that solids in the "soupy" water also included significant amounts of ferric hydroxide. It is also hypothesized that Al present as a suspended solid was in the form of hydroxides.

6.1.2 Limestone Chambers

Analytical results for total metals for aqueous samples collected at the bottom of the limestone chambers of Bioreactors II and IV resemble those obtained for aqueous samples of the cobble sections, as documented in Figure 6-5 for Bioreactor II. Thus, despite the lack of analytical data for dissolved metals concentration for this section of the bioreactors, it is assumed that the concentrations of suspended metal compounds in the limestone section were similar to those for the cobble section.

6.1.3 Sulfate and Sulfide Analyses

Table 6-1c includes analytical data for sulfate and sulfide in the aqueous sample collected during the autopsy of the bioreactors. The table shows that sulfate concentrations were as high as 352 mg/L in Bioreactor III. The highest concentration of sulfide, 4.2 mg/L, was found in Bioreactor IV.

6.2 Bioreactor Solid Matrix Samples

Analytical data for solid matrix samples collected during the autopsy of the bioreactors are included in Table 6-3 (a, b, c, d), and they are also presented in graphical form in the figures that are individually referred to in this section. Also presented in this section are analytical data of material that was found plugging the manifold for the AMD distribution system.

6.2.1 Metals Concentrations in the Bioreactors

Figures 6-6, 6-7, and 6-8 present the distribution of metals in the solid matrix of the first three chambers of Bioreactors II, III, and IV, respectively. These values are also compiled in Table 6-3a. Concentration of metals within the organic matter (denoted in the figures as organic matter chambers 1 and 2) was determined with respect to the total dry weight of the sample collected. For the limestone chamber, the precipitate present on the limestone was scraped, and metal concentrations were determined with respect to the dry weight, not including the weight of the limestone. As stated in Section 4.4.1, the cobbles that comprised the last chamber of each reactor did not have a visually discernible film of chemical precipitate, thus the metal concentrations in the solid matrix of these chambers were considered null.

Concentrations of metals that accumulated within the solid matrix of the bioreactors were measured in thousands of milligrams per kilogram with the exception of those for Cd and As. These high metals concentrations demonstrated that a large load of metals was retained within the organic carbon material, thus indicating the bioreactors were efficient in removing metals from the influent AMD. Unfortunately, the analyses performed using the inductively coupled argon plasma (ICP) method do not allow for distinguishing of metals that were adsorbed by organic matter from those that precipitated as chemical compounds due to SRB activities.

Evidence of the biological removal of metals by the SRB comes from other analytical results conducted for the project. First, the high concentrations of sulfide in the organic matter, as reported in Section 6.2.2, need to be stoichiometrically balanced. Metals that form amorphous metal sulfides, a chemical compound that can only be biogenic, probably provided this balance. Secondly, as stated in Section 5.5, Mn that is not biologically removable at the low pH level was efficiently removed only at the beginning of the operation when it was sorbed to the organic matter. After the sorption capacity of the organic matter was exhausted, Mn stayed in the aqueous phase while other metals (Zn, Cu, and Cd) were still being removed. Removal of these metals, with no organic-matter adsorption capacity present, can only be explained by formation of biogenic metal sulfides.

Because of a small population of samples collected, the diagrams in Figure 6-6 through 6-8 may be affected by spatial variability of metal concentrations. Nevertheless, the diagrams indicate some general features and trends that might not be explainable and/or clearly conclusive and are worth noting as general observations.

- Aluminum concentrations decrease along the flow paths within the first organic matter (Or1) and limestone chambers, then slightly increased in the second organic matter chamber (Or2) for Bioreactors II and IV.

- In general, Fe demonstrated a gentle trend of decreasing concentrations, though not without exceptions (mostly in Bioreactor IV). High concentrations of Fe in the first lift for all bioreactors agree with visual observations during the autopsy process when the orange-color precipitate was observed on the HDPE walls of the CCS and within the organic matter itself. This precipitate, iron hydroxide, was most abundant within the first CCS lift.

- Zinc seemed to be evenly distributed within Or1 of Bioreactor II, but its concentration peaked in the centers of Or1 of Bioreactors III and IV.

- There is a distinct trend of decreasing concentration for Cu along the flow direction for all three bioreactors.

- There seems to be an increasing trend for Mn concentration along the flow path, with the exception of a noticeable decrease in Mn concentration in the limestone chamber of Bioreactor IV.

- Cadmium concentrations behaved erratically for Bioreactors III and IV but had a decreasing trend for Bioreactor II. In all three bioreactors, Cd concentrations varied the most of all the metals presented.

- There was a decreasing trend for As concentrations within Bioreactors II and III and a similar but less obvious trend for Bioreactor IV. All bioreactors demonstrate much higher concentration of As in the most upgradient CCS lift (No. 1) than in the rest of the solid matrix sampled. For Bioreactor II, these much-elevated As concentrations extend to the second sampling point, lift No.3.

- The elevated concentrations of As at the very front portion of the bioreactors correlate very well with high concentrations of Fe at the same location.

Statistical correlation coefficients calculated for all possible pairs of the aforementioned metals are presented in Table 6-4. Outlined with heavy lines are three correlation coefficients that because of their high values need to be considered meaningful despite possible spatial variability of the metal concentrations that might have been missed by a small population of samples. These correlations coefficients are for Al and Cu, As and Fe, and Cd and Zn.

High correlation of Al and Cu may be explained by known observations that they both precipitate quickly when aqueous conditions change to a less acidic and more reducing environment. In such a case, Al precipitates as hydroxides, and Cu precipitates as sulfide.

A strong dependancy of As and Fe has already been explained in Section 5.5 by referring to a statement by Robins and Huang (Ref. 6) that As effectively adsorbs to $Fe(OH)_3$. As mentioned earlier in this section, observations during the autopsy indicated an abundance of $Fe(OH)_3$ precipitant in the bioreactors. The high correlation coefficient between As and Fe in the bioreactors support findings of Robins and Huang.

High correlation coefficient for concentrations of Cd and Zn were expected due to the similarity in chemical behavior of these two elements. As it appears, a two orders of magnitude difference in their concentrations in the aqueous solutions (Figures 5-11 and 5-13) corresponds to their precipitates in the bioreactors where they also differ by two orders of magnitude.

6.2.2 Sulfate and Sulfide Analyses

Figures 6-9 and 6-10 present diagrams for sulfate and sulfide profiles in the solid matrix of the bioreactors, respectively. These diagrams were plotted based on values included in Table 6-3c that were obtained from acid base accounting (ABA) analyses. The sulfide diagram (Figure 6-10) was assembled by adding values for insoluble sulfide to values of pyritic sulfides (both present in Table 6-3c) and converting the sum from percent to mg/kg.

Figure 6-9 shows different changes of sulfate concentrations within the first organic matter chamber of each bioreactor. However, considering the high value for lift No. 7 in Bioreactor IV as an outlier or a sulfate "nugget," there is some resemblance of concentration of sulfates in Bioreactors II and IV. In both bioreactors, the concentration of sulfates decrease toward the limestone chambers and then rise to a similar level in the second organic matter chamber. Sulfate concentrations in Bioreactor III, which does not include a limestone chamber, shows a general rising trend throughout the organic matter chamber. These features of the sulfate profiles show the advantage of placing a limestone chamber in the bioreactors.

Sulfide concentrations (Figure 6-10) within the first organic matter chamber are generally similar, except for CCS lift No. 9 in Bioreactor II. In general, sulfide concentrations in the organic matter chambers are above the 4,000 mg/kg level and are twice as high as concentrations of sulfates. The decrease of sulfide concentration in lift No. 9 of Bioreactor II to 3,300 mg/kg remains a conundrum. Its explanation can be sought in the fact that the relatively small number of samples collected (one every second lift) increases the chance for some samples not being representative of the actual hydrochemical conditions prevailing in the sampled medium.

Numerical values for sulfide concentrations in the solid matrix of organic matter are more than three orders of magnitude greater than those for the aqueous phase in the limestone and cobbles chambers (Table 6-1c). The actual difference was even greater because of the conversion of mg/kg for the solid matrix to mg/L for the aqueous phase. This is because 1 cubic decimeter of solid matrix, which equals 1 liter in volume, weighs more than 1 kilogram.

Sulfide load in organic matter, together with metal concentrations as addressed in Section 6.2.1, is indicative of metal sulfides precipitating in the organic matter chamber due to SRB activities. This postulate together with the observation of no precipitated metal in the cobble chambers of the bioreactors, indicates again that the role of the cobble section was limited to a sump for a small mass of precipitates that escaped from the organic matter chambers.

6.2.3 Total Organic Carbon

Numerical data for TOC concentration in the bioreactors solid matrix are included in Table 6-3b. As expected, they indicate that the lowest TOC concentrations were associated with the limestone chambers: 8.7 % and 1.2% by weight for Bioreactors II and IV respectively. The TOC concentrations within the organic matter chambers ranged from 17% to 24% with the majority of values above 20%. These values of TOC concentrations document that there was plenty of organic carbon remaining in the organic matter chambers after 32 months of bioreactor operation. Although, TOC concentrations for the initial fresh organic matter were not measured, the high TOC values measured during the autopsy may indicate that the depletion of organic carbon would not be a factor for the efficient removal of metals even if these bioreactors were operating for several more years.

6.2.4 Plugs in the Outlets of the AMD Distribution System Manifold

Many inlets in all three bioreactors were plugged with chemical precipitates located within the last 1 inch of the L-shaped inlet. Because the outlets (also called bioreactor inlets) were 2 inches in diameter, so were the plugs. The plugs had the appearance and consistency of gel. The upper half of a plug, the one facing the incoming AMD, was black. The lower half, which was in contact with the organic matter, was light brown. Analytical data for a plug collected from one of the outlets in Bioreactor III are tabulated in Table 6-3d and presented in graphical form in Figure 6-11.

As implied by a sharp boundary between black and brown portions of the plug, the concentrations of metals in each portion were different. The main differences were the concentrations of Al, Fe, Ca, and Mg (the last two are not shown in the figures). The Al concentration in the black portion of the plug was much higher (approximately 540% higher) than the Al concentration in the brown portion. Conversely, the concentrations of Fe, Ca, and Mg were lower in the black portion (i.e., approximately 45%, 26%, and 15%, respectively, of those in the brown portion).

