FINAL REPORT

Determination of Antimicrobial Effectiveness of Energy Wall Core Material

Submitted to

Dustin Eplee, President
Energy Wall

By

James Freihaut, Ph.D., P.E.,
William Bahnfleth, Ph.D.
Paul Kremer, Research Associate
Stephen Martin, Graduate Research Assistant

Titus O, Undergraduate Research Assistant
Jay Patel, Undergraduate Research Assistant
David Blum, Undergraduate Research Assistant
Geoffrey Kim, Undergraduate Research Assistant

Executive Summary

Energy Wall enthalpy exchange cores are expected to encounter airstreams with various levels of microbial species. Thus, it is desirable that the cores do not support microbial growth. Given the high surface area to treated air volume ratio and short air diffusion paths within the channels, the Energy Wall device has the potential to remove significant fractions of air entrained bioaerosols from treated air in addition to the explicit sensible and latent energy recovery functions of the device. Two aspects of viable bioaerosol air treatment properties were investigated in controlled, laboratory conditions – bioaerosol removal potential and, once a microbe is removed from the airstream and deposited on the device surfaces, the biocide effectiveness of the Energy Wall substrate material itself on the microbe.

The bioaerosol removal capability (i.e., bioaerosol filtration), of an Energy Wall unit was investigated in a full scale ASHRAE 52.2 compliant test rig operated by the Pennsylvania State University Indoor Environment Center. Viable *Bacillus subtilis* spores were used as the challenge microbe for the equivalent filtration characterization of an Energy Wall core. Bacteria spores are generally smaller and have aerodynamic properties that tend to enhance air entrainment relative to vegetative forms of bacteria. The Energy Wall device removed on average 82% of viable spores contained in the challenging air stream which when corrected with the 47% removal observed for no-core control tests, yields a 35% relative reduction. Given the 2 – 3 micron nominal diameter size of the Bacillus Subtilis spores the Energy Core and rig yielded the equivalent performance of a MERV 12 type filter.

The intrinsic, biocide effectiveness of the Energy Wall material itself, was also investigated by performing a series of controlled, viable microbe deposition tests using samples of Energy Wall, LiCl-treated paper substrates and an equivalent set of control, deposition tests using blank, no LiCl-treated, paper substrate samples. Three different microbe species were utilized – *Bacillus subtilis* spores, vegetative *Pseudomonas aeruginosa* and vegetative *Staphylococcus aureus*. Viable samples of the three types of microbes were deposited on the paper substrates for 15 minutes and then removed and cultured to determine viability after the surface interaction. The *Bacillus subtilis* spores displayed an average of 79% deactivation on blank paper and about 76% on the LiCl-impregnated paper. *Pseuduomonas aeruginosa* displayed about an 83% deactivation on either type of paper substrate. Thus, the benchtop data for *Bacillus subtilis and Pseudomonas aeruginosa* reveal no statistically significant difference in biocidal effect between the LiCl-treated and untreated paper for either species. However, these results do suggest that the base paper in the Energy Wall cores may have inherent antibacterial properties due to high concentrations of magnesium hydroxide and other proprietary ingredients not directly evaluated in this study. Follow-up control tests on paper with no additives are warranted.

In contrast, the *Staphylococcus aureus* samples were much more resistant to short time (15 minutes) surface contact deactivation than these two other microbe types, but showed an order of magnitude decrease in viability upon exposure to the LiCl-impregnated paper relative to the

blank paper. Whereas only ~ 5% of the *Staphylococcus aureus* samples were deactivated by the blank paper, ~53% of the *Staphylococcus aureus* were deactivated upon short term exposure to the LiCl-impregnated paper. The results indicate that disperse, monolayer, contact of viable microbes on paper for short periods of time can result in deactivation of viable microbes, and the Energy Wall LiCl treated paper has a greater biocide effect on surface contact-resistant type organisms than non-impregnated paper. Materials and procedures used for these tests and a summary of the test results are presented in this report.

Determination of Antimicrobial Effectiveness of Energy Wall Core Material

Three bacterial species are used to test potential biocidal effects of the LiCl-impregnated Energy Wall cores: *Pseudomonas aeruginosa* (vegetative form), *Bacillus subtilis* (spore form), and *Staphylococcus aureus* (vegetative form). Essentially, the vegetative and spore bacterial species are deposited on samples of the core material, allowed to remain for a fixed length of time, and finally swabbed and cultured to check for viability. Samples are cultured on nutrient agar plates using the spread plate technique [Eaton *et al.*, 1995; Nedeljkovic-Davidovic, 2008].

In order to plate bacterial species properly, they need to be diluted to a number that is statistically significant when counted. This number needs to fall between 20 and 250 colonies per plate. Three 2 x 2 in. squares of LiCl-treated and non-treated paper are cut out and mixed with the right concentration of microbes. Each treated paper specimen is then placed in a vial and afterwards, three plates per vial are prepared. The vial containing the paper is then further diluted, and again plated onto three plates.

