FIGURE 2. ASSESSMENT OF *BACILLUS* SPORE SURVIVAL DURING AGI-30 OPERATION

Table 5 shows survival of BSN as CFU and as a per cent recovery as compared to the control impingers. At 20 mins, mean BSN spore survival was 26%, falling to 5% at 40 mins and 11% at 60 minutes. These data indicate that *B. subtilis* var. *niger* spores do not remain viable in AGI-30 impingers for extended sampling periods, and that as sampling time increases, so also does collection fluid loss.

Table 5. Survival of Uninjured Spores of *B. subtilis* var. *niger* in All Glass Impingers

Sample	Counts (CFU)	CFU/ml	Vol (ml)	Total CFU	% Recovery	
					trial[a]	mean[b]
C T_0	111/140	1255	20.0	25100		
C T_{60}	141/118	1295	20.0	25900		
A T_{20}	52/59/59	567	14.0	7940	31	
B T_{20}	34/47/35	387	13.9	5380	21	26
A T_{40}	9/7/6	73	9.5	690	3	
B T_{40}	21/20/20	203	9.8	1990	8	5
A T_{60}	93/104/85	940	5.1	4790	19	
B T_{60}	20/11/16	157	4.8	750	3	11

[a] = Total CFU of trial/mean of total CFU of C T_0 and C T_{60}
[b] = Mean of total CFU A and B/mean of total CFU of C T_0 and C T_{60}

B. stearothermophilus spore survival is given in Table 6. There is little loss of viability until after 40 mins of operation of the impingers. At 60 mins, 62% of the spores were still viable.

Table 6. Survival of Uninjured Spores of *B. stearothermophilus* in All-Glass Impingers

Sample	Counts (CFU)	CFU/ml	Vol (ml)	Total CFU	% Recovery	
					trial[a]	mean[b]
C T_0	93/118	910	20.0	21100		
C T_{60}	61/84/144	963	20.0	19300		
A T_{20}	115/114/145	1297	14.0	17500	87	
B T_{20}	125/155/122	1340	13.9	18600	92	89
A T_{40}	184/153/180	1723	9.5	16300	81	
B T_{40}	N.D./138/146	1420	9.8	13900	69	75
A T_{60}	214/230/251	2317	5.1	11800	58	
B T_{60}	279/N.D.	2790	4.8	13400	66	62

[a] = Total CFU of trial/mean of total CFU of C T_0 and C T_{60}
[b] = Mean of total CFU A and B/mean of total CFU of C T_0 and C T_{60}
N.D. = No data

7.2 Survival of Injured Spores

As it is assumed that microorganisms emitted from medical waste treatment processes are injured to some degree, the survival of injured spores in the impingers was investigated. This experiment was identical to the survival experiment with fresh, uninjured spores with a few exceptions. Both control impingers (C T_0 and C T_{60}) were sampled before and after the vacuum pumps were operated. The control samples were plated in triplicate, rather than in duplicate. Based on the results of BSN survival above, the sample periods were shortened to 5, 10, and 20 minutes. To simulate

spore injury by thermal treatment, the spore suspension (4.5 ml @1 x 10^5 CFU/ml) was heated to 100°C for 20 mins immediately prior to the experiment.

BSN spores did not survive the heat treatment. Two CFU were recovered from control impinger samples, and two CFU were recovered from the six trial impinger samples (data not shown). As expected, BST spores were very resistant to moist heat inactivation. Following heat treatment of the BST stock, 84% of the spores remained viable. Survival of heat-treated spores in operating impingers was also high. Table 7 shows that after 5 mins of impinger operation, no spore viability was lost. After 10 and 20 mins of operation, spore recovery was 86% and 82%, respectively.

Table 7. Survival of Heat Treated Spores of *B. stearothermophilus* in All-Glass Impingers

Sample	Counts (CFU)	CFU/ ml	Vol (ml)	Total CFU	% Recovery	
					trial[b]	mean[c]
CA T_0	92/N.D./115	1035	20.0	20700		
CB T_0	110/100/89	997	20.0	19900		
A T_5	104/89/151	1147	18.8	21600	107	
B T_5	142/96/105	1143	18.2	20800	103	105
A T_{10}	89/111/87	957	18.0	17200	85	
B T_{10}	105/98/85	960	18.3	17600	87	86
A T_{20}	117/103/105	1083	15.3	16600	82	
B T_{20}	100/89/134	1077	15.3	16500	82	82
CA T_{20}	85/101/112	993	20.0	19900		
CB T_{20}	100/99/105	1013	20.0	20300		

[a] = (Total CFU of trial/mean of total CFU of all controls)x 100
[b] = (Mean of total CFU A and B/mean of all controls)x 100
N.D. = No data

7.3 Spore Slippage from AGI-30s

In addition to verifying spore survival during sampler operation, design of a monitoring approach requires a determination of degree of spore slippage through the impingers. Such slippage has been reported as 0.3 - 0.5% of BSN for the AGI-4 (Harstad and Filler, 1969; Tyler and Shipe, 1959), although no data have been found for BST or BSN slippage through AGI-30s. Because of the similar design, slippage was expected to be low for the AGI-30. To assess the rate of slippage, pairs of impingers were connected in series with the first impinger containing a spore suspension, and the second, or rear impingers, containing only PBDW. The impingers were operated for 20 mins followed by assay of the contents of the front and rear impingers.