With the exception of Fe, Al, and As, the metal concentrations in the plug were in the same range as those determined for samples collected from the CCS lift. Iron concentration in the brown portion of the plug was nine-fold of that found in the adjacent CCS lift and constituted 12.7% of the weight of the plug. Since it is expected that the majority of the Fe present in that place in the system was associated with $Fe(OH)_3$, this compound made up 24% of weight for the lower portion of the plug. An elevated (five times higher than in adjacent CCS lift) concentration of As was certainly the result of its adsorption to $Fe(OH)_3$, as explained earlier in this report.

Aluminum concentration in the upper portion of the plug was more than six-fold of that determined for the adjacent CCS lift. Assuming its presence as a hydroxide, this compound made up 16% of weight for the upper portion of the plug.

The high percentage of Fe and Al hydroxides in the plug indicates a high probability that the bioreactor inlets were plugging mainly due to chemical reactions; the sediment carried by the unfiltered AMD was a secondary reason for plugging.

6.3 Flow Pattern

Visual observations made during the autopsy of the bioreactors indicated no preferential flow paths developed during the 32-month operation period. This means that the AMD flowed and was treated throughout the entire cross section of the organic matter. Most of the individual cells of the CCS were found full of organic matter. Probably less than 5% of cells were voided of the organic matter in their top portions, with voids never taller than 2 inches. There was no discernible pattern of these voids that seemed to be uniformly distributed throughout each individual CCS lift.

6.4 Sulfate-Reducing Bacteria Population on the Solid Matrix

Although the SRB population in the aqueous phase of the bioreactors was measured during the operating period, no measurements of SRB population attached to the solid matrix of the bioreactors were made. To measure the final population of SRB within the solid matrix, samples of both the organic matter and limestone were collected during the autopsy and submitted to a bacteriological laboratory for an SRB count. The results returned were expressed in MPN of SRB per gram (MPN/g) of wet weight of the solid matrix for organic matter and in MPN of SRB per sample for the limestone.

Therefore, the results needed to be converted and expressed as a function of the volume of the matrix. This conversion was needed to compare the SRB population in the aqueous and solid phases using the same dimensions (i.e., MPN per volume).

To make the aforementioned conversion for the organic matter, an attempt was made to determine its particle density. This presumably simple task was not successful because standard methods used for determination of particle density of solids did not seem to be applicable for organic matter. Therefore, the SRB population per unit volume was determined as a function of particle density. To accomplish this, a geometric mean of MPN/g for SRB populations in eight samples collected from all three bioreactors was calculated. This value, 1.83E+06 MPN/g, and the average volumetric moisture content of 58.5% were used to determine SRB populations as a function of particle density. Thus, the values for SRB populations were expressed as MPN per unit volume of wet organic matter, and they ranged from 1.45E+06 MPN per cubic centimeter (MPN/cc) to 2.63E+6 MPN/cc for the particle density ranging from 0.5 grams per cubic centimeter (g/cc) to 2.05 g/cc, respectively. Assuming particle density of 1.3 g/cc, the MPN for SRB population in 1 cc of wet organic matter was 2.06E+6 MPN/cc. This population of SRB attached to the solid matrix of the organic matter is two orders of magnitude greater than the value of 2.81E+4 MPN/milliliter (mL) calculated as a geometric mean for three aqueous samples collected during the last sampling event on July 9, 2001.

To determine SRB population per unit volume of the limestone chamber, an average surface area for the limestone was calculated. This was necessary because the SRB populations on the limestone were determined by the bacteriological laboratory by scraping the biofilm from the sampled limestone and then expressing the population of SRB as MPN per sample. The calculations performed resulted in a determination of SRB population at 8.2E+4 MPN/cc. This SRB population is of the same order of magnitude as for the aqueous samples. The unit of cubic centimeter for the limestone population estimate is used in this case for comparison purposes only. Actually, a more representative unit of volume would have been cubic meter since the limestone ranged in size from 5 centimeters (cm) to 15 cm in diameter.

6.5 Toxicity Characteristic Leaching Procedure

The results of TCLP testing demonstrated that the solid matrix of the bioreactors did not exhibit characteristics of toxicity as it was documented by concentrations of Cd and As, the only metals listed in *40 Code of Regulations* that might be of concern for the project. Cadmium and As concentrations in leachate from samples of organic carbon matter were 7.6 and 136 times lower, respectively, than the regulatory levels that would classify this matrix as hazardous waste.

Tables 6-1. Analytical Results of the Aqueous Samples Collected During the Autopsy

Table 6-1a. Total and Dissolved Metals

				Dissolved metals (Fg/L)					
Location	**Al**	**As**	**Cd**	**Ca**	**Cu**	**Fe**	**Mg**	**Mn**	**Zn**
Bioreactor II cobble chamber	1380	29.3	4.8	13500	86.8	2910	29400	1760	506
Bioreactor III cobble chamber	353	16.8	4.8	87000	220	2830	20000	2720	1030
Bioreactor IV cobble chamber	162	8.5	4.8	124000	38.7	981	33300	1980	329
				Total metals samples (Fg/L)					
Location	**Al**	**As**	**Cd**	**Ca**	**Cu**	**Fe**	**Mg**	**Mn**	**Zn**
Bioreactor II limestone chamber	34700	251	86.1	1040000	2450	43000	55100	7270	20400
Bioreactor II cobble chamber	37100	53.1	5.3	152000	951	52200	45100	3000	2150
Bioreactor III cobble chamber	34300	53.1	5.9	109000	592	42300	36200	5280	3770
Bioreactor IV limestone chamber	7300	30	11.4	539000	506	13200	42800	3160	6390
Bioreactor IV cobble chamber	9240	20	4.8	122000	159	12700	35200	2170	856

Table 6-1b. Iron speciation

	Iron Species (mg/L) (nonfiltered samples)			
Location	**Fe II**	**Fe III**	**Fe Total**	**% Fe Recovery**
Bioreactor II limestone chamber	1.9	0.17	2.1	100
Bioreactor II cobble chamber	3.6	<0.05	3.5	100
Bioreactor III cobble chamber	2	<0.05	2.1	100
Bioreactor IV limestone chamber	0.93	0.057	0.99	100
Bioreactor IV cobble chamber	1	0.076	1.1	100

Table 6-1c. Sulfate and Sulfide

	Sulfate and Sulfide concentrations (mg/L) (nonfiltered samples)	
Location	**Sulfate**	**Sulfide**
Bioreactor II limestone chamber	309[1]	3.2
Bioreactor II cobble chamber	240[1]	3.9
Bioreactor III cobble chamber	352	2.2
Bioreactor IV limestone chamber	225	3
Bioreactor IV cobble chamber	159[1]	4.2

[1]Corrected values obtained using inductive coupled plasma (ICP) method

Table 6-2. Comparison of the Dissolved Metals from the Autopsy and Monthly Sampling

Bioreactor	Sample location	Date	Dissolved metal concentration (µg/L)						
			Zn	Cu	Mn	Cd	As	Al	Fe
II	AII[1]	07/9/01	899	110	996	5	4	118	707
	Bottom[2]	07/17/01	506	87	1,760	5	29	1,380	2,910
III	AIII[1]	07/9/01	1,010	122	2,470	6	3	80	638
	Bottom[2]	07/17/01	1,030	220	2,720	5	17	353	2,838
IV	AIV[1]	07/9/01	292	18	1,270	5	1	42	572
	Bottom[2]	07/16/01	329	39	1,980	5	9	162	981

[1] See Figure 3-5 for location
[2] Bottom of the cobble chamber adjacent to the organic matter chamber

Tables 6-3. Analytical Results of the Solid Matrix Samples Collected During the Autopsy

Table 6-3a. Metal Concentrations

Location	Metal concentrations of the solid matrix samples (mg/Kg)								
	Al	As	Cd	Ca	Cu	Fe	Mg	Mn	Zn
Bioreactor II manure chamber 1 lift 1	7830	42.1	23.4	8490	1470	11400	5170	931	4450
Bioreactor II manure chamber 1 lift 3	13700	31.4	31.9	10400	2970	9960	9890	902	3790
Bioreactor II manure chamber 1 lift 5	10900	23.6	21.6	6350	2260	6910	6990	387	3470
Bioreactor II manure chamber 1 lift 7	6880	22.8	28.6	12300	975	9040	7640	1200	4270
Bioreactor II manure chamber 1 lift 9	4700	15.4	12.8	38800	438	7010	6480	884	2720
Bioreactor II limestone chamber	2650	8.3	3.8	208000	163	4150	5110	740	1000
Bioreactor II manure chamber 2 lift 5	3730	10.1	2.4	11500	147	7280	5920	866	3010
Bioreactor III intake plug - brown	10300	400	25.7	5700	7340	127000	3980	25200	2530
Bioreactor III intake plug - black	55800	485	31.2	1480	8560	57800	613	35100	2100
Bioreactor III manure chamber 1 lift 1	8600	76.1	24.3	9560	1480	14000	6180	637	3630
Bioreactor III manure chamber 1 lift 3	4020	24	10.1	13100	241	7710	6670	940	3820
Bioreactor III manure chamber 1 lift 5	6060	19.1	35.5	10500	596	8280	7130	783	5790
Bioreactor III manure chamber 1 lift 7	5500	17.8	5	15400	579	8970	6680	912	2900
Bioreactor III manure chamber 1 lift 9	4550	15.8	18.7	12600	203	8370	5210	1180	4880
Bioreactor IV manure chamber 1 lift 1	15100	109	10.5	2740	2400	22900	4750	263	1660
Bioreactor IV manure chamber 1 lift 3	13000	18.2	10.8	6130	2340	7390	6130	402	1600
Bioreactor IV manure chamber 1 lift 5	10900	14.9	59.3	9460	1670	6580	7600	668	7690
Bioreactor IV manure chamber 1 lift 7	5680	22	19.4	16600	562	7830	5290	655	6400
Bioreactor IV manure chamber 1 lift 9	3890	14.4	0.92	19100	107	9210	4930	800	1610
Bioreactor IV limestone chamber	2400	4.2	0.91	274000	58.9	4050	4800	185	566

Table 6-3a. Metal Concentrations

Location	Metal concentrations of the solid matrix samples (mg/Kg)								
	Al	As	Cd	Ca	Cu	Fe	Mg	Mn	Zn
Bioreactor IV manure chamber 2 lift 5	4700	19.9	0.92	18500	178	10300	7110	1500	583

