7.2 Microbial Challenge

All four potential emissions points were sampled during the processing of both non-spiked and spiked medical waste as the treatment system is continually fed in batch mode. Bioemissions sampling took place during the continuous processing of medical waste. For the bioemissions sampling during treatment of spiked waste, four consecutive waste batches were seeded with the indicator spores. Each waste batch was seeded with ten vials of BST (each with 4.8×10^7 BST spores), and ten vials of BSN (each with 2.2×10^7 BSN spores). Thus, total spore challenge for forty vials of each indicator organism for the four waste batches was at least 1.9×10^9 for *B. stearothermophilus*, and at least 8.8×10^8 for *B. subtilis* var. *niger*.

7.3 Air Sampling

All air sampling was conducted as applicable, according to ASTM Standard Practice E884-82. Ambient air sampling was conducted in duplicate for each of two sampling days near emission points 1, 3, and 4, and consisted of:

Emission Point

1,4	4 x 1 min AND-2 (2 @ 37°C and 2 @ 55°C)
	2 x 5 min M/G (37°C and 55°C)
3	2 x 1 min AND-1 (37°C and 55°C)

Air sampling during both spiked and non-spiked waste processing was be conducted in duplicate at:

Emission Point

1	2 x 1 min AND-2 (37°C and 55°C) for each of 4 door openings
3	1 x 1 min AND-1 for each of 4 door openings (2 @ 37°C and 2 @ 55°C)
2	3 x 15 min AGI (sequential, 37°C and 55°C)
4	4 x 15 min AGI (sequential, 37°C and 55°C)

7.4 RESULTS

Results of air bioemissions sampling are shown in Tables 12 and 13. *B. subtilis* var. *niger* was not recovered from ambient air nor from air sampled during treatment of non-spiked medical waste. During the spiked run, one CFU of BSN was recovered from an AND-2 sampler positioned adjacent to the hydraulic door during the opening of the door to deposit a cart load of untreated medical waste into the grinding chamber. No BSN was found at the HEPA filter exhaust. A single BSN colony was also recovered from sample point 2, the transport screw access port between the grinder and the microwave units. Three CFU of BSN were recovered from the sample point 4, the tube where treated waste was expelled from the microwave unit into the dumpster.

A single CFU of *B. stearothermophilus* was recovered from ambient air above the dumpster, and one CFU was also recovered during a non-spiked treatment run above the hydraulic doors. Two CFU of BST were recovered from the waste exit tube during the non-spiked run. At the transport screw access port, 28 CFU of BST were recovered from impinger samples during the spiked run, and 17 CFU were recovered from exit tube air samples. There was also heavy growth of non-indicator *Bacillus* sp. in one sample taken at the access port during the non-spiked run, and ~20 CFU of *Bacillus* sp. taken from the exit tube during the same run. No other reports of bioaerosol emissions were identified. While there is documentation of the effectiveness of microwave radiation to inactivate microorganisms in the presence of moisture (Latimer and Matsen, 1977; Lechowich *et al*, 1969), it was recognizable that *B. stearothermophilus*, because of its intrinsic thermal resistance, would not be inactivated by microwave treatment. There are no human pathogen bacterial spores as heat resistant as BST.

Biological emission points were primarily: the access port to destroyed, but untreated waste, as well as the treated waste exit tube. The transport auger access port is not normally open during operation. However, recovery of 5 CFU of BSN and 45 CFU of BST over 0 and 4 CFU background levels, respectively, illustrate the potential for bioemissions when open during routine operations (e.g. inserting spore test packs for effectiveness testing, or cleaning a waste jam).

Table 12. Indicator Organism Recovery from Impinger Fluids from an On-site Microwave 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	0,0	0	0,0	0
Auger Port								
A	0,0	0	0,0	1	0,0	0	0,0	0
B	0,0	0	0,1	19	0,0	0	0,0	1
C	0,0	0	0,0	7	0,0	0	0,0	0
Exit Tube								
A	0,0	0	0,0	2	0,0	0	NA	1
B	0,0	0	0,0	1	0,0	0	0,0	1
C	0,0	1	0,0	14	0,0	0	0,0	0
D	1,0	0	0,0	0	0,0	0	0,1	0

NA = Data Not Available
[1] = Four waste batches seeded with 1.9×10^9 spores in suspension
[2] = Four waste batches seeded with 8.8×10^8 spores in suspension
[3] = 15 min; 20 ml PBDW: 0.1 ml plated; 0.2 µm filtration; Trypticase soy agar
Mean temperature 27.0°C during non-spiked sampling
Mean temperature 27.4°C during spiked sampling
Mean relative humidity 56.1% during non-spiked sampling
Mean relative humidity 67.2% during spiked sampling