These bacterial species require adherence to a strict protocol when handling. Moreover, proper personal protective equipment (e.g., VWR Micrograp purple nitrile power-free gloves) and the use of biosafety cabinets when handling the species is important to ensure lab personnel safety and to help prevent contamination. It is also very important to have a clear labeling system for labeling all labware used ahead of time to help ensure that the potential for cross contaminations is minimized.

Materials

Supplies and apparatus used for the tests are described below.

Glass Rods (Figure 1): Used to spread solutions evenly onto agar plates. Glass rods (VWR Kimble Chase Kimax Glass Stirring rods) are sterilized before use by an open flame from the butane burner for ten seconds.

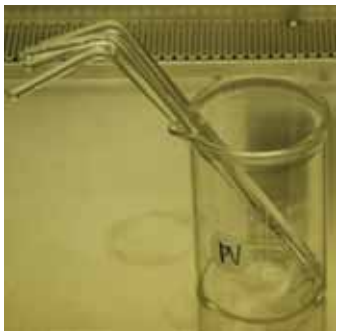


Figure 1: Glass rods

Butane Cartridge Burner (Figure 2): An IBS Integra Biosciences FIREBOY eco burner with a Camping Gaz CV360 butane cartridge is used to sterilize the glass rods.

Figure 2: Butane cartridge burner

Micropipettes (Figure 3): Used to draw up specific amounts of liquids indicated by each micropipette (Eppendorf 100, Eppendorf 1000, Eppendorf 5000). Micropipettes are color coded to indicate the volume of liquid they can handle (purple: 500 – 5000 µl; blue: 100 - 1000 µl; yellow: 10 - 100 µl). Micropippetes and their color-coded micropipette tips (Eppendorf epT.I.P.S) used for this study are shown in Figure 3. Tips are disposed into a biohazard bag after use and the micropipettes are cleaned with 70% isopropyl alcohol.

Figure 3: Micropipettes and micropippete tips

Autoclave (Figure 4): A machine used to sterilize equipment with pressurized steam (Tuttnauer EZ10, Tuttnauer). The autoclave has four settings. The first setting is for unwrapped instruments and reaches 134^{o}C for 4 minutes. The second setting is for wrapped instruments and reaches 134^{o}C for 8 minutes. The third setting is for glassware and reaches 121^{o}C for 30 minutes. The last setting is a drying cycle that blows HEPA filtered air for 60 minutes.

Figure 4: Autoclave

Forceps (Figure 5): Fisher Scientific Busse Hosp Disposables Sterile Posi-Grip plastic forceps are used to transfer LiCl-treated or non-treated squares. Forceps are wrapped in aluminum foil, sealed with autoclave tape, and sterilized using the wrapped instrument setting on the autoclave.

Figure 5: Forceps

Biohazard Bags (Figure 6): Used to collect all wastes generated from the conduct of these experiments.

a. b.

Figure 6: a. Biohazard bag; b. biohazard sharps and general waste containers.

Vortex mixer (Figure 7): A VWR Vortex Mixer 58816-121 is used to mix solutions thoroughly by spinning at high speed. The speeds can vary depending on the controls. For these experiments, each solution is vortexed at a medium speed setting for 30 seconds.

Figure 7: Vortex mxer

Agar (Figure 8): A culture medium for microbial growth, Difco tryptic soy agar soybean casein digest agar (BD Biosciences, cat. No. 236950). The procedure for making plates with this agar is located in appendices A-C. Using this procedure, agar culture plates were made using VWR 25384-342 sterile petri dishes.

Figure 8: Agar powder

Incubator (Figure 9): A Fisher Scientific Isotemp Incubator is used to set and control precise temperature conditions for microbial growth.

Figure 9: Incubator

E count (Figure 10): A Heathrow Scientific eCount™ Colony Counter holds a sharpie and counts the number of times the sharpie contacts the surface of the plate while enumerating colony forming units.

Figure 10: eCount™

Other: VWR BD Falcon 15ml serile, poystyrene cntrifuge tubes; VWR borosilicate glass vials with phenolic screw caps; and other disposable supplies listed in appendices A-C.

Procedures

Each species was supplied as an aqueous suspension of biological species at a concentration of 1 x 10^9 per ml as shown in Figure 11a. Overall, 108 plates were prepared according to the procedures detailed below to ensure experimental accuracy.

Step 1: The initial concentration of 1 x 10^9 spores or vegetative cells per ml needs to be diluted to yield a concentration on the order of 1 x 10^6. This is done easily by first mixing one ml of the initial concentration solution with 9 ml of reagent grade water to yield a 10-fold dilution of 1 x 10^8 concentration. This process is repeated two more times to create a 1 x 10^6 concentration. (Note: it is possible to dilute from a concentration of 1 x 10^9 to 1 x 10^7 by adding 10 ml of reagent grade water to a tube and replacing one micro liter of the reagent grade water with one micro liter of the 1 x 10^9 solution as shown in Figure 11 b and c.)

a. b. c.

Figure 11: a. Bacterial aqueous suspensions used; b and c. 100-fold dilution using the 10 ml exchange method described above.