Table 6-3b. Nitrogen, Phosphorus, and TOC

Location	Analytical chemistry for solid matrix samples (mg/Kg)		
	Total Nitrogen	Phosphorous	TOC (%)
Bioreactor II manure chamber 1 lift 1	10251	58.2	20.0
Bioreactor II manure chamber 1 lift 3	14623	86.1	20.0
Bioreactor II manure chamber 1 lift 5	12259	53	22.3
Bioreactor II manure chamber 1 lift 7	12141	62.1	23.4
Bioreactor II manure chamber 1 lift 9	12983	124	17.2
Bioreactor II limestone chamber	3056	144	8.7
Bioreactor II manure chamber 2 lift 5	13086	65.9	23.3
Bioreactor III manure chamber 1 lift 1	10917	74.4	22.6
Bioreactor III manure chamber 1 lift 3	15533	104	21.6
Bioreactor III manure chamber 1 lift 5	10406	44.1	20.7
Bioreactor III manure chamber 1 lift 7	11074	119	21.2
Bioreactor III manure chamber 1 lift 9	6689	72.4	21.4
Bioreactor IV manure chamber 1 lift 1	8308	60.4	24
Bioreactor IV manure chamber 1 lift 3	13528	90.4	22.1
Bioreactor IV manure chamber 1 lift 5	11063	78.9	23.5
Bioreactor IV manure chamber 1 lift 7	10501	66.1	21.6
Bioreactor IV manure chamber 1 lift 9	9920	68.4	23.9
Bioreactor IV limestone chamber	3963	22	1.2
Bioreactor IV manure chamber 2 lift 5	10682	168	16.9

Table 6-3c. Sulfide Acid-Base Accounting

Location	Sulfide Analysis by Acid-Base accounting				
	Total % Sulfur	Hot Water Extractable S % Sulfate	HCl Extractable S % Insoluble Sulfide	HNO_3 Extractable S % Pyritic Sulfide	Residual S % Organic
Bioreactor II manure chamber 1 lift 1	0.79	0.20	0.04	0.48	0.08
Bioreactor II manure chamber 1 lift 3	0.70	0.19	0.03	0.44	0.05
Bioreactor II manure chamber 1 lift 5	0.63	0.03	0.14	0.41	0.06
Bioreactor II manure chamber 1 lift 7	0.69	0.18	0.05	0.41	0.05
Bioreactor II manure chamber 1 lift 9	0.46	0.07	<0.01	0.33	0.07
Bioreactor II limestone chamber	0.15	0.07	<0.01	0.17	0.02
Bioreactor II manure chamber 2 lift 5	0.87	0.25	0.06	0.50	0.06
Bioreactor III manure chamber 1 lift 1	0.76	0.23	0.04	0.37	0.12
Bioreactor III manure chamber 1 lift 3	0.82	0.28	0.08	0.32	0.14
Bioreactor III manure chamber 1 lift 5	1.12	0.44	0.13	0.41	0.14
Bioreactor III manure chamber 1 lift 7	1.02	0.33	0.11	0.41	0.17
Bioreactor III manure chamber 1 lift 9	1.10	0.52	<0.01	0.56	0.06

Table 6-3c. Sulfide Acid-Base Accounting

Location	Total % Sulfur	Hot Water Extractable S % Sulfate	HCl Extractable S % Insoluble Sulfide	HNO_3 Extractable S % Pyritic Sulfide	Residual S % Organic
	Sulfide Analysis by Acid-Base accounting				
Bioreactor IV manure chamber 1 lift 1	0.68	0.21	0.06	0.32	0.10
Bioreactor IV manure chamber 1 lift 3	0.72	0.23	0.02	0.42	0.05
Bioreactor IV manure chamber 1 lift 5	0.84	0.27	0.03	0.46	0.08
Bioreactor IV manure chamber 1 lift 7	1.19	0.81	<0.01	0.47	0.14
Bioreactor IV manure chamber 1 lift 9	0.90	0.29	0.06	0.49	0.07
Bioreactor IV limestone chamber	<0.01	<0.01	<0.01	<0.01	<0.01
Bioreactor IV manure chamber 2 lift 5	0.69	0.29	<0.01	0.32	0.08

Table 6-3d. Analytical Results for Plugging Material in the Manifold of Bioreactor III

Location	Al	As	Cd	Ca	Cu	Fe	Mg	Mn	Zn
	Total metals in plugging material (mg/Kg)								
Reactor III intake plug - brown	10300	400	25.7	5700	7340	127000	3980	25200	2530
Reactor III intake plug - black	55800	485	31.2	1480	8560	57800	613	35100	2100

Table 6-4. Correlation Coefficients (k) for Total Metals Concentrations in the Solid Matrix of Bioreactors II, III, and IV

	Al	As	Cd	Cu	Fe	Mn	Zn
Al	1	0.602	0.475	0.9703	0.5622	-0.376	0.1745
As		1	0.095	0.5278	0.9434	-0.281	-0.0387
Cd			1	0.476	0.0175	0.0003	0.8375
Cu				1	0.4464	-0.39	0.1559
Fe					1	-0.096	-0.0747
Mn						1	0.1254
Zn							1

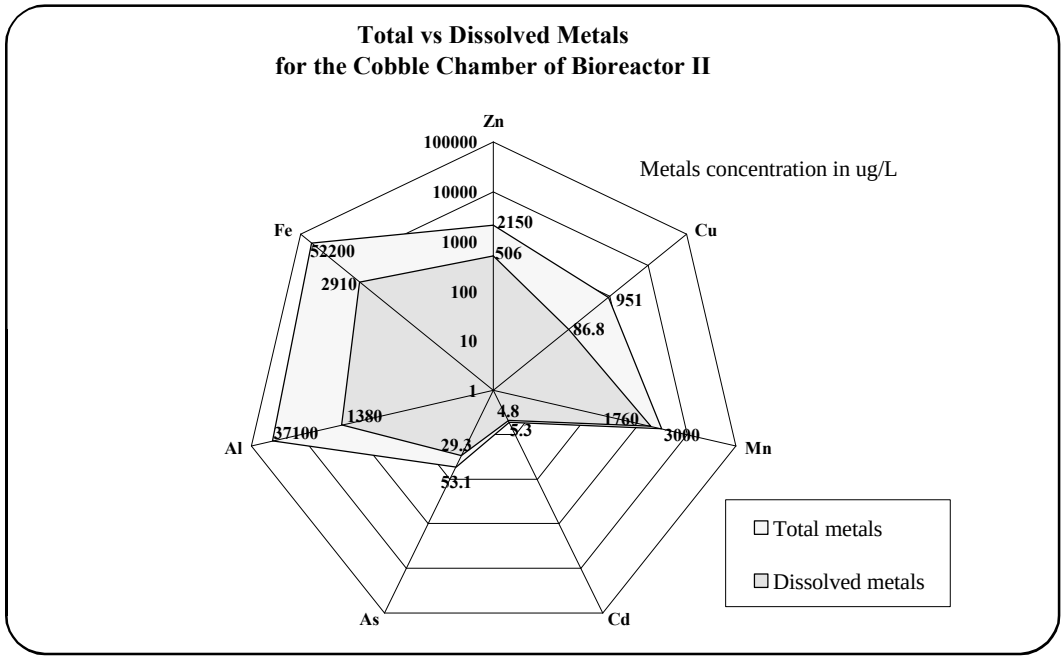

Figure 6-1a. Total vs. dissolved metals for the cobble chamber of Bioreactor II.

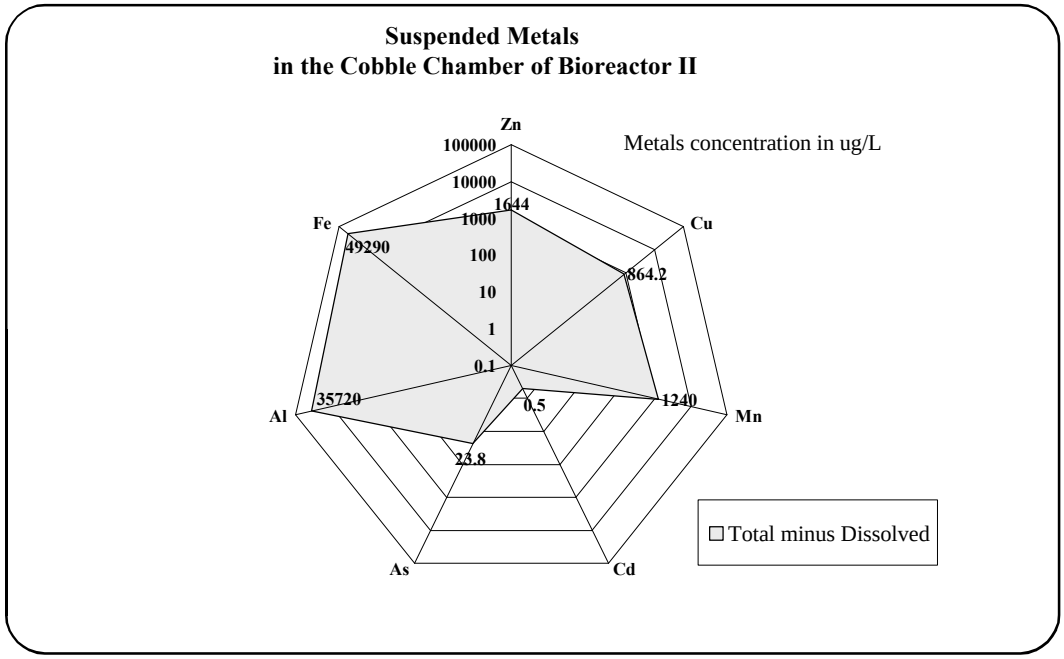

Figure 6-1b. Suspended metals in the cobble chamber of Bioreactor II.

