Table 15. Indicator Organism Recovery from Impinger and Fluid Samples from an On-site Mechanical/Chemical Medical Waste Treatment System

	Colony Forming Units (CFU) Recovered							
	Bacillus stearothermophilus (@ 55°C)				*Bacillus subtilis* var. *niger* (@ 37°C)			
AIR SAMPLES	**Non-spiked**		**Spiked**[1]		**Non-spiked**		**Spiked**[2]	
	Plates	Filter	Plates	Filter	Plates	Filter	Plates	Filter
AGI-30 [3]								
Blank	0,0	0			0,0	0		
Drum Conveyor								
1-A	0,0	0	0,0	1	0,0	0	0,0	1
1-B	0,0	0	0,0	5	0,0	0	1,0	2
2-A	0,0	0	0,0	0	0,0	0	0,0	0
2-B	0,0	0	0,0	0	0,0	0	0,0	2
HEPA Exhaust								
-1	0,0	0	0,0	4	0,0	0	0,0	0
-2	0,0	0	0,0	0	0,0	0	0,0	0

FLUID SAMPLES	**Non-spiked** Filter	**Spiked** Filter	**Non-spiked** Filter	**Spiked** Filter
Sedimentation Tank Water [4]				
Time (min)				
Blank	0	0	0	0
1	0	0	0	0
5	0	0	0	0
10	0	0	0	0
15	0	0	0	0
20	0	0	0	1
25	0	0	0	0
30	0	28	0	47
35	0	ND	0	ND

ND = Not Done
[1] = Twelve waste bags seeded with 4.3×10^9 spores in suspension
[2] = Twelve waste bags seeded with 2.2×10^9 spores in suspension
[3] = 15 min; 20 ml PBDW; 0.1 ml plated; 0.2 µm filtration; Trypticase soy agar
[4] = 50 ml duplicate; 0.1 ml plated; 0.2 µm filtration; Trypticase soy agar

9.0 OFF-SITE STEAM AUTOCLAVE SAMPLING PLAN

This large commercial medical waste treatment facility has the capability of treating 5,000 pounds of medical waste per hour. There are two large single door autoclaves eight feet in diameter and thirty feet long. Each accommodates five stainless steel waste bins per treatment cycle, and is set to operate at 163°C and 80 psi. The steam used in the treatment is generated by an on-site boiler and is piped into each autoclave through an overhead system. Air and condensed steam are removed from the chamber of the autoclaves by means of a pre-vacuum system. Treatment temperature and pressure remain constant for 22 minutes. Prior to completion of the 55 minute cycle, air is removed from the chambers by a vacuum system. All exhausted air is cycled through a condenser and exhausted to the roof through a six inch vent. The condensate flows through a carbon filter/ion exchange water treatment system prior to discharge to the sanitary sewer. Treated waste is then removed from the autoclave and shredded prior to disposal in a landfill.

9.1 Sampling Points

Three potential emission points were identified, two for air and one for fluid emissions. They are described below and are shown is Figures 11-13.

1. The chamber vacuum exhaust. (Fig. 11) A six inch diameter vent on the roof provides for vacuum exhausted chamber air after passage through the condenser before the treatment cycle.

2. The chamber vacuum exhaust. The same, six inch diameter vent on the roof also provides for the exhaust of autoclave chamber air during the post-treatment cycles.

3. The autoclave door exhaust door. (Fig. 12) Following treatment, when the door to each autoclave is opened automatically and in one motion, a plume of steam is emitted and rises above the unit where it exits through a large, fan assisted vent (~4 ft. dia.) in the building roof.

4. The autoclave condensed treatment system. (Fig. 13) Fluid samples for bioemissions were collected from the condensate fluid treatment line prior to discharge to the sanitary sewer.