Step 2: Once the 1 x 10^6 solution is made, three, 2 x 2 in. squares each of the LiCl-impregnated paper and the non-LiCl impregnated paper are cut out from the Energy Wall core stock supplied. Each square is then placed into a clean, empty petri dish. Petri dishes are labeled "with LiCl" or "no LiCl" as shown in Figure 12 b.

a. b.

Figure 12: a. LiCl-impregnated paper and non-LiCl-impregnated paper; b. paper placed into an empty petri dish.

Step 3: Pipet 0.25 ml (250 µl) of the 1 x 10^6 solution and transfer it onto the paper square in each petri dish as shown in Figure 13. Use the glass rods to spread out the solution evenly over the paper squares whilst attempting to leave as little of the solution on the glass rod as possible in an attempt to reduce error in the final count. Use a different rod for each dish and sterilize the rods afterwards. (Note: the LiCl leaves a salt residue so it is best to wipe it off with a paper towel before sterilizing it on the burner. Dispose of the used paper towel into the biohazard bag.)

a. b. c.

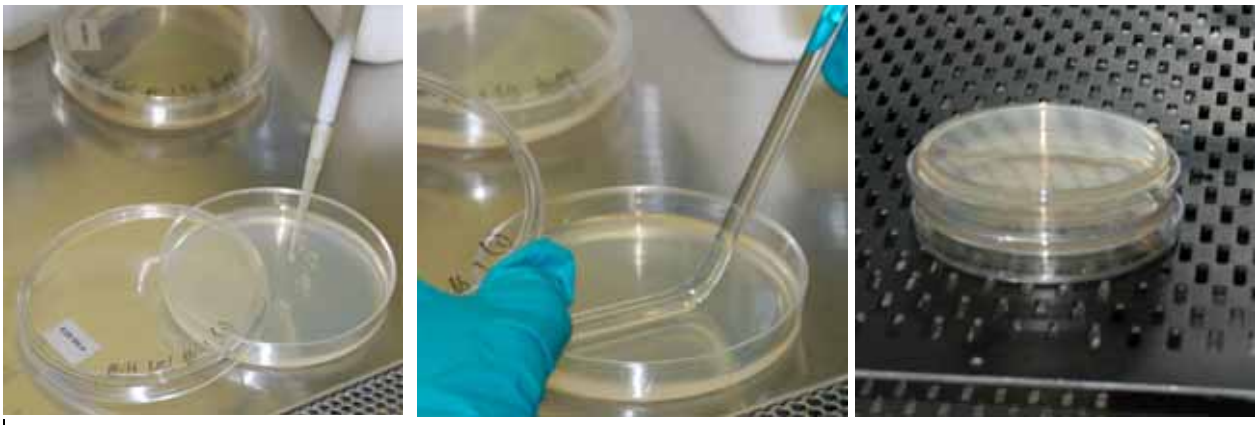
Figure 13: a. Transferring the 1 x 10^6 concentration to the LiCl paper; b. spreading the solution with a glass rod; c. sterilizing the glass rod with a burner.

Step 4: Allow the microbes fifteen minutes to interact with the paper. Afterwards, use sterile forceps to carefully place the squares into individual glass vials labeled according to the sample (e.g., "With LiCl Staph 1", "With LiCl Staph 2", etc.) as shown in Figure 14. In this step take care not to touch the paper at any point to prevent contamination. Pipet 25 ml of reagent grade water into each vial and vortex at a medium speed for 30 seconds. The 25 ml of reagent grade water further dilutes the solution to 1 x 10^4 so that the number of colony forming units (CFUs) on the pate will be a manageable number to count and lead to statistically significant counts.

Step 5: Prepare three plates (appendices A-C) from the solution in each vial as shown in Figure 15. Label each plate (e.g., "With LiCl Staph 1 (1)", "With LiCl Staph 1 (2)", etc.). Transfer 10 ml (10,000 µl) of the newly made solution onto the correct plate. Use a sterile glass rod to evenly distribute the solution over the surface of the agar. (Note: if the agar is punctured when spreading the solution on the agar surface, a new plate will have to be made.) Afterwards sterilize the glass rod over the butane burner. Repeat this same step for all the plates, then place the plates into the incubator set to 35^o C for 24 hours.

a. b. c.

Figure 14: a. Glass vials used; b. using forceps to transfer solution-infused paper to a glass vial; c. after transfer, 25 ml of reagent grade water is added to the vial.



a. b. c.

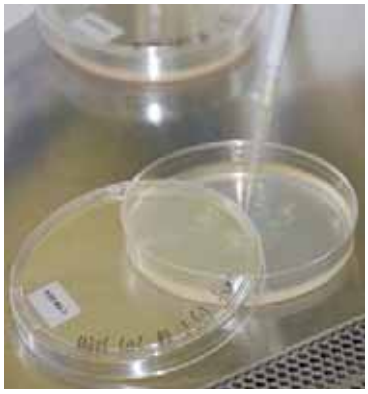
Figure 15: a. Transfer solution to tryptic soy agar (TSA) plates; b. spread the solution over the TSA plate surface; c. place the prepared plates in the incubator.