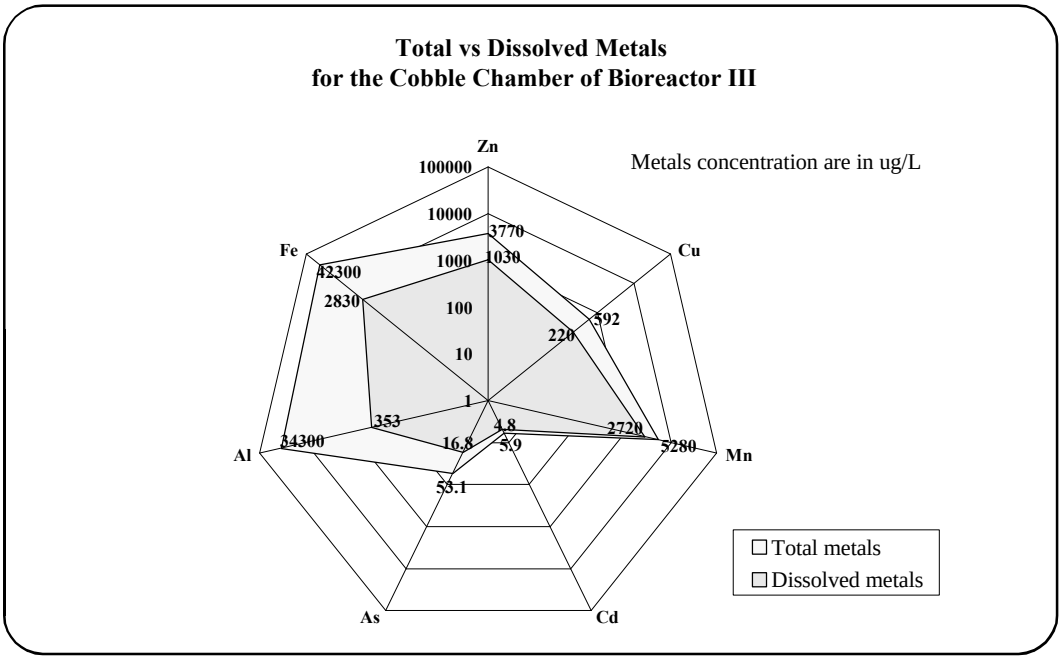

Figure 6-2a. Total vs. dissolved metals for the cobble chamber of Bioreactor III.

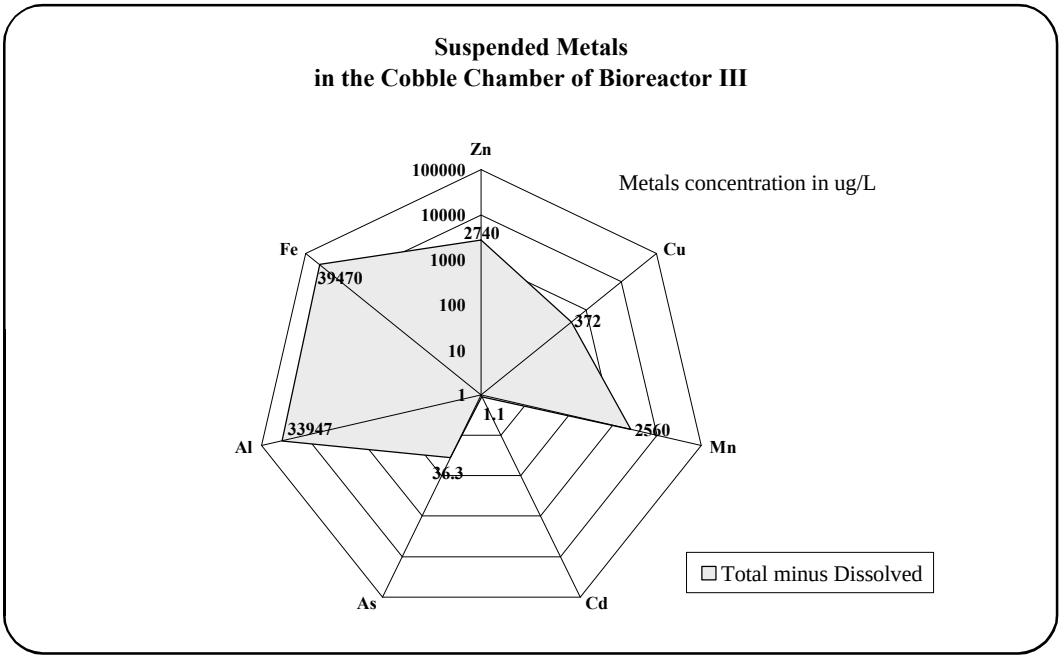

Figure 6-2b. Suspended metals in the cobble chamber of Bioreactor III.

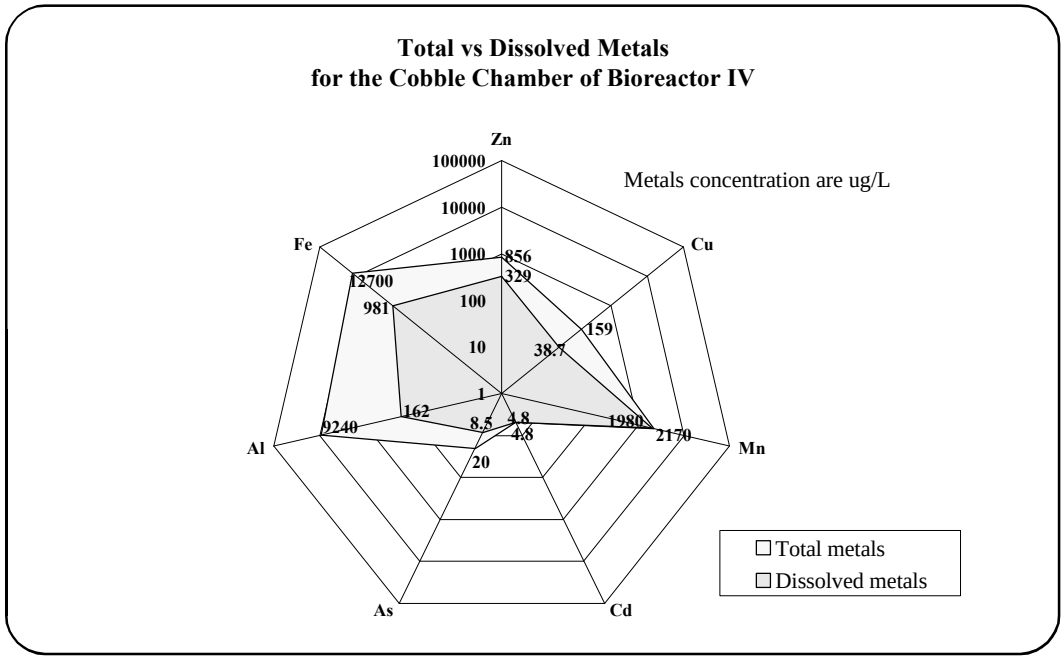

Figure 6-3a. Total vs. dissolved metals for the cobble chamber of Bioreactor IV.

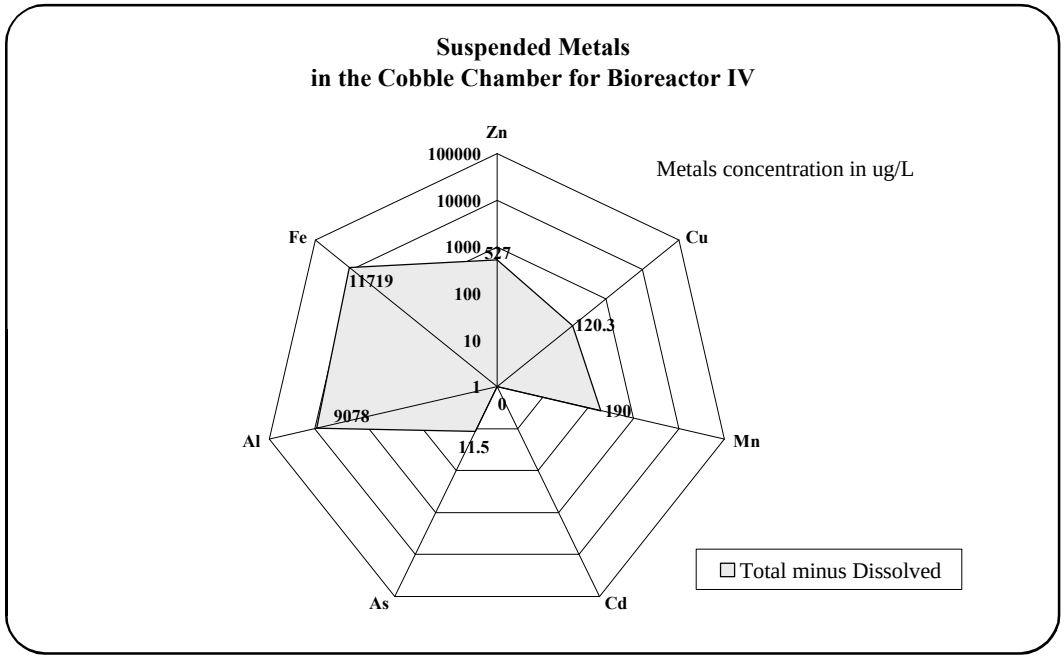

Figure 6-3b. Suspended metals in the cobble chamber of Bioreactor IV.

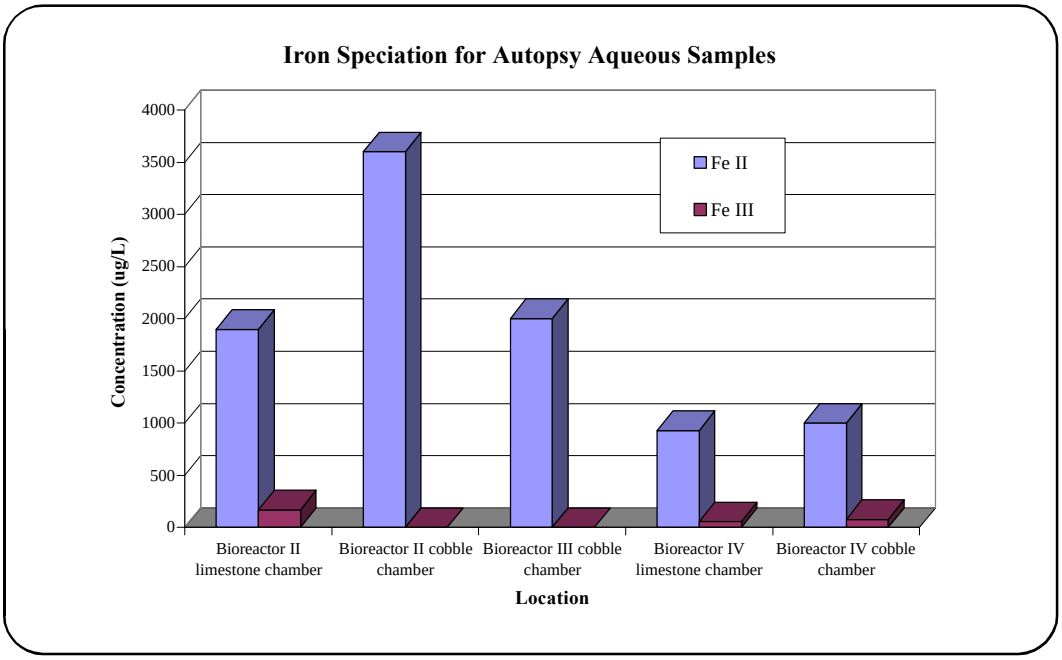

Figure 6-4. Iron speciation for autopsy aqueous samples.