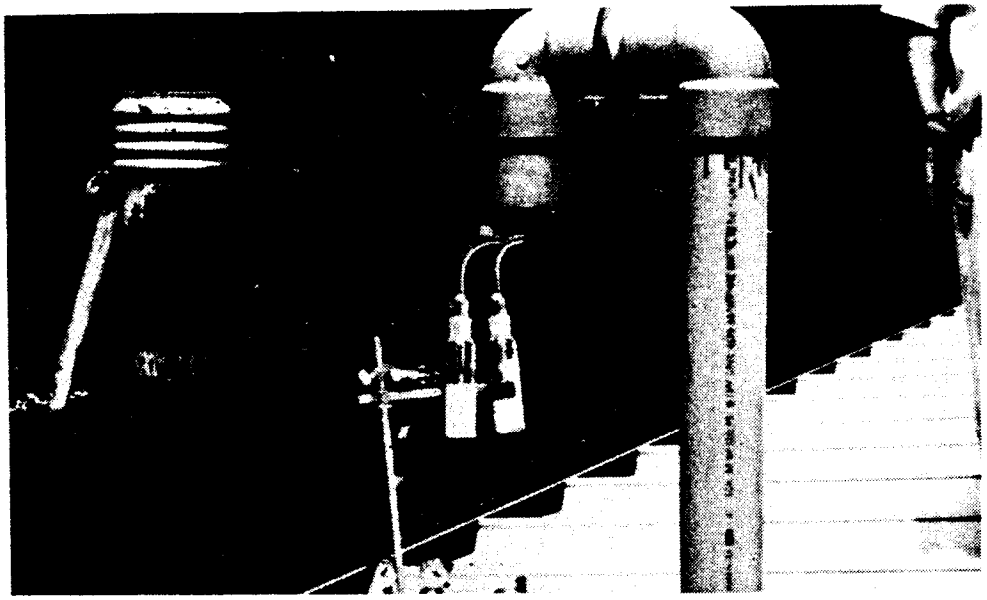

FIGURE 11. ROOF VENT FOR VACUUM EXHAUST AIR FROM STEAM AUTOCLAVE CHAMBER

FIGURE 12. ROOF VENT FOR EXHAUST AIR FROM STEAM AUTOCLAVE DOOR

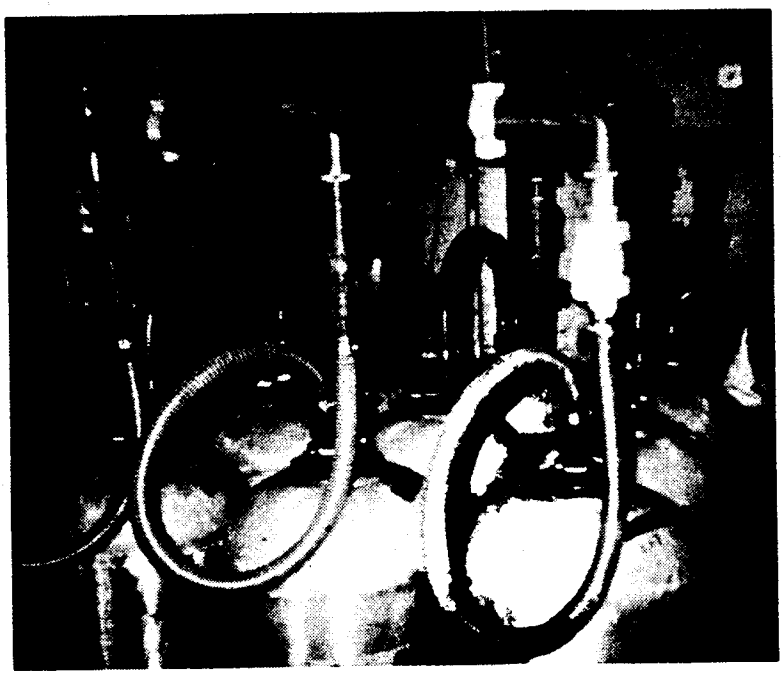

FIGURE 13. STEAM AUTOCLAVE CONDENSATE TREATMENT SYSTEM

9.2 Microbial Challenge

Both air and fluid-sampling points were sampled during the processing of both non-spiked and spiked medical waste. For the bioemissions sampling following treatment of spiked waste, the five waste bins in a treatment cycle were seeded with dried BST and BSN spores. For each waste bin, ten open petri dishes containing previously dried spores of BST, and ten containing spores of BSN, were attached to the top of the un-treated waste. Each BST dish contained 4.0×10^7 spores, and each BSN dish 1.5×10^7 spores. Total spore challenges for 50 BST plates was at least 2.0×10^9; while for 50 BSN plates, the challenge was at least 7.4×10^8.

9.3 Air and Fluid Sampling

Ambient air sampling was conducted in the vicinity of the autoclaves before sampling of the spiked and non-spiked waste treatment cycles. Sampling during treatment was at the six inch vent during the pre-treatment vacuum exhaust phase, and again at that vent during the post-treatment vacuum exhaust, and finally at the conclusion of the cycle, at the large roof vent as the door was opened.