Step 6: Use a polypropylene conical tube in conjunction with a micropipette to transfer 9 ml of reagent grade water and 1 ml of the 1 x 10^4 solution to create a new 1 x 10^3 solution as shown in Figure 16. Plate three plates per vial of the new 1 x 10^3 solution using the same procedure discussed at step 5.

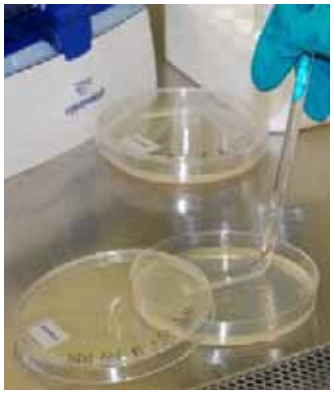
a. -

b.

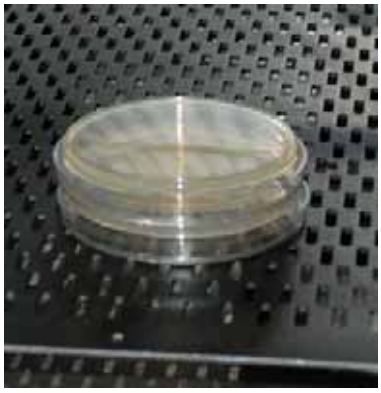
c.



d.



e.



f.

Figure 16: a. Fill a polystyrene conical tube with reagent grade water up to 9 ml; b. transfer 1 ml of the 1 x 10^4 solution to the conical tube; c. vortex the solution for 30 seconds; d, e, and f. plate the 1 x 10^3 solution as described at step 5.

Step 7: Count each plate after 24 hours. Care is taken to ensure that the spots on the plate are the actual microbes and not contaminates. This process is guided by references detailing what each microbe should look like when cultured in agar. For example, *Pseudomonas aeruginosa* look like tiny white spots; *Bacillus subtillis* generates larger 2 to 3 mm diameter spots; and *Staphylococcus aureus* generate slightly larger spots than the *Pseudomonas aeruginosa*. The

aforementioned eCount™ device is used to count the cultures as shown in Figure 17. Each plate is disposed of in a biohazard bag after enumeration.

Figure 17: Use an eCount™ to enumerate cultured bacteria.

Bacillus subtilis Energy Wall Core Benchtop Test Results

Achieved concentrations for the three stock solutions used for the *Bacillus subtilis* benchtop tests on the Energy Wall core material with and without LiCl treatment are presented in Table 1. Resulting concentrations after following the procedures for benchtop tests on Energy Wall material with and without the LCl are presented in Tables 2 and 3. These concentrations allow the computation of survival fractions of the microbial populations exposed to the treated and untreated paper: $S_{with} = N_{with}/ N_0$ and $S_{without} = N_{without}/ N_0$, where N_{with} and $N_{without}$ represent the number of viable microorganisms after contact with the Energy Wall core material, and N_0 represents the number of viable organisms prior to exposure to the treated and untreated core material. An overall comparative inactivation efficiency percentage is then calculated as $(1- S_{with} /S_{without})*100$.

Mean, standard deviation, coefficient of variation, quartiles and median descriptive statistics for the groupings in Tables 2 and 3 are given in Table 4. In addition, results of an Anderson-Darling test for normality for each grouping are presented. AD is the test statistic for the Anderson-Darling test. A reported p-value ≥ 0.1 suggests that data are normally distributed; 0.1 > p-value ≥ 0.05 suggests that data are near-normally distributed; and a p-value < 0.1 suggests that data

are not normally distributed. Means for various groupings were compared using two-sample t-tests assuming equal variances, and confidence intervals for the difference in means at a confidence level of 95% and 99% are presented in Table 5. A "p-value" was calculated in conjunction with the two-sample t-tests to determine the significance level achieved for the test. A significance level of 0.05 (i.e., 95% confidence level) was chosen as the acceptance criterion for statistical significance. A p-value≦ 0.05 indicates that the hypothesis that the means are equal should be rejected at the 95% confidence level (i.e., there are significant differences in the means for the groupings being compared). A p-value > 0.05 indicates that the hypothesis of equal means should be accepted at the 95% confidence level.

The first listing in Table 5 compares the mean computed from all nine tests with untreated paper (BS-no(all)) to the mean computed from all nine tests with treated paper (BS all). As shown, no statistical significance is shown through this comparison (p-value = 0.558). Interestingly, some of the comparisons between the treated and untreated paper for groupings derived from the same stock solution (i.e., BS1 and BS2) do show statistical significance.

S_{with} is 0.76, $S_{without}$ = 0.79, and the comparative inactivation efficiency percentage is -14.29% for the overall grouping of results, which suggests that when viewed as a whole, the benchtop data for *Bacillus subtilis* reveal no statistically significant improvement in biocidal effect for the LiCl-treated paper as compared to the untreated paper.