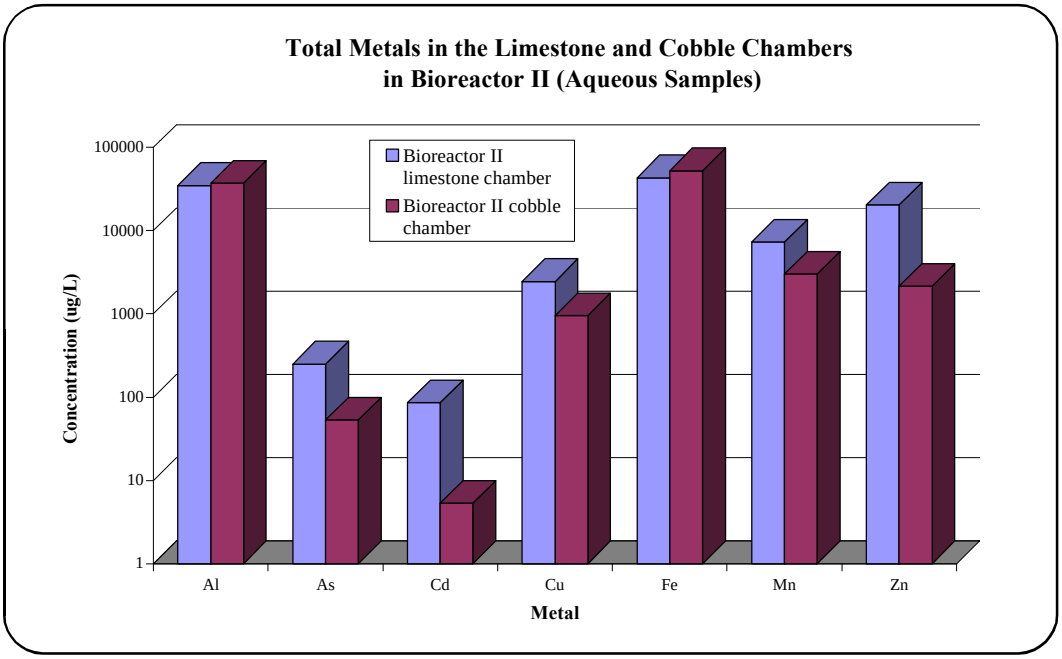

Figure 6-5. Total metals in the limestone and cobble chambers in Bioreactor II (aqueous samples).

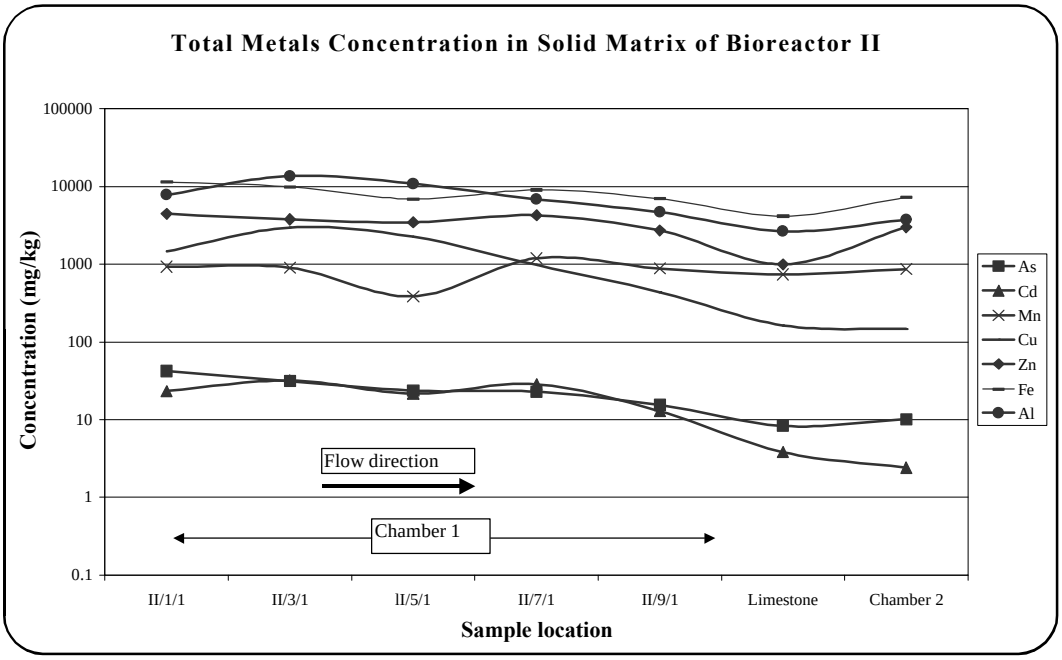

Figure 6-6. Total metals concentration in the solid matrix of Bioreactor II.

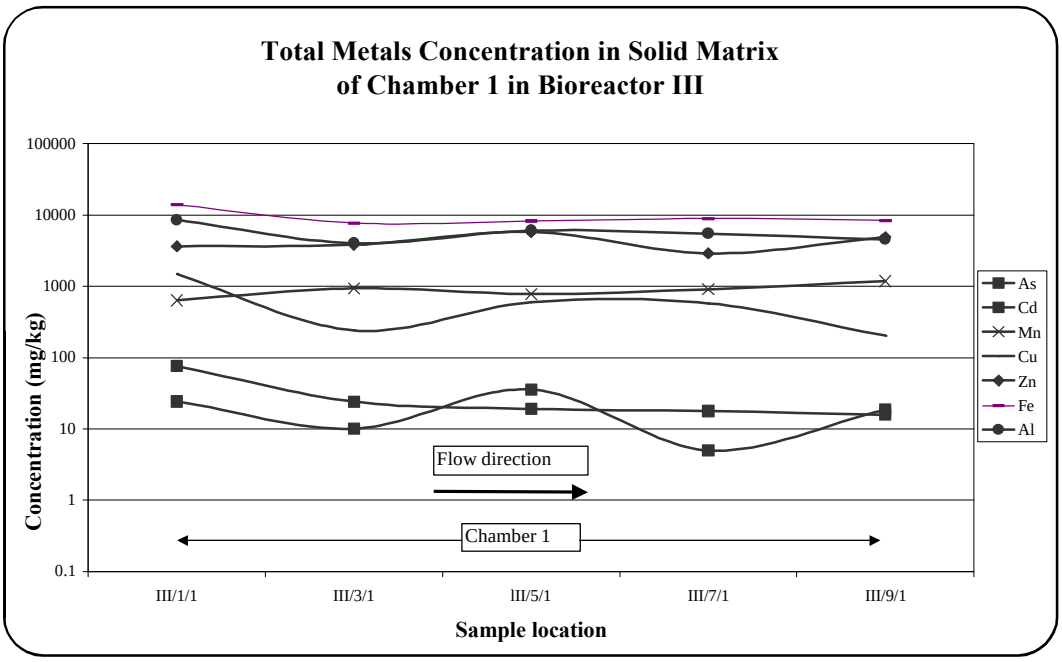

Figure 6-7. Total metals concentration in the solid matrix of Chamber 1 in Bioreactor III.

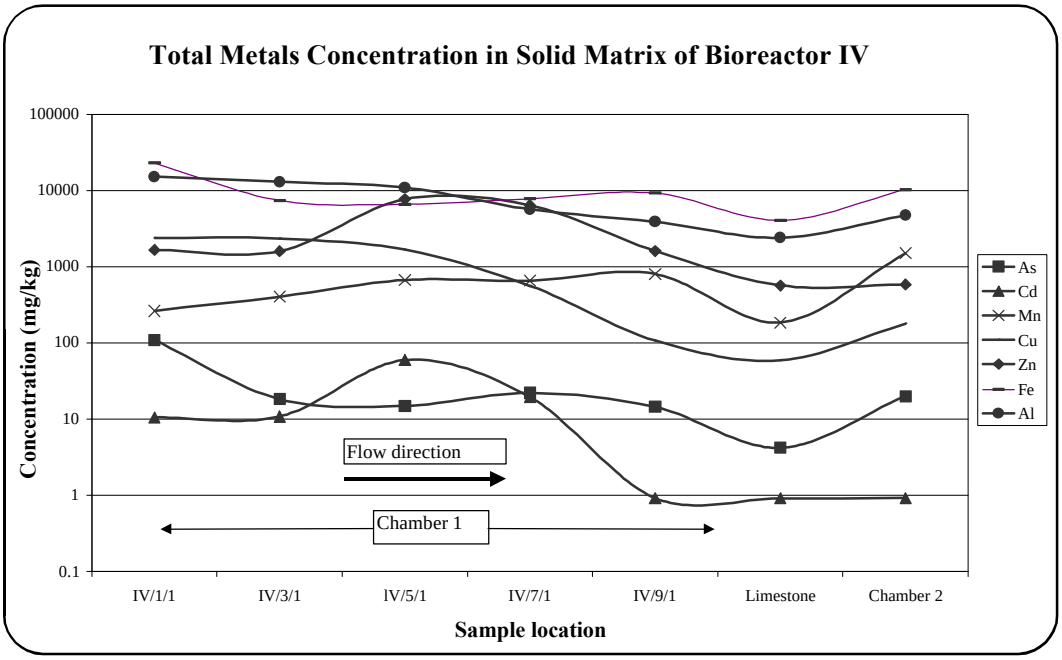

Figure 6-8. Total metals concentration in the solid matrix of Bioreactor IV.