Fresh stocks of BSN and BST spores were diluted and mixed to yield a suspension containing ~1 x 10^5 CFU/ml of each organism. Four impingers were labelled "front" and filled with 19.7 ml PBDW, and four were labelled "rear" and filled with 20 ml PBDW. Stock spore suspension was added to the front impingers (0.3 ml each) and sampled for enumeration as a control. Four pairs of front and rear impingers were connected in series with Tygon tubing and then connected to vacuum pumps, which were operated for 20 mins at 41 mm Hg. Front impingers were sampled and plated in triplicate on TSA for each organism as described above. The volume of impinger fluid remaining was measured and recorded. Impinger fluid from the rear impingers was divided into two portions, measured, and filtered through separate 0.2 µm membranes. The membranes were placed on TSA for incubation at 37°C and 55°C for growth of BSN and BST retained on the membranes.

Results showed very low slippage of spores from the front impingers for both organisms (Tables 8, 9). Slippage can be calculated based on the spores recovered from the front impinger (Surviving) or on the amount inoculated into the front impinger (Total). Using the more conservative calculation, slippage of surviving spores, BSN shows a mean slippage of 0.2% (Table 8), while mean BST slippage was 0.3% in four trials.

Table 8. Slippage of *B. subtilis* var. *niger* Through AGI-30 Impingers

	Spore Counts (CFU)		Slippage (%)	
Sample	Front	Rear	Surviving[a]	Total[b]
A	22000	4	0.02	0.01
B	7400	17	0.20	0.04
C	4700	<2	<0.04	<0.005
D	2700	16	0.60	0.04
Mean			.20	.02

[a] = (Rear CFU / Front recovered spores CFU) x 100
[b] = (Rear CFU / Applied Spores 3.8 x 10^4 CFU) x 100
< = At detection limit of assay, no colonies counted

Table 9. Slippage of *B. stearothermophilus* Through AGI-30 Impingers

	Spore Counts (CFU)		Slippage (%)	
Sample	Front	Rear	Surviving[a]	Total[b]
A	26900	73	0.3	0.2
B	23500	82	0.3	0.3
C	24800	38	0.2	0.1
D	20400	65	0.3	0.2
Mean			0.3	0.2

[a] = (Rear CFU / Front recovered spores CFU) x 100
[b] = (Rear CFU / Applied Spores 3.2 x 10^4 CFU) x 100

8.0 DISCUSSION

Methods for field evaluation of biological emissions from medical waste treatment devices can be selected based upon the above results. Bacterial endospores can be grown on Trypticase Soy Agar (TSA) and thermophilic *B. stearothermophilus* can be selectively grown at elevated temperature with no interference from other spore-forming bacteria. Similarly, a pigmented spore former, *B. subtilis* var. *niger*, can be grown and differentiated from endogenous organisms by its coloration on three different agars. BSN forms orange colonies on TSA, is more intensely pigmented on beef extract glucose agar, and becomes black on tyrosine agar. TSA provided the highest counts of the three agars.

Because the primary spore sampling method is impingement, spore survival and slippage were also determined. Slippage was minimal (≤0.6%), and consistent with published results for the AGI-4. BSN spores proved more labile than BST spores, with loss of viability approaching 90% after one hour of cycling in the impingers compared to a 38% loss for BST. At shorter cycling times, losses of both spores were less. After 20 mins, 89% of BST spores remained viable, as did 26% of BSN spores. Heat treatment inactivated nearly all BSN spores, but did not affect BST. At least 80% of heat treated BST spores were recovered after twenty mins of cycling in impingers. Although both organisms survived simulated sampling in the impingers, BST was clearly more stable than BSN, particularly after heat treatment. For field monitoring of emissions, *B. stearothermophilus* is the better choice for thermal treatment devices.

9.0 RECOMMENDATIONS

Based upon the data generated from the studies described, it is recommended that Trypticase Soy Agar be used as the primary medium for recovery of indicator spores from air and fluid monitoring of potential medical waste treatment bioemissions. Confirmation of *B. subtilis* var. *niger* recovery and growth can be made by subculture to Tyrosine Agar and/or Beef Extract/Glucose Agar.

In order to maximize airborne spore recovery, it is recommended that air monitoring with AGI-30s not exceed 20 minutes. Large volume air samples should be collected with replicate samplers.

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