.

Table 1: Plate counts of *Bacillus subtilis* bacterial stock suspensions

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)	N_0
BSstock-1	05/19/2009	100	05/20/2009	142	2	1.42E+05	1.74E+05
BSstock-2	05/19/2009	100	05/20/2009	178	2	1.78E+05	
BSstock-3	05/19/2009	100	05/20/2009	202	2	2.02E+05	

Table 2: Plate counts of *Bacillus subtilis* after exposure of Energy Wall material without lithium chloride (LiCl) treatment

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)
BS1-1no	05/19/2009	100	05/20/2009	21	2	2.10E+04
BS1-2no	05/19/2009	100	05/20/2009	20	2	2.00E+04
BS1-3no	05/19/2009	100	05/20/2009	21	2	2.10E+04
BS2-1no	05/19/2009	100	05/20/2009	46	2	4.60E+04
BS2-2no	05/19/2009	100	05/20/2009	39	2	3.90E+04
BS2-3no	05/19/2009	100	05/20/2009	62	2	6.20E+04
BS3-1no	05/19/2009	100	05/20/2009	39	2	3.90E+04
BS3-2no	05/19/2009	100	05/20/2009	42	2	4.20E+04
BS3-3no	05/19/2009	100	05/20/2009	39	2	3.90E+04

Table 3: Plate counts of *Bacillus subtilis* after exposure of Energy Wall material with lithium chloride (LiCl) treatment

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)
BS1-1	05/19/2009	100	05/20/2009	74	2	7.40E+04
BS1-2	05/19/2009	100	05/20/2009	59	2	5.90E+04
BS1-3	05/19/2009	100	05/20/2009	72	2	7.20E+04
BS2-1	05/19/2009	100	05/20/2009	33	2	3.30E+04
BS2-2	05/19/2009	100	05/20/2009	40	2	4.00E+04
BS2-3	05/19/2009	100	05/20/2009	41	2	4.10E+04
BS3-1	05/19/2009	100	05/20/2009	17	2	1.70E+04
BS3-2	05/19/2009	100	05/20/2009	20	2	2.00E+04
BS3-3	05/19/2009	100	05/20/2009	20	2	2.00E+04

[1] actual counts on plate

Table 4: Descriptive statistics and Anderson Darling normality test results for *Bacillus subtilis*

Variable	Total Count	Mean	SE Mean	StDev	Variance	CoefVar	Q1	Median	Q3	AD	Pvalue
BS-no(all)	9	36556	4628	13884	192777778	37.98	21000	39000	44000	0.519	0.134
BS(all)	9	41778	7352	22055	486444444	52.79	20000	40000	65500	0.398	0.288
BS1-no	3	20667	333	577	333333	2.79	20000	21000	21000	0.488	0.057
BS1	3	68333	4702	8145	66333333	11.92	59000	72000	74000	0.366	0.158
BS2-no	3	49000	6807	11790	139000000	24.06	39000	46000	62000	0.234	0.434
BS2	3	38000	2517	4359	19000000	11.47	33000	40000	41000	0.373	0.148
BS3-no	3	40000	1000	1732	3000000	4.33	39000	39000	42000	0.488	0.057
BS3	3	19000	1000	1732	3000000	9.12	17000	20000	20000	0.488	0.057

Table 5: Two-sample t-test results and confidence intervals for *Bacillus subtilis*

Means	95% CI	99% CI	T-Value	P-Value	DF
BS-no(all), BS(all)	(-23990, 13546)	(-31391, 20946)	-0.60	0.558	13
BS1-no, BS1	(-67950, -27384)	(-94453, -881)	-10.11	0.010	2
BS2-no, BS2	(-20225, 42225)	(-61026, 83026)	1.52	0.269	2
BS3-no, BS3	(17074, 24926)	(14489, 27511)	14.85	0.000	4

Pseudomonas aeruginosa Energy Wall Core Benchtop Test Results

Achieved concentrations for the three stock solutions used for the P*seudomonas aeruginosa* benchtop tests on the Energy Wall core material with and without LiCl treatment are presented in Table 6. Resulting concentrations after following the procedures for benchtop tests on Energy Wall material with and without the LiCl are presented in Tables 7 and 8.

Descriptive statistics for the groupings in Tables 7 and 8 are given in Table 9 and results of an Anderson-Darling test for normality for each grouping are presented. Means for various groupings were compared using two-sample t-tests assuming equal variances, and confidence intervals for the difference in means at a confidence level of 95% and 99% are presented in Table 10.

The first listing in Table 10 compares the mean computed from all nine tests with untreated paper (PA-no(all)) to the mean computed from all nine tests with treated paper (PA all). As shown, no statistical significance is shown through this comparison (p-value = 0.508). However, as with the *Bacillus subtilis*, some of the comparisons between the treated and untreated paper for groupings derived from the same stock solution (i.e., PA1 and PA2) do show statistical significance.