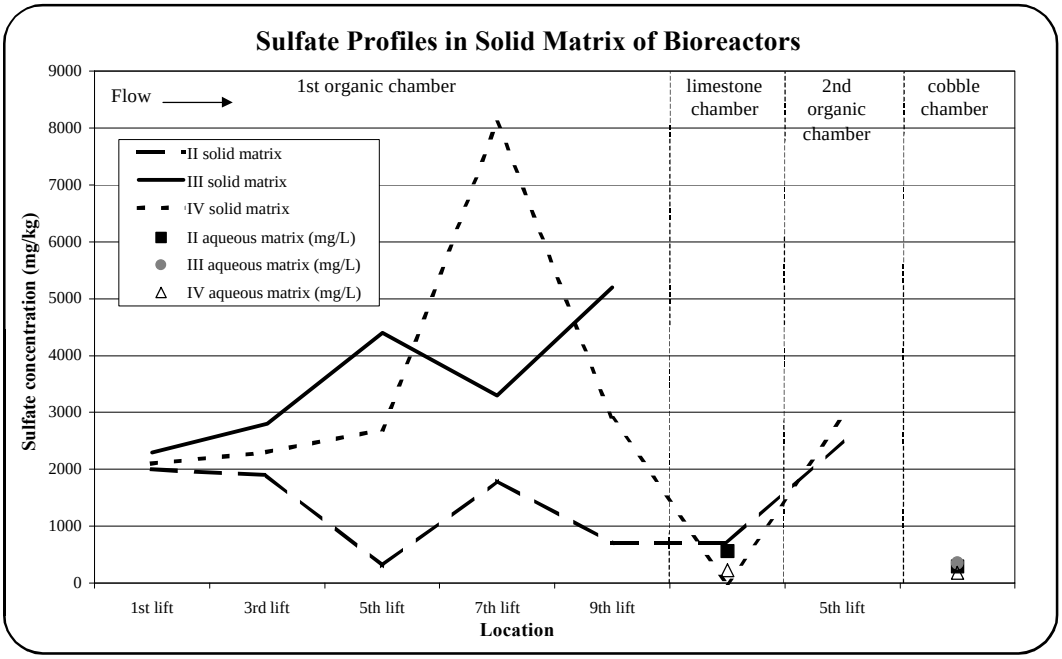

Figure 6-9. Sulfate profiles in the solid matrix of the bioreactors.

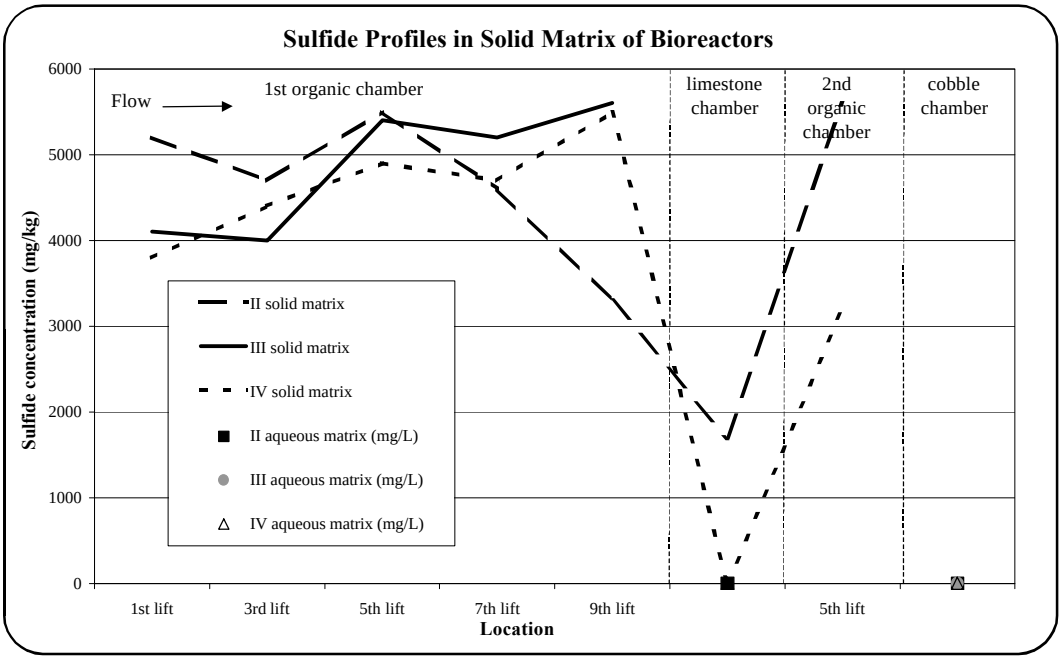

Figure 6-10. Sulfide profiles in the solid matrix of the bioreactors.

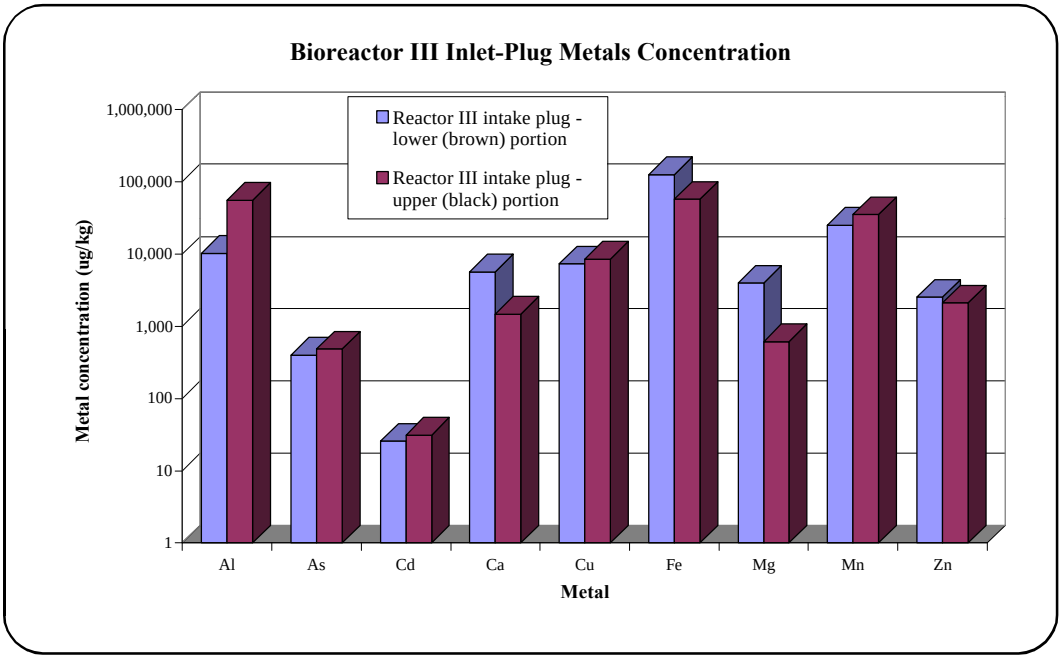

Figure 6-11. Bioreactor III inlet-plug metals concentrations.

7. Quality Assurance/Quality Control

7.1 Background

Following is a summary of the Quality Assurance (QA) activities associated with the project. Samples were collected according to the schedule outlined in the approved project-specific QAPP document. Performance data were collected monthly for 32 months. All field and laboratory data available had been evaluated to determine the usability of the data. Dissolved metals (Al, Cd, Cu, Fe, Mn, and Zn) analysis and field pH measurements were classified as critical analyses for this project. A critical analysis is an analysis that must be performed in order to determine if project objectives were achieved. Data from noncritical analyses were also evaluated.

7.2 Project Reviews

During the project, the following evaluations were performed:

- internal field systems review at the demonstration site; and
- external Technical Systems Audit (TSA).

7.2.1 Internal Field Systems Review at the Demonstration Site

A field systems review was performed on October 10, 1999, at the Calliope Mine. The field systems review included a review of the following items:

- personnel, facilities, and equipment;
- documentation (chain-of-custody, logbooks);
- calibration of equipment; and
- sampling procedures.

No concerns were identified during the audit.

7.2.1.1 Personnel, Facilities, and Equipment

Personnel present during the audit included John Trudnowski, MSE Project Engineer; Rod Schwab, MSE Sampler; and Ken Reick, MSE Project QA Officer. The Project Engineer and Sampler were knowledgeable about the demonstration and their duties and responsibilities at the demonstration site.

7.2.1.2 Documentation

Chain-of-custodies (COC) were reviewed at the demonstration site, and all COC procedures were being followed. The project logbooks were also reviewed. The sampling logbook was very thorough and included spaces where specific information was required. Sampling personnel were familiar with the logbook format and COC procedures. The sampling logbook did not conform to the Standard Operating Procedures (SOP) because the pages of the logbook were not numbered consecutively as stated in the SOP.

7.2.1.3 Calibration of Equipment

Field equipment was used to measure pH, dissolved oxygen, temperature, and E_H potential. This information was recorded in the project logbooks. All meters were properly calibrated prior to performing measurements. Standard operating procedures were available at the demonstration site and explained how to calibrate/operate the meters. Sampling personnel were familiar with the SOPs and requirements for routine calibration of the various meters.

Measurement of pH in the water samples is a critical measurement and, therefore, has had quality control (QC) objectives already assigned. The quantitative objectives are accuracy, precision, and completeness. The absolute difference between the measurement and the buffer pH is reported as accuracy. Precision is based on consecutive determinations of accuracy. During sampling, accuracy was determined by measuring the appropriate buffer; however, it was performed only once (this QC check was well within the required limits). Because consecutive determinations were not performed, precision could not be determined. For future sampling events, the samplers were

required to determine the accuracy and precision of the pH measurements.

As a corrective action for the audit observations, an amended QAPP was prepared. The need for accuracy and precision measurements associated with critical field measurements were reiterated in annual MSE internal sampling refresher training.

7.2.1.4 Sampling Procedures

A review of sampling activities was also performed during the systems review. All sample collection procedures and equipment decontamination procedures were followed by sampling personnel.

7.2.2 External Technical Systems Audit

In addition to the internal field systems review by MSE, an external TSA of both the project and the HKM Laboratory was performed by Joe Evans of Science Applications International Corporation (subcontractor to EPA) during the week of September 25, 2000. There were no findings resulting from the audit; however, there were three observations. All three observations related to making minor changes to the QAPP to more accurately reflect field procedures. An amended project-specific QAPP was prepared as a result of the audit.