Table 13. Indicator Organism Recovery from Air Impactor Samples from an On-site Microwave Medical Waste Treatment System

		Colony Forming Units (CFU) Recovered							
		Bacillus stearothermophilus (@ 55°C)				*Bacillus subtilis* var. *niger* (@ 37°C)			
AIR SAMPLES		Ambient (Day 1)	Non-Spiked	Ambient (Day 2)	Spiked[1]	Ambient (Day 1)	Non-Spiked	Ambient (Day 2)	Spiked[2]
M/G [3]									
Hydraulic Door		0	*	0	*	0	*	0	*
Exit Tube		0	*	0	*	0	*	0	*
Andersen [4]									
Hydraulic Door	-1	0	*	0	*	0	*	0	*
	-2	0	*	0	*	0	*	0	*
Roof Vent	-1	0	*	0	*	0	*	0	*
Exit Tube	-1	1	*	0	*	0	*	0	*
	-2	0	*	0	*	0	*	0	*
Hydraulic Door	-1	*	0	*	0	*	0	*	0
	-2	*	0	*	0	*	0	*	0
	-3	*	1	*	0	*	0	*	0
	-4	*	0	*	0	*	0	*	0
	-5	*	0	*	0	*	0	*	1
Roof Vent [5]	-1	*	0	*	0	*	0	*	0
	-2	*	0	*	ND	*	0	*	ND

ND = Not Done (sample not taken)
* = No sample collected according to sampling plan
1 = Four waste batches seeded with 1.9×10^9 spores in suspension
2 = Four waste batches seeded with 8.8×10^8 spores in suspension
3 = Slit-to-agar sampler; 30 min; Trypticase soy agar
4 = 2-Stage sampler; 5 min (1 min on hydraulic door); Trypticase soy agar
5 = 1-Stage sampler; 1 min; Trypticase soy agar

8.0 MECHANICAL/CHEMICAL SAMPLING PLAN

The indoor flow-through chemical treatment system tested is located at a large metropolitan medical center and is used to treat all categories of medical waste, including pathological waste. It is a large, continuous feed process that combines sodium hypochlorite treatment with shredding and then grinding of the waste by means of a hammermill. The device has a waste treatment capacity of 2,000 pounds per hour, and a liquid flow through of 30 gallons per minute. The waste bags are moved automatically by conveyor belt into the shredding/grinder chamber where the chemical is sprayed as the waste is shredded and ground by the hammermill. After the grinding, the solids are separated from the chemical by means of a revolving drum screen. The drum screen deposits the "dewatered" solids onto a conveyor that carries them to a compactor.

8.1 Sampling Points

Four potential emission points were identified. They are described below, and are shown in Figures 7-10. There are three air and one fluid emission points.

1. **The conveyor for continuous waste feeding.** (Fig. 7) At this location, an operator places the waste to be treated onto the conveyor that transports it to the shredding and grinding chamber. The system is designed so that once the waste is contained in the enclosed portion of the conveyor, a negative pressure draws air away from the operator.

2. **The drum screen/conveyor interface.** (Fig. 8) The system separates the solids from the liquids by means of the drum screen, and deposits them onto another conveyor for exit from the machine into an outside waste compactor. This interface is not completely enclosed.

3. **Treatment system exhaust air.** (Fig. 9) Air is mechanically exhausted from the grinding chamber, drumscreen, and sedimentation tank, and is sent through a HEPA filtration unit that is exhausted to a large stack located outdoors. As the exhaust duct exits the building and turns upward, there is a port for access to the exhaust air stream.

4. **Sewer drain at sedimentation tank.** (Fig. 10) Treatment chemical and water flow from the drum screen dewatering point into the sedimentation tank. The tank momentarily holds the water by means of a baffle in order to allow particulates to sediment. The fluid can then pass through to the sewer drain.