S_{with} is 0.844, $S_{without}$ = 0.823, and the comparative inactivation efficiency percentage is 9.29% for the overall grouping of results, which suggests that when viewed as a whole, the benchtop data for *pseudomonas aeruginosa* reveal no statistically significant improvement in biocidal effect for the LiCl-treated paper as compared to the untreated paper.

.

Table 6: Plate counts of *Pseudomonas aeruginosa* Bacterial stock suspensions

	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)	N_0
PAstock-1	05/20/2009	100	05/21/2009	135	3	1.35E+06	1.38E+06
PAstock-2	05/20/2009	100	05/21/2009	195	3	1.95E+06	
PAstock-3	05/20/2009	100	05/21/2009	84	3	8.40E+05	

Table 7: Plate counts of *Pseudomonas aeruginosa* after exposure of Energy Wall material without lithium chloride (LiCl) treatment

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)
PA1-1no	05/20/2009	100	05/21/2109	214	2	2.14E+05
PA1-2no	05/20/2009	100	05/21/2109	216	2	2.16E+05
PA1-3no	05/20/2009	100	05/21/2109	173	2	1.73E+05
PA2-1no	05/20/2009	100	05/21/2109	30	3	3.00E+05
PA2-2no	05/20/2009	100	05/21/2109	29	3	2.90E+05
PA2-3no	05/20/2009	100	05/21/2109	25	3	2.50E+05
PA3-1no	05/20/2009	100	05/21/2109	223	2	2.23E+05
PA3-2no	05/20/2009	100	05/21/2109	228	2	2.28E+05
PA3-3no	05/20/2009	100	05/21/2109	248	2	2.48E+05

Table 8: Plate counts of *Pseudomonas aeruginosa* after exposure of Energy Wall material with lithium chloride (LiCl) treatment

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)
PA1-1	05/20/2009	100	05/21/2109	31	3	3.10E+05
PA1-2	05/20/2009	100	05/21/2109	37	3	3.70E+05
PA1-3	05/20/2009	100	05/21/2109	30	3	3.00E+05
PA2-1	05/20/2009	100	05/21/2109	181	2	1.81E+05
PA2-2	05/20/2009	100	05/21/2109	143	2	1.43E+05
PA2-3	05/20/2009	100	05/21/2109	159	2	1.59E+05
PA3-1	05/20/2009	100	05/21/2109	199	2	1.99E+05
PA3-2	05/20/2009	100	05/21/2109	125	2	1.25E+05
PA3-3	05/20/2009	100	05/21/2109	156	2	1.56E+05

[1] actual counts on plate

Table 9: Descriptive statistics and Anderson Darling normality test results for *Pseudomonas aeruginosa*

Variable	Total Count	Mean	SE Mean	StDev	Variance	CoefVar	Q1	Median	Q3	AD	Pvalue
PA-no(all)	9	238000	13124	39373	1550250000	16.54	215000	228000	270000	0.292	0.523
PA (all)	9	215889	29245	87736	7697611111	40.64	149500	181000	305000	0.594	0.085
PA1-no	3	201000	14012	24269	589000000	12.07	173000	214000	216000	0.444	0.082
PA1	3	326667	21858	37859	1433333333	11.59	300000	310000	370000	0.358	0.169
PA2-no	3	280000	15275	26458	700000000	9.45	250000	290000	300000	0.312	0.249
PA2	3	161000	11015	19079	364000000	11.85	143000	159000	181000	0.199	0.588
PA3-no	3	233000	7638	13229	175000000	5.68	223000	228000	248000	0.312	0.249
PA3	3	160000	21455	37162	1381000000	23.23	125000	156000	199000	0.199	0.586

Table 10: Two-sample t-test results and confidence intervals for *Pseudomonas aeruginosa*

Means	95% CI	99% CI	T-Value	P-Value	DF
PA-no(all), PA (all)	(-48442, 92664)	(-77446, 121669)	0.69	0.508	11
PA1-no, PA1	(-208295, -43039)	(-277318, 25985)	-4.84	0.017	3
PA2-no, PA2	(59066, 178934)	(9001, 228999)	6.32	0.008	3
PA3-no, PA3	(-24990, 170990)	(-153031, 299031)	3.21	0.085	2

Staphylococcus aureus Energy Wall Core Benchtop Test Results

Achieved concentrations for the three stock solutions used for the *Staphylococcus aureus* benchtop tests on the Energy Wall core material with and without LiCl treatment are presented in Table 11. Resulting concentrations after following the procedures for benchtop tests on Energy Wall material with and without the LiCl treatment are presented in Tables 12 and 13.

Descriptive statistics for the groupings in Tables 12 and 13 are given in Table 14 and results of an Anderson-Darling test for normality for each grouping are presented. Means for various groupings were compared using two-sample t-tests assuming equal variances, and confidence intervals for the difference in means at a confidence level of 95% and 99% are presented in Table 15.

The first listing in Table 15 compares the mean computed from all nine tests with untreated paper (SA-no(all)) to the mean computed from all nine tests with treated paper (SA all). Statistical significance is shown through this comparison (p-value = 0.000) and for all of the comparisons between the treated and untreated paper for groupings derived from the same stock solution.