7.3 Data Evaluation

The data quality indicator objectives for field pH measurements and dissolved metals analysis were outlined in the QAPP and were compatible with project objectives and the methods of determination being used. The data quality indicator objectives were method detection limits (MDLs) for accuracy, precision, and completeness. Control limits for each of these objectives are summarized in Table 7-1.

In addition to the data quality indicators listed in Table 7-1, HKM also analyzed internal QC checks, including calibration, calibration verification checks, calibration blanks, method blanks, and laboratory control samples. These QC checks have also been evaluated for the purpose of this data review.

7.4 Validation Procedures

Data that were generated throughout the project were validated. The purpose of data validation is to determine the usability of data generated during a project. Data validation consists of two separate evaluations: 1) an analytical evaluation and 2) a program evaluation.

7.4.1 Analytical Evaluation

An analytical evaluation is performed to determine the following:

- that all analyses were performed within specified holding times;
- that calibration procedures were followed correctly by field and laboratory personnel;
- that laboratory analytical blanks contain no significant contamination;
- that all necessary independent check standards were prepared and analyzed at the proper frequency and that all remained within control limits;
- that duplicate sample analysis was performed at the proper frequency and that all Relative Percent Differences (RPD) were within specified control limits;
- that matrix spike sample analysis was performed at the proper frequency; and
- that all spike recoveries (%R) were within specified control limits.

Measurements that fall outside of the control limits specified in the QAPP, or for other reasons were judged to be outlier, were flagged appropriately to indicate that the data were judged to be estimated or unusable.

An analytical evaluation was performed to determine the usability of data that were generated by the HKM Laboratory for the project. Laboratory data validation was performed using

Reference 7 as a guide. The QC criteria outlined in the QAPP were also used to identify outlier data and to determine the usability of the data for each analysis. A summary of QC check results for the critical dissolved metals and pH analyses are presented in Table 7-2. All data requiring flags are summarized in Table 7-3. In addition to the analytical evaluation, a program evaluation was performed.

7.5 Program Evaluation

Program evaluations include an examination of data generated during the project to determine:

- that all samples, including field QC samples, were collected, sent to the appropriate laboratory for analysis, and were analyzed and reported by the laboratory for the appropriate analyses;
- that all field blanks contained no significant contamination; and
- that all field duplicate samples demonstrated precision of field as well as laboratory procedures by remaining within control limits established for RPD.

Program data that were inconsistent or incomplete and did not meet the QC objectives outlined in the QAPP were viewed as program outliers and were flagged appropriately to indicate the usability of the data.

7.5.1 Field QC Samples

In addition to internal laboratory checks, field QC samples were collected to determine overall program performance.

7.5.2 Field Blanks

None of the field blanks collected for the project showed significant contamination for dissolved metals analysis, with two exceptions. The field blank collected on June 3, 1999, showed significant contamination (greater than the Contract Required Detection Limit under the Contract Laboratory Program). Iron samples with concentrations less than 10 times the contamination concentration in the field blank were flagged "U." A "U" flag indicates the data are undetected below the associated value. Another field blank collected on August 11, 1999, showed significant contamination for manganese analysis; however, all of the sample concentrations were greater than 10 times the contamination, so no samples required a flag. Early in the project, several field blanks showed significant contamination for cadmium and zinc. The problem was investigated and traced to the holding tank being used to store deionized (DI) water. A clean tank solved the problem. Samples were not flagged because the contamination source was not linked to contamination problems resulting from sampling procedures.

7.5.3 Field Duplicates

All field duplicates collected were within control limits for all analyses, with the four exceptions. A field duplicate collected on January 8, 2001, was out of control for aluminum analysis. While EPA does not specify control limits for field duplicates, the data reviewer is allowed discretion when evaluating field duplicates. For this project, precision control limits of #35% RPD were used for field duplicates. As a result, the samples from the January 8, 2001, event were flagged "J." A "J" flag indicates that the associated value is estimated. Field duplicates samples collected on April 5, 1999, and February 12, 2001, were out of control for copper analyses, resulting in samples from these events being flagged "J" for copper. A field blank collected on February 3, 1999, was out of control for iron analysis, and associated samples were flagged "J."

7.6 Summary

All data from the HKM Laboratory were validated according to EPA guidelines and the project-specific QAPP. Some of the data were flagged for various reasons and are summarized in Table 7-3.

The importance of calibration of field meters should be reiterated to sampling personnel because the lack of calibration resulted in critical pH data from two sampling events being discarded. On a positive note, the data were very organized, which made the data evaluation process much easier.

MWTP, Activity III, Project 12 presented unique challenges for the sampling and analytical team. While several of the data points were qualified for various reasons, this multi-year project produced high quality data.

Table 7-1. Data Quality Indicator Objectives

Parameter	Matrix	Unit	MDL[a]	Precision[b]	Accuracyc[c]	Completeness[d]
pH	Aqueous	SU	N/A	±0.1[e]	±0.1[f]	95%
Dissolved Metals	Aqueous	µg/L	5	#20%	75-125%	95%

[a]MDLs were based on what is achievable by the methods and what is necessary to achieve project objectives and account for anticipated dilutions to eliminate matrix interferences. MDLs were adjusted as necessary when dilutions of concentrated samples are required.
[b]Relative percent difference of analytical sample duplicates.
[c]Percent recovery of matrix spike, unless otherwise indicated.
[d]Based on number of valid measurements compared to the total number of samples.
[e]Precision of pH measurements was based on consecutive determinations of accuracy.
[f]Accuracy of pH measurements was based on the absolute difference between accepted value of the buffer and the measured value of the buffer.

Table 7-2. Summary of QC Checks for Critical Field pH Measurements and Dissolved Metals Analysis

Analysis	**Mean Absolute Difference (Precision)**	**Range of Absolute Differences (Precision)**
Field pH Measurements	**0.02 SU**	**0–0.09 SU**
Analysis	Mean RPD for Sample Duplicates	Range of RPDs for Sample Duplicates
Dissolved Al	9.9%	0–28.3%
Dissolved As	7.4%	1.3–18.8%
Dissolved Cd	2.8%	0.9–8%
Dissolved Cu	8.4%	0–50.6%
Dissolved Fe	4.6%	0–12.6%
Dissolved Mn	2.6%	0–8.4%
Dissolved Zn	8.2%	0–70.3%
Analysis	**Mean Absolute Difference (Accuracy)**	**Range of Absolute Differences (Accuracy)**
Field pH Measurements	**0.02 SU**	**0–0.09 SU**
Analysis	Mean Matrix Spike Recovery	Range of Matrix Spike Recoveries
Dissolved Al	101%	82–131%
Dissolved As	95.5%	58.9–114%
Dissolved Cd	99.6%	88–110%
Dissolved Cu	100.4%	84–115%
Dissolved Fe	101.1%	82.2–134%
Dissolved Mn	102.2%	79.3%–127%
Dissolved Zn	98.2%	69.6–114.8%

Table 7-3. Summary of Qualified Data for MWTP Activity III, Project 12

Date[1]	Sample ID	Analysis	QC Criteria	Control Limit	Result	Flag[2]	Comment
12/23/98	BDRB D-Blank IAD IILD IIIA IVKD IVKDD IVKDS	Dissolved Cu	Analytical Duplicate	#20% RPD	50.6	J	Samples were flagged "J" due to an out-of-control field duplicate.
01/04/99	II1D IIIJD IVKD IIIA	Alkalinity (forms)	Initial Calibration Verification	90-110% recovery	63% recovery	R	Initial calibration verification had <75% recovery; therefore, the data should be removed from consideration.
02/03/99	ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved Fe	Field Duplicate	#35% RPD	47.4% RPD	J	Flag samples "J" for out-of-control field duplicate.
04/05/99		Field pH	Holding Time	Analyze immediately	Hours elapsed	J	Because pH readings were not performed immediately in the field but instead analyzed in the laboratory following transport to Butte, the pH readings from this sampling event should be considered estimated and flagged with a "J."
04/05/99	ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved Cu	Field Duplicate	#35%RPD	165% RPD	J	Flag samples "J" for out-of-control field duplicate.
06/03/99	ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved Fe	Field Blank	#100 ppb	128 ppb	U	Flag samples with concentrations less than 10 times the contamination concentration "U" due to out-of-control field blank.

Date[1]	Sample ID	Analysis	QC Criteria	Control Limit	Result	Flag[2]	Comment
7/12/99	BDRB FB ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved Zn	Matrix Spike Analytical Duplicate	75-125% recovery #20% RPD	69.6% 70.3%	J	Zinc results should be flagged "J" as estimated for out-of-control spike and duplicate.
08/11/99	BDRB FB ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved Zn	Matrix Spike	75-125% recovery	72.8	J	Zinc results should be flagged "J" as estimated for out-of-control matrix spike.
08/11/99		Field pH	Calibration	Required each sampling event	Not performed	R	Flag samples "R" as unusable due to lack of calibration documentation.
04/05/00	BDRB FB ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved As	Matrix Spike	75-125% recovery	58.9	J	Arsenic results should be flagged "J" as estimated for out-of-control matrix spike.
06/07/00	BDRB FB ID IIIA IIIAFC IIIJD IILD IVKD	Dissolved Zn	Serial Dilution	#10% difference	15.7%	J	Zinc results should be flagged "J" as estimated due to out-of-control serial dilution analysis.
12/04/00	Intake IIBD IIIBD IVB IILD IIIJD IVKD	Field pH Field ORP	Calibration	Required each sampling event Required each sampling event	Not performed Not performed	R	Flag samples "R" as unusable due to lack of calibration documentation.

Date[1]	Sample ID	Analysis	QC Criteria	Control Limit	Result	Flag[2]	Comment
1/8/01	Intake IIBD IIIBD IVB IILD IIIJD IVKD	Dissolved Al	Field Duplicate	#35% RPD	40.1% RPD	J	Flag samples "J" for out-of-control field duplicate.
2/12/01	Intake IIBD IIIBD IVB IILD IIIJD IVKD	Dissolved Cu	Field Duplicate	#35% RPD	40.1% RPD	J	Flag samples "J" for out-of-control field duplicate.
3/14/01	IIBD	Sulfate	Outlier	Rosner's Test for Outliers	1,400 mg/L is an outlier at a 5% significance level	R	Sample is outlier according to Rosner's test. Data were transformed to achieve normal distribution prior to performing outlier test. The data should be removed from further consideration.
4/09/01	Intake	Sulfate	Outlier	Rosner's Test for Outliers	8 mg/L is an outlier at a 5% significance level	R	Sample is outlier according to Rosner's test. Data were transformed to achieve normal distribution prior to performing outlier test. The data should be removed from further consideration.
7/20/01	RCIIC OB RCIIIC OB RCIVC OB	Dissolved Al Dissolved Fe Dissolved Mn	Matrix Spike	75-125% recovery	130.7% 134.5% 127.5%	J	Flag samples "J" for out-of-control matrix spike.