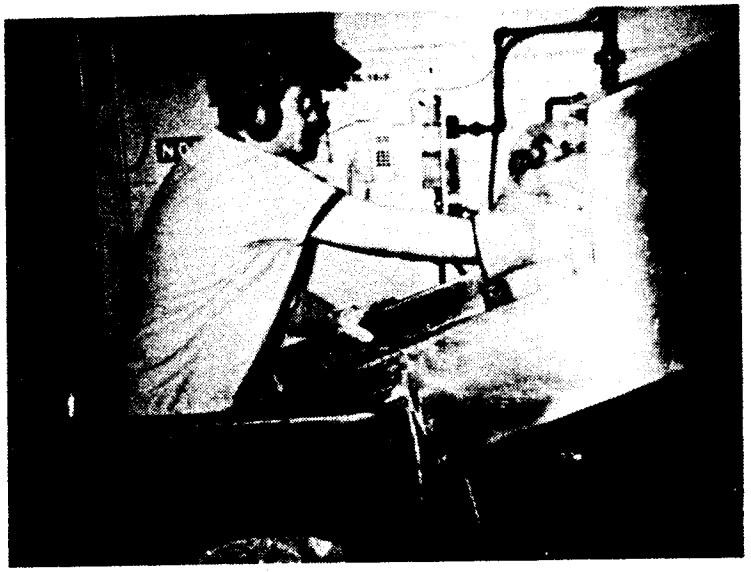

FIGURE 7. WASTE CONVEYOR FOR MECHANICAL/CHEMICAL TREATMENT SYSTEM

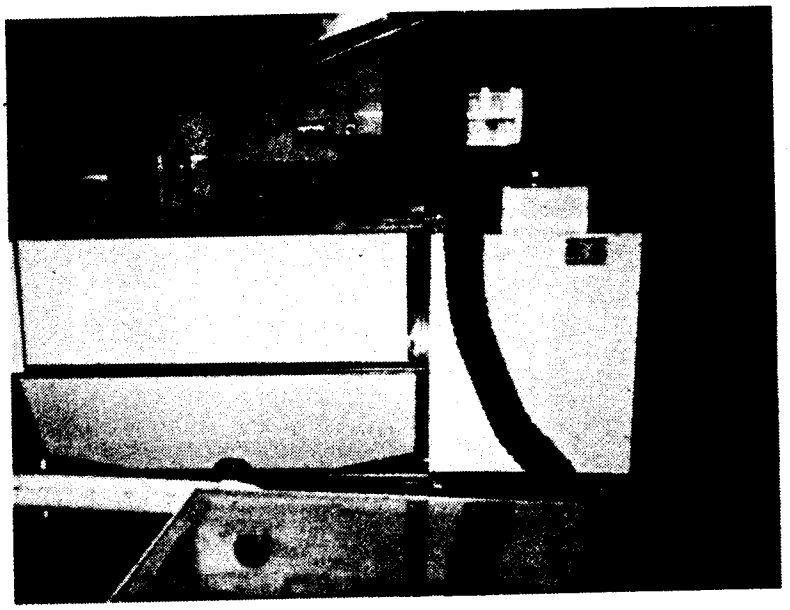

FIGURE 8. DRUM SCREEN TO SEPARATE SOLIDS FROM LIQUIDS DURING MECHANICAL/CHEMICAL TREATMENT

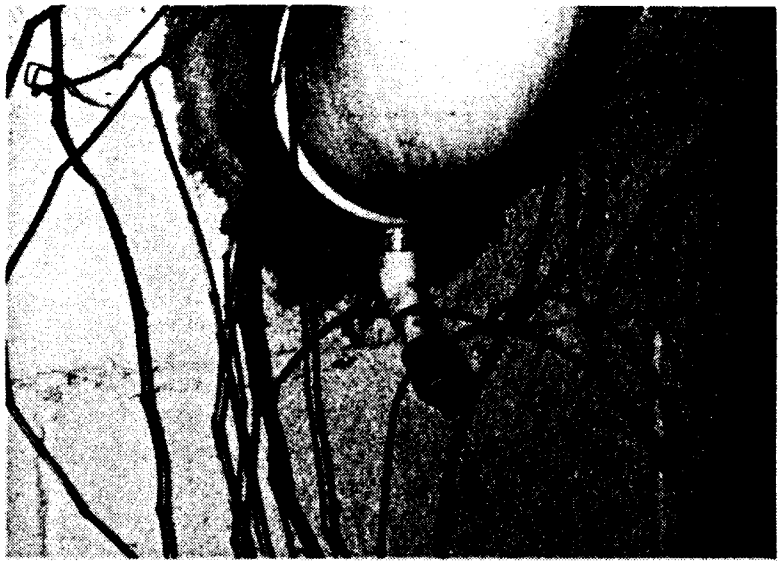

FIGURE 9. ACCESS PORT TO HEPA AIR EXHAUST FROM MECHANICAL/CHEMICAL TREATMENT SYSTEM

FIGURE 10. SEDIMENTATION TANK FOR LIQUID EFFLUENT FROM MECHANICAL/CHEMICAL TREATMENT SYSTEM

8.2 Microbial Challenge

All four potential emission points were monitored during the processing of both non-spiked and spiked medical waste. Bioemissions sampling was conducted during the continuous processing of medical waste. For the bioemissions sampling during treatment of spiked waste, 24 standard waste bags were seeded with indicator spores. A tube containing five vials of BST spores (7.2×10^7/vial) was placed into each of twelve of the waste bags (4.3×10^9 total BST spores) in 60 vials, while a tube containing five vials of BSN spores (3.7×10^7/vial) was attached to each of another twelve waste bags (2.2×10^9 total BSN spores). The spore challenge was greater than that for on-site steam autoclave or microwave treatment because the mechanical/chemical treatment system is a continuous feed, rapid, flow-through system. Approximately 35 minutes was alloted for the treatment of 24 waste bags and the concomitant sampling.

8.3 Air and Fluid Sampling

All air sampling was conducted as applicable, according to ASTM Standard Practice E884-82. Ambient air sampling was conducted prior to waste treatment at three locations: 1) Near the end of the machine by the grinding chamber, and approximately 36 inches above the floor; 2) at the beginning of the waste loading conveyor, approximately 36 inches from the floor; and 3) outdoors. Sampling at each of the first two locations consisted of:

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

Ambient sampling outdoors consisted of:

2 x 5 min M/G (37°C and 55°C)

Air sampling during both spiked and non-spiked waste processing were conducted at:

Emission Point

1	4 x 5 min M/G (sequential 2 @ 37°C and 2 @ 55°C) 4 x 5 min AND-2 (sequential 2 @ 37°C and 2 @ 55°C)
2	4 x 15 min AGI (2 simultaneous & sequential, 37°C/55°C)
3	4 x 5 min M/G (2 @ 37°C and 2 @ 55°C) 2 x 15 min AGI (simultaneous, 37°C/55°C)