Ambient sampling consisted of the following:

Indoor	4 x 15 min M/G (2 @ 37°C and 2 @ 55°C) 4 x 5 min AND-2 (2 @ 37°C and 2 @ 55°C)
Outdoor	4 x 5 min M/G (2 @ 37°C and 2 @ 55°C) 4 x 1 min AND-2 (2 @ 37°C and 2 @ 55°C)

Sampling during waste treatment consisted of the following for each of the two cycles sampled:

Emission Point

1	2 x 15 min M/G (simultaneous, 37°C and 55°C) 2 x 10 min AGI (simultaneous, 37°C and 55°C)
2	2 x 10 min AGI (simultaneous, 37°C and 55°C)
3	2 x 5 min M/G (37°C and 55°C) 2 x 10 min AGI (simultaneous, 37°C and 55°C)

For fluid sampling during each cycle, ten 50 ml samples were collected in duplicate from the condensate discharge drain during the treatment process (20 samples total).

9.4 RESULTS

Results of air and fluid bioemissions sampling are shown in Tables 16 and 17. No *B. subtilis* var. *niger* was recovered from ambient air, or air and water samples from spiked or non-spiked treatment runs. One CFU of *B. stearothermophilus* was recovered from the pre-vacuum, exhaust air in the non-spiked run, but BST was not found in other water or air samples. This contrasts with previous work that found BSN spore, coliphage T1, and *Serratia marcescens* in the pre-sterilization vacuum exhaust of a high vacuum autoclave (Barbieto and Brookey, 1976). The authors disseminated dry BSN spores onto the waste loads or sprayed liquid aerosols of the three test organisms into the chamber immediately prior to the vacuum cycle. Because chamber evacuation does not take place at operating temperature and pressure, this maximized the likelihood of aerosolization of the test organisms. The present tests were designed to mimic spilled, dried organisms on the surfaces of medical waste materials.

Based upon the assessment of the steam autoclave units as described in this study, there appears to be limited potential for biological emissions during medical waste treatment under normal operating conditions.

Table 16. Indicator Organism Recovery from Air Impactor Samples from an Off-site Medical Waste Treatment Steam Autoclave

		Colony Forming Units (CFU) Recovered					
		Bacillus stearothermophilus (@ 55°C)			*Bacillus subtilis* var. *niger* (@ 37°C)		
AIR SAMPLES		Ambient	Non-Spiked	Spiked[1]	Ambient	Non-Spiked	Spiked[2]
M/G [3]							
Indoor	-1	0	*	*	0	*	*
	-2	0	*	*	0	*	*
Outdoor	-1	0	*	*	0	*	*
	-2	0	*	*	0	*	*
Pre-Vac	-1	*	1	0	*	0	0
Post-Vac	-2	*	*	*	*	*	*
Door Vent		*	0	0	*	0	0
Andersen [4]							
Indoor	-1	0	*	*	0	*	*
	-2	0	*	*	0	*	*
Outdoor	-1	0	*	*	0	*	*
	-2	0	*	*	0	*	*

* = No sample collected according to sampling plan
1 = Waste loads seeded with 2.0×10^9 dried spores
2 = Waste loads seeded with 7.4×10^8 dried spores
3 = Slit-to-agar sampler; 15 min ambient indoor, 5 min ambient outdoor; 15 min chamber exhaust (pre and post-vac); 5 min autoclave door vent; Trypticase soy agar
4 = 2-Stage sampler; 5 min indoor, 1 min outdoor; Trypticase soy agar

Table 17. Indicator Organism Recovery from Impinger and Fluid Samples from an Off-site Medical Waste Treatment Steam Autoclave

	Colony Forming Units (CFU) Recovered							
	Bacillus stearothermophilus (@ 55°C)				*Bacillus subtilis* var. *niger* (@ 37°C)			
AIR SAMPLES	**Non-spiked** Plates[3]	Filter	**Spiked**[1] Plates[3]	Filter	**Non-spiked** Plates[3]	Filter	**Spiked**[2] Plates[3]	Filter
AGI-30 [3]								
Blank	0,0	0			0,0	0		
Pre-Vac								
-1	0,0	0	0,0	0	0,0	0	0,0	0
-2	0,0	0	0,0	0	0,0	0	0,0	0
Post-Vac								
-1	0,0	0	0,0	0	0,0	0	0,0	0
-2	0,0	0	0,0	0	0,0	0	0,0	0
Door Vent								
-1	0,0	0	0,0	0	0,0	0	0,0	0
-2	0,0	0	0,0	0	0,0	0	0,0	0
FLUID SAMPLES	**Non-spiked** Plates	Filter	**Spiked** Plates	Filter	**Non-spiked** Plates	Filter	**Spiked** Plates	Filter
Condensate Drain Water [4]								
Time (min)								
Blank	0,0	0			0,0	0		
1	0,0	0	0,0	0	0.0	0	0,0	0
6	0,0	0	0,0	0	0.0	0	0,0	0
12	0,0	0	0,0	0	0.0	0	0,0	0
30	0,0	0	0,0	0	0.0	0	0,0	0
36	0,0	0	0,0	0	0.0	0	0,0	0
42	0,0	0	0,0	0	0.0	0	0,0	0
48	0,0	0	0,0	0	0.0	0	0,0	0
54	0,0	0	0,0	0	0.0	0	0,0	0
60	0,0	0	0,0	0	0.0	0	0,0	0
65	ND	ND	0,0	0	ND	ND	0,0	0