S_{with} is 0.54, $S_{without}$ = 0.053, and the comparative inactivation efficiency percentage is 51.37% for the overall grouping of results, which suggests that when viewed as a whole, the benchtop data for S*taphylococcus aureus* reveal a statistically significant improvement in biocidal effect for the LiCl-treated paper as compared to the untreated paper.

Table 11: Plate counts of *Staphylococcus aureus* Bacterial stock suspensions

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)	N_0
SAstock-1	05/26/2009	100	05/27/2009	94	2	9.40E+04	8.13E+04
SAstock-2	05/26/2009	100	05/27/2009	94	2	9.40E+04	
SAstock-3	05/26/2009	100	05/27/2009	56	2	5.60E+04	

Table 12: Plate counts of *Staphylococcus aureus* after exposure of Energy Wall material without lithium chloride (LiCl) treatment

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)
SA1-1no	05/26/2009	100	05/27/2709	71	2	7.10E+04
SA1-2no	05/26/2009	100	05/27/2709	77	2	7.70E+04
SA1-3no	05/26/2009	100	05/27/2709	69	2	6.90E+04
SA2-1no	05/26/2009	100	05/27/2709	81	2	8.10E+04
SA2-2no	05/26/2009	100	05/27/2709	82	2	8.20E+04
SA2-3no	05/26/2009	100	05/27/2709	78	2	7.80E+04
SA3-1no	05/26/2009	100	05/27/2709	79	2	7.90E+04
SA3-2no	05/26/2009	100	05/27/2709	65	2	6.50E+04
SA3-3no	05/26/2009	100	05/27/2709	91	2	9.10E+04

Table 13: Plate counts of *Staphylococcus aureus* after exposure of Energy Wall material with lithium chloride (LiCl) treatment

Plate #	Date Plated	Vol Plated (µL)	Date Counted	Counts[1]	Dilution	Conc (#/ml)
SA1-1	05/26/2009	100	05/27/2709	44	2	4.40E+04
SA1-2	05/26/2009	100	05/27/2709	37	2	3.70E+04
SA1-3	05/26/2009	100	05/27/2709	35	2	3.50E+04
SA2-1	05/26/2009	100	05/27/2709	37	2	3.70E+04
SA2-2	05/26/2009	100	05/27/2709	31	2	3.10E+04
SA2-3	05/26/2009	100	05/27/2709	23	2	2.30E+04
SA3-1	05/26/2009	100	05/27/2709	42	2	4.20E+04
SA3-2	05/26/2009	100	05/27/2709	46	2	4.60E+04
SA3-3	05/26/2009	100	05/27/2709	42	2	4.20E+04

[1] actual counts on plate

Table 14: Descriptive statistics and Anderson Darling normality test results for *Staphylococcus aureus*

Variable	Total Count	Mean	SE Mean	StDev	Variance	CoefVar	Q1	Median	Q3	AD	Pvalue
SA-no(all)	9	77000	2598	7794	60750000	10.12	70000	78000	81500	0.277	0.738
SA(all)	9	37444	2399	7196	51777778	19.22	33000	37000	43000	0.312	0.482
SA1-no	9	72333	2404	4163	17333333	5.76	69000	71000	77000	0.277	0.334
SA1	3	38667	2728	4726	22333333	12.22	35000	37000	44000	0.296	0.285
SA2-no	3	80333	1202	2082	4333333	2.59	78000	81000	82000	0.277	0.334
SA2	3	30333	4055	7024	49333333	23.16	23000	31000	37000	0.197	0.596
SA3-no	3	78333	7513	13013	169333333	16.61	65000	79000	91000	0.192	0.620
SA3	3	43333	1333	2309	5333333	5.33	42000	42000	46000	0.488	0.057

Table 15: Two-sample t-test results and confidence intervals for *Staphylococcus aureus*

Means	95% CI	99% CI	T-Value	P-Value	DF
SA-no(all), SA (all)	(32019, 47092)	(29136, 49975)	11.19	0.000	15
SA1-no, SA1	(22095, 45239)	(12428, 54906)	9.26	0.003	3
SA2-no, SA2	(31802, 68198)	(8023, 91977)	11.82	0.007	2
SA3-no, SA3	(2169, 67831)	(-40730, 110730)	4.59	0.044	2

