Colonies from the TSA at 55°C that were presumptively identified as *B. stearothermophilus* were subcultured on TSA for growth at 40°C and 65°C as one confirmation of *B. stearothermophilus*. A second confirmation was performed with API 50 CHB and API 20E biochemical test strips. The biochemical test strips were able to confirm *B. stearothermophilus* at a 90.0 to 99.9% probability.

It is important to note that recovered spores are expressed as colony forming units (CFU) that are an imprecise form of microbial measurement. A CFU may originate from a single spore, or more typically, in the natural environment from an aggregation of spores. Therefore, CFU data will actually underestimate the total number of recovered organisms.

5.0 TREATMENT TECHNOLOGIES, SITES AND SAMPLING SCHEDULE

Three treatment technologies were monitored for potential bioemissions at four sites in the eastern United States. These included: 1) On-site microwave treatment at a large medical center; 2) On-site steam autoclave treatment at a large medical center; 3) Off-site, commercial, steam autoclave treatment; and, 4) On-site mechanical/chemical treatment at a large medical center.

Initial visits were made to each site to identify potential bioemissions/sampling points for each participating technology. On June 2, 1993, all initial medical waste treatment site visits were completed. The following lists the bioemissions sampling schedule and types of participating facilities.

- 6/8/93 - on-site steam autoclave treatment facility at a large university medical center.
- 6/14-16/93 - on-site microwave treatment facility at a large county hospital in the southeastern U.S.
- 6/23-24/93 - on-site mechanical/chemical treatment facility at a large medical center in the northeastern U.S.
- 6/29/93 - off-site, commercial steam autoclave treatment facility in the eastern U.S.

6.0 ON-SITE STEAM AUTOCLAVE SAMPLING PLAN

Two gravity displacement steam autoclaves are located in a 350 ft^2 room dedicated to medical waste treatment in a hospital microbiology laboratory facility. Each unit is capable of holding 300-400 pounds of waste. Only clinical laboratory waste is treated. The units operate at 121°C/15 psi for 45 minutes. A complete cycle takes one hour.

6.1 Sampling Points

Two potential emission points were identified for each autoclave. They are described below, and are shown in Figures 1 and 2. They include both air and fluid emissions.

1. **The autoclave door.** (Fig. 1) At the completion of each treatment cycle, the door was opened slightly for about one minute (per facility operating procedure) to insure pressure equalization. The door was then opened wide, allowing air and steam from the chamber to escape and rise above the autoclave in a visible plume.

2. **The chamber exhaust.** (Fig. 2) At the start of the treatment process, steam entering the autoclave chamber forces air surrounding the waste out through the chamber exhaust. The exhaust continues to function during processing as the system works to maintain a constant level of temperature and pressure throughout the cycle. The autoclaves exhausted into a floor drain that is shared by both units.

6.2 Microbial Challenge

Both autoclaves were monitored during, and immediately following, the treatment of both spiked and non-spiked wastes. For bioemissions sampling during treatment of spiked waste, the waste loads of both autoclaves were seeded with dried BST and BSN spores. For each waste load, open petri dishes containing 0.2 μm polycarbonate filters with previously dried spores of each of the indicator organisms were placed on top of the waste and in a drain pan directly under the waste. Each waste batch was seeded with ten dishes of BST spores (each with at least 5.3×10^7) totalling 5.3×10^8, and ten dishes of BSN spores (each with 1.1×10^7) totalling 1.1×10^8. The use of dried spores simulated infectious agents that might potentially be aerosolized (and exhausted during initial autoclave pressurization).