ND = Not Done
[1] = Waste loads seeded with 2.0×10^9 dried spores
[2] = Waste loads seeded with 7.4×10^8 dried spores
[3] = 10 min; 20 ml PBDW; 0.1 ml plated; 0.2 µm filtration; Trypticase soy agar
[4] = 50 ml duplicates; 0.1 ml plated; 0.2 µm filtration; Trypticase soy agar

10.0 DISCUSSION

This study, while limited in scope due to time and funding constraints, has provided valuable information relative to the identification of emission points as well as the potential for biological emissions from steam autoclave, microwave, and mechanical/ chemical medical waste treatment technologies. Evaluation of the treatment systems' potential for bioemissions focused on the analysis of air and fluid samples from specific emission points. Samples were analyzed for the presence or absence of *Bacillus stearothermophilus* and *Bacillus subtilis* var. *niger* spores collected during the treatment of spiked medical waste. Bioemission potential was assessed after such data were compared to background levels of the selected indicator organisms in ambient air in the vicinity of the devices, as well as to background levels in air and fluid samples collected from potential emission points during the treatment of non-spiked medical waste.

As it was beyond the scope of this study to characterize all bioemissions and airflow from the treatment units, the results should be regarded only as indicative of bioemission potential on a "demonstrated/not demonstrated" basis. The collection and analysis of bioaerosol grab samples during the treatment of spiked medical waste showed that recovery of both types of indicator spores was demonstrated in emissions from specific points in the microwave and mechanical/chemical units. Similar sampling did not indicate bioemissions from the steam autoclave units during treatment of spiked waste, when compared to background levels.

While this study was not intended to assess environmental or occupational health risks from bioemissions during medical waste treatment, the results can be used to focus attention on the potential for bioemissions from certain areas or phases of operation from different treatment technologies. In particular, those situations with any technology where access is afforded to a chamber during the grinding and shredding of untreated medical waste, or where a phase of operation in the treatment process remains open to the environment, would appear to have the greatest potential for the generation of bioemissions.

11.0 FUTURE RESEARCH

This study has identified the indicator organisms, samplers, and monitoring techniques needed to evaluate additional medical waste treatment technologies, and can serve as a basis for the development of a quantitative approach to the assessment of environmental and occupational health risks from medical waste treatment.

12.0 QUALITY ASSURANCE PLAN

Microbiological quality assurance procedures followed American Public Health Association standard method 9020 Quality Assurance, as contained in *Standard Methods For The Examination Of Water And Wastewater,* 17th edition, 1989. Guidance for airborne bioemissions sampling was provided by the ASTM *Standard Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities*, E884-82, American Society for Testing Materials, Philadelphia. The following quality control performance evaluations were performed in the assessment of potential bioemissions from medical waste treatment technologies. Specific materials used and their controls are shown in Table 18.

12.1 Microorganisms

Microorganisms were purchased from reputable suppliers (e.g. ATCC, AMSCO, DIFCO), inspected and logged upon receipt according to date, lot number, expiration, and stored at 2° - 8°C until ready to use. Organisms were received as bacterial spore suspensions. Commercially prepared spore suspensions were quantified by duplicate serial dilutions and plating to confirm manufacturer's minimum specified concentration. Spore suspensions were stored under refrigeration until used, according to manufacturers' directions.

12.2 Microbiological Media

Microbiological culture media was prepared in the RTI laboratories. Media consisted of agar prepared in petri plates. Laboratory media was made according to the manufacturers' directions. All media containers were dated when first opened. Ingredients were weighed on calibrated laboratory balances and suspended in distilled, deionized 18 megohm water prepared from a Corning Mega-Pure DF system. Media was sterilized in a steam autoclave operated at 121°C and 15-16 psi. Each autoclave cycle was logged with date, time of cycle, type of exhaust, and temperature. Autoclave cycles were monitored with calibrated temperature and pressure gauges built into the sterilizer and also with a digital thermocouple and temperature chart recorder. Items to be autoclaved were also tagged with temperature sensitive indicator tape. All media was incubated following preparation as a sterility check, with representative samples inoculated with known microorganisms as growth controls. The media was also inspected before use, and if found contaminated, was discarded. All media was transported to and from sites in insulated containers.