[1] Date that the samples were collected.
[2] Data Qualifier Definitions:

U-The material was analyzed for but was not detected above the level of the associated value (quantitation or detection limit).
J-The sample results are estimated.
R-The sample results are unusable.
UJ-The material was analyzed for but was not detected, and the associated value is estimated

8. Recommended Design Improvements

- To minimize AMD stratification with respect to ORP and thus increasing bioreactors efficiency, the bioreactors need to be covered with a plastic liner that would minimize oxygen intrusion either directly from the atmosphere or through atmospheric precipitation. The layer of two lifts of straw bails used at the demonstration site was not sufficient.

- Because the biochemical reactions take place within the organic matter where they also precipitate, there is no need for a large cobble chamber.

- If a preventive "trap sump" to collect a small mass of precipitates that might escape from the organic matter chamber needs to be included in the design, it should have its outlet placed high above its bottom. A trap sump could be designed as a flow-through container or a small lined retention pond filled with cobbles supporting a plastic liner covering the pond. Other designs could also be considered, provided they would minimize the possibility of agitation of the collected effluent of the bioreactor by atmospheric conditions (wind and precipitation) or human and animal encounters.

- The abundance of TOC present in the organic matter chamber at the end of the project demonstrates that the bioreactors would have worked equally efficiently with a much smaller supply of organic carbon, provided the same residence time of AMD was maintained. Since most of the organic matter mass inhibits permeability, it is prudent to reduce the ratio of organic matter and the permeability enhancing component (e.g., gravel, shell, etc.) and have a more permeable medium.

- Although not explicitly indicated by the demonstration project results, the straw added to the organic matter does not seem to be an appropriate material to increase the permeability of the medium. More rigid material like walnut shells or even gravel, added in high proportion to the organic matter, is needed to effectively increase permeability.

- Since most of the plugging material that restricted the flow was found within and adjacent to the outlets of the AMD distribution system, there is a need to devise a system that would allow for occasional breakdown and removal of the plugging material. Such a system may need to involve only a few outlets rather than the three dozen used in this demonstration. It might include ports extended to the ground surface that would facilitate blowing in combustion engine exhaust to destroy plugging material that would then be removed by bailing.

- If any instrumentation for automatic measurements of the AMD quality and quantity is used, it is recommended that a filter be installed upstream of the sensor.

9. Conclusions

- A CCS built in the bioreactors worked very well in preventing settling of organic matter and ensuring uniform flow of AMD throughout the entire cross section of the organic matter with no preferential flow paths (channeling).

- Most of the individual cells of the CCS were found full of organic matter, with probably less than 5% of the cells void of the organic matter in their top 2-inch portions. This indicates that the organic matter could have been packed less tightly and still conform to the design parameters that allowed approximately 3 inches of settling.

- Configuring the bioreactors to accommodate flow in a horizontal plane (rather than in the vertical direction) was successful. Problems that were experienced with reduction in the flow rate turned out to be associated with the AMD distribution system that plugged with chemical precipitates. However, this hindrance is common to both configurations.

- It takes some time for the SRB to be established in a new bioreactor. Once established and supplied with organic carbon, they maintained a population of E+4 MPN/mL or higher in an aqueous phase at temperatures ranging from 2 EC to 16 EC.

- Winter freezing of a well-established SRB population has little or no effect on their activity for the remainder of the year.

- The SRB average population of 2.06E+6 MPN/cc attached to solid matrix of organic matter was two orders of magnitude greater than the value of 2.81E+4 MPN/mL calculated as an average SRB population in the aqueous samples collected during the last sampling event on July 9, 2001.

- The SRB population attached to the limestone was of the same order of magnitude (E+4) as for the aqueous samples.

- High sulfide load in the organic matter, together with high concentrations of metals, is indicative of metal sulfides precipitating in the organic matter chamber due to SRB activities.

- A drop in the SRB population in July of 2000, which paralleled the 100% increase of the flow rates, might indicate flushing out of the bacteria at that flow velocity.

- Although it appeared a limestone chamber slightly increased effluent pH, its role was not dominant for the overall performance of the bioreactors.

- The E_H values measured in the most downgradient piezometer show that with the exception of a few periods, the E_H values were positive. However, data acquired during the E_H profiling of the bioreactor document that the E_H values in the deep portion of the organic matter were approximately 50 mV and 150 mV lower for Bioreactors II and III respectively. This is an important observation because it confirms that reduced conditions, which are required for the SRB activity, were present within the portions of the bioreactors where most of the biochemical reactions took place and where most metals precipitated.

- The bioreactors were notably stratified with respect to E_H. In Bioreactor II, it was up to 400 mV lower at the bottom of the bioreactor than close to the surface. This difference, thus also stratification, diminished downgradient and close to the outlet from the bioreactors due to mixing of water flowing through the cobble section. It is postulated that the E_H

stratification was caused by an inadequate isolation of atmospheric air at the top of the bioreactors.

- The alkalinity buildup in the effluents is a good indication of biochemical reactions (i.e., sulfate reduction) taking place in the bioreactors.

- Data acquired from the project indicate that only Zn, Cu, and Cd were being removed as sulfides due to SRB activities. This statement is based on the stoichiometric balance that includes analytical data for metals and sulfide and is also supported by Mn data that indicate that the adsorptive capacity of the organic matter was exhausted after 8 months of operation.

- Changes in concentrations of other metals, which precipitated not necessarily as sulfides, seem to be affected by the SRB only in an indirect manner, by responding to changes of chemical conditions caused by the SRB.

- Zinc removal thresholds of 500 µg/L for Bioreactors II and IV and 800 µg/L for Bioreactor III seem to be independent of influent concentrations.

- Copper removal thresholds of 50 µg/L for Bioreactors II and IV and 80 µg/L for Bioreactor III also seem to be independent of influent concentrations.

- Cadmium removal thresholds of 5 µg/L that prevailed for most of the operating time decreased when the Cd concentration of the influent dropped to 1 Fg/L.

- Different Zn and Cu removal thresholds for Bioreactor III than the respective removal thresholds for Bioreactors II and IV indicate that the thresholds depend on the configuration of the bioreactors but are not affected by the closing up and freezing of a bioreactor during winter.

- A slightly lower metal removal efficiency in Bioreactor III that contained only one chamber with organic matter may indicate that the residence time of 10 hours within the organic carbon matter is close to minimal. This residence time may vary for different climates.

- Bioreactor III, with only one organic matter chamber and no limestone chamber, was noticeably less efficient in creating a reducing environment and also less efficient in removing dissolved metals. It is precarious to discriminate whether Bioreactor III was less efficient due to the absence of a limestone chamber or a second organic matter chamber.

- Evidence of metal sulfides precipitating in the organic matter chambers, together with the observation of no precipitate in the cobble chambers, indicates that the cobble chamber was not essential for biochemical reaction.

- For this demonstration, the role of the cobble chamber was limited to a collection sump for a small mass of precipitates that escaped from the organic matter chambers.

- The autopsy on the bioreactors revealed a convoluted biochemical environment that was probably caused by a dramatic change in the AMD chemistry after the first 10 months of operation. The environment examined during the autopsy included mixed results of processes that were occurring first at a low pH and a reasonably high load of metals with the subsequent reactions that were characteristic for water with a neutral pH laden with much less dissolved metals.

- Aqueous samples collected during the autopsy indicated that only a small portion of the dissolved metals in reduced form were balanced in the solution by sulfides. These were probably Zn, Cu, Cd, and a small portion of Fe. Other metals dissolved in solution, including high

concentrations of Ca, were electronically balanced by high concentration of sulfate, bicarbonate, and assumed-present hydroxide.

- It is hypothesized, based on the analytical data and visual observation during the autopsy on the bioreactors, that water accumulated at the bottom of the bioreactors contained large amounts of suspended ferric and Al hydroxides.

- The high concentration of metals that accumulated with the solid matrix of the bioreactors demonstrated that a large load of metals was retained within the organic matter material, thus indicating the bioreactors were efficient in removing metals from the influent AMD.

- For the autopsy data, there was a trend of decreasing concentrations for some metals as they were retained along the flow path within the first organic matter chamber. These metals include Al, Fe, Cu, and As. The reason behind such a behavior remains a conundrum.

- A high correlation coefficient ($k = 0.9434$) for concentration trends for As and Fe in the solid matrix of the bioreactors seem to support the inference that the higher As concentration in the effluents rather than influent was controlled by sorption processes of As to $Fe(OH)_3$.

- In general, sulfide concentrations in the organic matter chambers were above the 4,000 mg/kg level and were twice as high as concentrations of sulfates. This large sulfide load together with high metal concentrations is indicative of metal sulfides precipitating in the organic matter chamber due to SRB activities.

- Although TOC concentrations for the initial fresh organic matter were not measured, the high TOC values measured during the autopsy strongly indicate that the depletion of organic carbon would not be a factor for the efficient removal of metals even if these bioreactors were operating for several more years.

- Plugging of the bioreactors took place within or immediately adjacent to the AMD distribution system. This statement is supported by the water level decrease between the inlet sump and first organic matter chamber. Also supporting this conclusion were the observations during the autopsy that more chemical precipitate was present in the front portion of the bioreactors than downstream.

- The high percentage of Fe and Al hydroxides in the plug removed from the AMD inlet to Bioreactor III indicates a high probability that bioreactor inlets were plugging mainly due to chemical reactions; the sediment carried by the AMD was a secondary reason for plugging.

- The reliability of transducer-/sensor-generated measurements was poor especially for the pH sensors that were coated with precipitate within several weeks. Part of the problem was a very slow flow that did not allow for dynamic cleaning of the sensor. The low flow rate also inhibited measurements because of the necessity of using a small diameter vortex that was prone to plugging.

- If sulfide concentrations in aqueous samples from the influent and effluent are to be indicative of the bioreactor performance, they need to be filtered prior to laboratory analysis. In addition, the initial and final concentrations of sulfate and sulfide in the organic matter need to be known.

10. References

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