FIGURE 1. ON-SITE STEAM AUTOCLAVES WITH SAMPLING EQUIPMENT IN PLACE

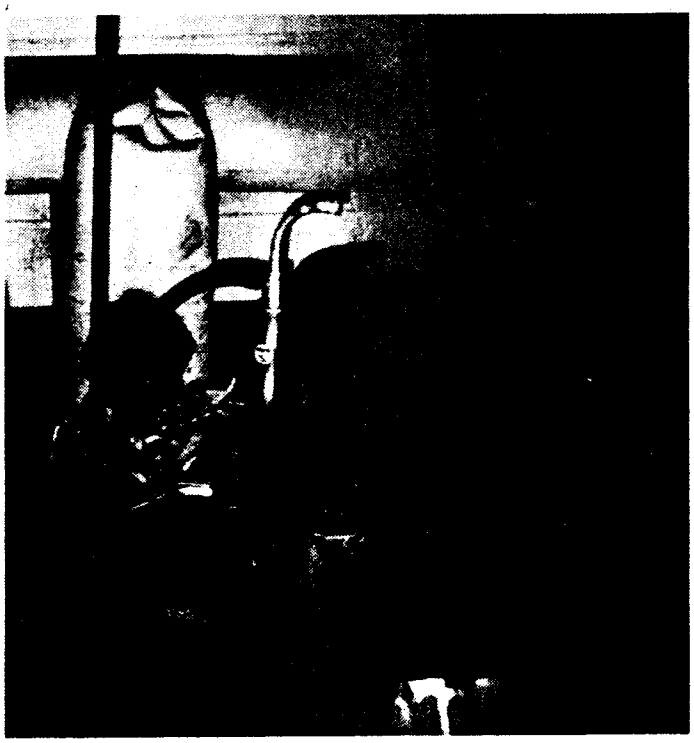

FIGURE 2. STEAM AUTOCLAVES EXHAUSTING TO FLOOR DRAIN AS IMPINGER COLLECTS AIR SAMPLE

6.3 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 -- above the door of each autoclave, and at the space between the two autoclaves, approximately 36 inches from the floor. Sampling above the door of each autoclave consisted of:

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

Sampling at the front of and between the autoclaves consisted of:

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

Air sampling during both spiked and non-spiked waste processing above each autoclave door, following treatment, as doors were opened, consisted of:

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

Air sampling during both spiked and non-spiked waste processing at the exhaust drain consisted of:

1 x 10 min AGI (37C°/55°C) pre-treatment chamber exhaust
2 x 10 min AGI (37°C/55°C) post-treatment chamber exhaust

For fluid sampling, duplicate 50 ml samples were collected from the exhaust drain during the treatment process (approximately every six minutes), for a total of 22 samples from each cycle.

6.4 RESULTS

Results of the air and fluids bioemissions sampling are shown in Tables 10 and 11. Both test indicator organisms were recovered from ambient air samples. One CFU of *B. stearothermophilus* was recovered from ambient air samples, but none were found in air and water samples taken during spiked and non-spiked treatment runs.

Background levels of *B. subtilis* var. *niger* were slightly higher. Six CFU were recovered from non-spiked air samples. One CFU of BSN was recovered from the non-spiked run air samples, but none from the corresponding condensate drain water samples. Following the spiked run, single colonies of BSN were recovered from one water sample and one air sample.

Overall indicator occurrence was very low, and recovery of both BST and BSN following treatment of spiked waste was below background levels established from sampling ambient air and the potential emission points after treating waste that was not spiked with indicator organisms. Neither test organism was recovered from the initial evacuation cycle of these gravity displacement autoclaves. Because the evacuation occurs before temperature and pressure are at operational levels, this part of the cycle has the greatest potential for bioaerosol emissions. No previous study was found that addressed bioaerosol emissions from gravity displacement autoclaves. The potential for bioaerosol emissions from high vacuum type autoclaves has been demonstrated by Barbieto and Brookey (1976). When seeded with dry BSN spore preparations and liquid aerosols of BSN, *Serratia marcescens*, and coliphage T1, the test organisms were recovered from the chamber vacuum exhaust prior to the sterilization cycle. It is important to note the differences between that report and the present study. First, this study used gravity displacement rather than high vacuum sterilizers. Secondly, Barbieto and Brookey either directly aerosolized test organisms, or used a dry spore preparation apparently designed to maximize the likelihood of spore aerosolization. In this study, spores dried onto filters were intended to mimic spilled, dried organisms on the surface of 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 10. Indicator Organism Recovery from Air Impactor Samples from Two On-site Medical Waste Treatment Steam Autoclaves

	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]						
Above #2042	*	0	0	0	*	*
Above #2052	1	*	*	*	6	0
Room Air	0	*	*	1	*	*
Andersen [4]						
Above #2042	0	*	*	0	*	*
Above #2052	0	*	*	0	*	*
Room Air	0	*	*	0	*	*