For fluid sampling, duplicate 50 ml samples were collected from the sedimentation tank near the sewer drain. Samples were taken at one minute after waste treatment began, and at each five minute interval thereafter for 35 minutes, for a total of 16 samples.

8.4 RESULTS

Results of the air and fluids bioemissions sampling are shown in Tables 14 and 15 and show recovery of both *B. stearothermophilus* and *B. subtilis* var. *niger* spores only from samples collected during the processing of spiked medical waste. Neither BST nor BSN was recovered from ambient air samples or from air and water samples taken during the non-spiked treatment run of the mechanical/chemical medical waste treatment system. Aerosolized BST was recovered from the unenclosed drum screen/conveyor interface (6 CFU). BST was also recovered from the HEPA-filtered exhaust stack (4 CFU). One waste water sample contained 28 CFU of BST. Aerosolized BSN was recovered above the conveyor where waste is loaded into the machine (6 CFU), the drum screen/conveyor interface (6 CFU), and 47 CFU were found in one waste water sample and 1 CFU in another. Jetté and Lapierre (1992) used the vegetative bacterium *S. marcescens* to test a similar system and found no evidence of *S. marcescens* aerosolization when the system operated with disinfectant. The difference may be due to the fact that bacterial endospores are more environmentally stable and disinfectant resistant than vegetative forms. The BST found in HEPA-filtered exhaust stack samples may indicate HEPA filter failure. Proper maintenance of this or any treatment device incorporating HEPA filters is essential to minimize potential bioemissions.

Table 14. Indicator Organism Recovery from Air Impactor 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		Ambient	Non-Spiked	Spiked[1]	Ambient	Non-Spiked	Spiked[2]
M/G [3]							
Outdoor		0	*	*	0	*	*
Conveyer	-1	0	0	0	0	0	1
	-2	*	0	0	*	0	1
Drum Conveyor	-1	0	*	*	0	*	*
HEPA Exhaust	-1	*	0	0	*	0	0
	-2	*	0	0	*	0	0
Andersen [4]							
Conveyor	-1	0	0	0	0	0	1
	-1 dup	*	*	*	*	0	*
	-2	0	0	0	0	0	3
Drum Conveyor	-1	0	*	*	0	*	*
	-2	0	*	*	0	*	*

* = No sample collected according to sampling plan
[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] = Slit-to-agar sampler; 15 min ambient; 5 min during treatment; Trypticase soy agar
[4] = 2-Stage sampler; 5 min; Trypticase soy agar