[1] = Each waste load seeded with 5.3×10^8 dried spores
[2] = Each waste load seeded with 1.1×10^8 dried spores
[3] = Slit-to-agar sampler; 15 min (5 min room air); Trypticase soy agar
[4] = 2-Stage sampler; 5 min; Trypticase soy agar
* = No sample collected according to sampling plan
Mean temperature 28.4°C during sampling
Mean relative humidity 53.7% during sampling

Table 11. Indicator Organism Recovery from Impinger and Condensate Fluids from Two On-site Medical Waste Treatment Steam Autoclaves

	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		
Pre-vac	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	ND	ND	0,0	0	ND	ND	0,0	0
Door Exhaust								
#2042 -1	0,0	0	0,0	0	0,1	0	0,0	0
-2	0,0	0	0,0	0	0,0	0	0,0	1
Door Exhaust								
#2052 -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**		**Spiked**		**Non-spiked**		**Spiked**	
	Plates	Filter	Plates	Filter	Plates	Filter	Plates	Filter
Condensate Drain Water [4]								
Time (min)								
0	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
18	0,0	0	0,0	0	0,0	0	0,0	0
24	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	0,1	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

ND = Not done
[1] = Each waste load seeded with 5.3×10^8 dried spores
[2] = Each waste load seeded with 1.1×10^8 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

7.0 MICROWAVE SAMPLING PLAN

The outdoor microwave treatment system evaluated is located at a large hospital/ medical center and is used to treat general medical waste. It is designed to treat medical waste at the rate of 220 pounds per hour. The waste is fed by continuous batch mode into a grinding chamber where it is sprayed with steam and mechanically destroyed to render it unrecognizable. The waste is then treated with additional steam as it slowly moves via a transport auger under a series of microwave units. Following microwave exposure, the treated waste is conveyed via an auger tube to a waiting dumpster or compactor, for a total batch treatment time of 90 minutes.

7.1 Sampling Points

Four potential air emission points were identified. They are described below, and are shown in Figures 3-6. There are no fluid emissions from the treatment system.

1. **The top of the grinding chamber when the hydraulic door opens to receive a cart of waste.** (Fig. 3) Although the opening of the door activates a negative pressure airflow which moves air inward and through HEPA and charcoal filters, the potential exists for some air emissions from the grinding of the previous batch of untreated waste, as indicated by the escape of steam from the chamber as the door opens. The door opens for only 45 seconds.

2. **The access port to the untreated waste transport auger.** (Fig. 4) This small opening can be uncapped for maintenance and to insert packs of indicator spores when performing routine treatment effectiveness testing.

3. **The face of the roof vent which exhausts HEPA filtered air from the grinding chamber as each batch is dumped in for processing.** (Fig. 5) This exhaust is operative only when the door to the grinding chamber is open (45 seconds).

4. **The opening of the waste exit tube.** (Fig. 6) Treated waste exits from the machine into a general refuse compactor.

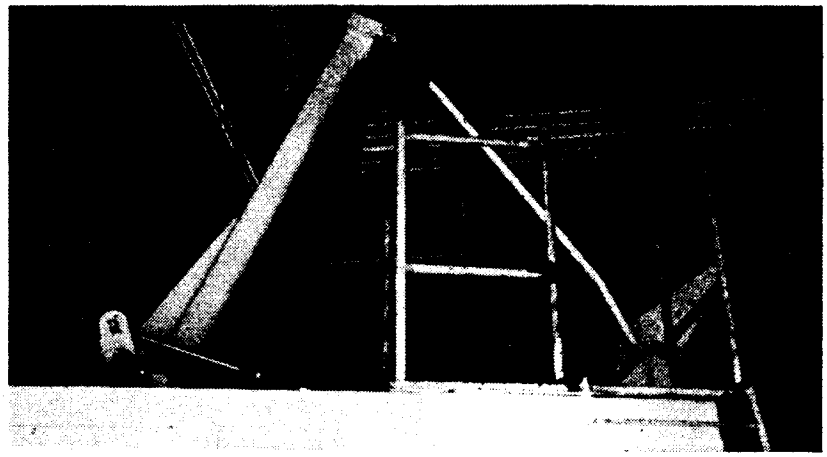

FIGURE 3. MICROWAVE UNIT WITH DOOR TO GRINDING CHAMBER OPEN TO RECEIVE WASTE

FIGURE 4. MICROWAVE UNIT ACCESS PORT TO UNTREATED WASTE TRANSPORT AUGER

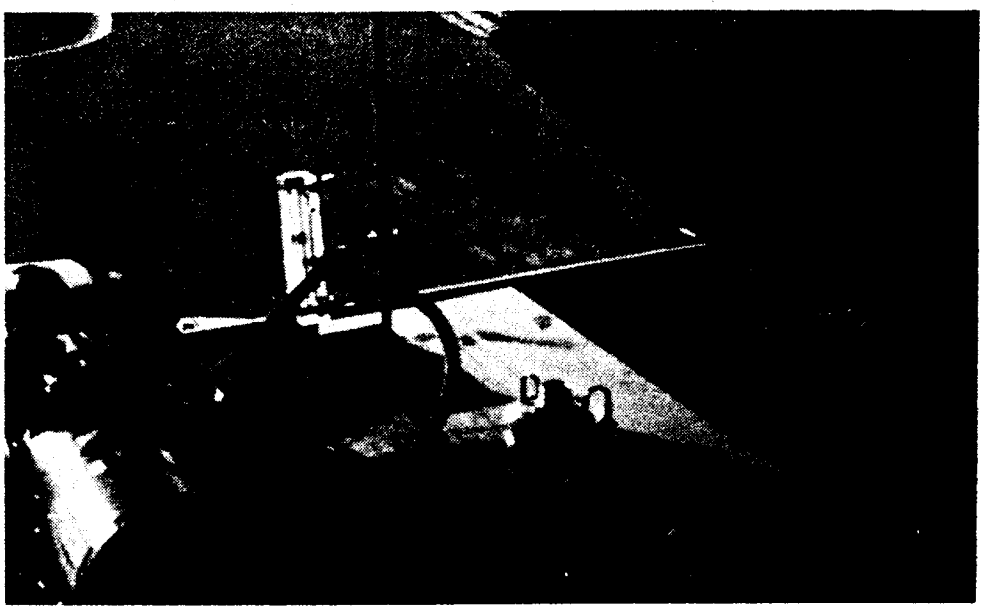

FIGURE 5. GRINDING CHAMBER HEPA EXHAUST VENT ON TOP OF MICROWAVE UNIT

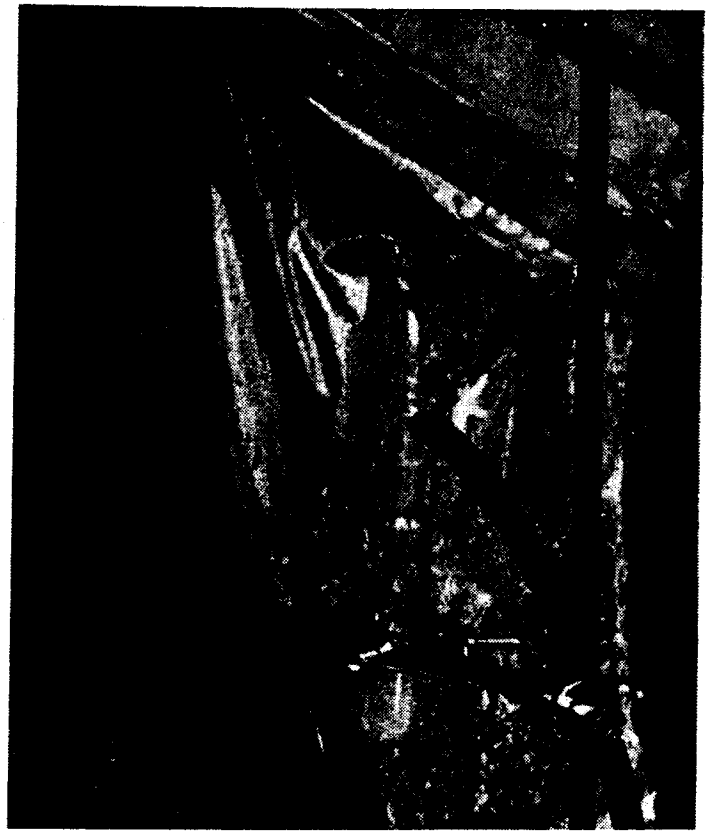

FIGURE 6. TREATED WASTE EXIT TUBE ON MICROWAVE UNIT WITH AGI-30 COLLECTING AIR SAMPLE