Full Flow ASHRAE 52.2-Compliant Rig Tests

52.2 Compliant Rig

An ASHRAE Standard 52.2-1999 (Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size) compliant rig in the Indoor Environment Center (IEC) laboratory at The Pennsylvania State University (PSU) is used to evaluate the full-flow capture efficiencies of the Energy Wall cores. *Bacillus subtilis* spore suspension at a concentration of approximately 1×10^5 spore/ml is the biological challenge used for the rig tests. Bacteria spores are generally smaller and have aerodynamic properties that tend to enhance air entrainment relative to vegetative forms of bacteria. Thus the spore microbe species is utilized for the filtration type tests as a type of worst case challenge organism. The vegetative forms of microbe would likely display greater cross section capture characteristics. Three separate runs are conducted for each of three prototype 12 in. x 12 in. Energy Wall cores (with treated core material) mounted in a special insert section fabricated for the rig as shown in Figure 18 to establish penetration of the challenge agent with the Energy Wall in place. To allow correction of the measured penetration with the Energy Wall installed and calculation of an inactivation efficiency that can be attributed to the Energy Wall, a number of tests are also conducted without the Energy Wall core inserted in the rig to characterize the penetration of the challenge agent without the Energy Wall. Moreover, runs with the Energy Wall core and nebulized, unchallenged reagent grade water are run as an extra level of control in the study. Nebulization, sampling, and sensor data acquisition is controlled and synchronized using LabVIEW 8.6 software and a customized National Instruments compact fieldpoint programmable automation controller hardware platform. Aforementioned microbiology equipment used to prepare

solutions, plate/incubate/enumerate cultures, sterilize equipment items and dispose of biological wastes generated are referred to without further description in this section.

BGI Six-Jet Collison Nebulizer

Collison MRE nebulizers are used to aerosolize particulates, including microorganisms, from liquid suspensions. One of the common nebulizers is the six-jet Collison MRE nebulizer shown in Figure 20. In the six-jet nebulizer, an aqueous suspension of particles (microorganisms included) is placed into a glass jar with the nozzle immersed no more than ⅜ in (1 cm) below the liquid level [BGI, 2002]. Compressed air is then supplied to the nebulizer, with various air pressures resulting in different aerosol concentration outputs. The clean air supplied to a nebulizer expands in 6 jets causing a drop in pressure which brings about a flow of liquid suspension into the air jets [Mercer *et al.*, 1968; BGI, 2002]. The liquid droplets suspended in the air jets represent a wide distribution of droplet sizes. These droplets are forcefully impacted against the glass wall of the nebulizer jar, which reduces droplet size. While a large fraction of the droplets in the jar after impaction are still too large, the smallest droplets are carried out of the jar by the flowing air stream. These liquid droplets evaporate rapidly (generally in much less than a second) when the aerosol stream in mixed with unsaturated air, leaving only the droplet nuclei (e.g., bacteria, fungal spore, latex sphere, etc.) for use in laboratory testing.

It has previously been shown that bioaerosols generated from Collison nebulizers typically have 99% of their mass resulting from particles less than 10 micrometers. Additionally, it has been shown that the bioaerosol size distribution is insensitive to the air pressure fed to the nebulizer and the viscosity of the liquid used to suspend the aerosol particles [May, 1973].

Thermo Two-Stage Viable Sampler

The Thermo Electron (Thermo Electron Corporation, Franklin, MA) Series 10-800 Two-Stage Viable Sampler is one of several multiple-jet impactor samplers used to collect and size-separate viable biological aerosols from the air. Single stage, six-stage, and eight-stage versions are also available from various manufactures that provide varying levels of separation of bioaerosols into distinct particle size ranges.

Air is pulled into the two-stage sampler through the inlet cone at a flow rate of 1 ACFM (28.3 L/min) using a vacuum pump (Figures 21 and 22). The sampler has a critical orifice built into the base plate that results in 1 ACFM (28.3 L/s) of air flow with any pump capable of generating ≥14 in. Hg (190 in. H_2O) of vacuum or greater [Thermo, 2003]. This eliminates the need for a separate flow control valve and flow meter in addition to the pump. The sampler separates particles into two size ranges using separate sampling stages for each. Each stage contains 200 tapered sampling holes through which the aerosol passes to deposit on nutrient agar plates installed under each stage. The hole sizes are different for each stage, so the air velocity through the holes are different resulting in aerosol separation by particle size. Stage 0 (top) uses 1.5 mm

holes and has a 50% effective cutoff diameter (d_{50}) of 8.0 μm for spherical particles with a density of 1.0 or other particles with the same aerodynamic diameter. Particles smaller than 8.0 μm remain in the air stream and pass around the Stage 0 agar plate to be collected in Stage 1, which incorporates 0.4 mm holes. It can be interpreted that the particles collected on Stage 0 are non-respirable and would deposit in the tracheobronchial region of the human respiratory system. Similarly, those particles collected by Stage 1 are respirable and would deposit in the deep alveolar region. The sampler collects 95-100% of the bioaerosol in the air above 0.8 μm [Thermo, 2003].

Once the samples are collected, the agar plates are incubated for colony enumeration. The resulting colonies can be counted and reported directly, or the observed number of colonies can be adjusted for the probability that more than one viable particle was collected through a sampling hole. A positive hole correction table was used with actual plate counts reported later in the results section to determine the average viable particle counts and the standard deviation determined from probability theory [Macher, 1989].

a.

b.

c.

Figure 18: a. 52.2-compliant rig insert section used to house the Energy Wall core under test; b. Energy Wall fitted in the middle of the insert section using GE Silicone II XST sealant along the core perimeter to eliminate bypass around the perimeter of the core; c. inside view of insert without Energy Wall core installed.

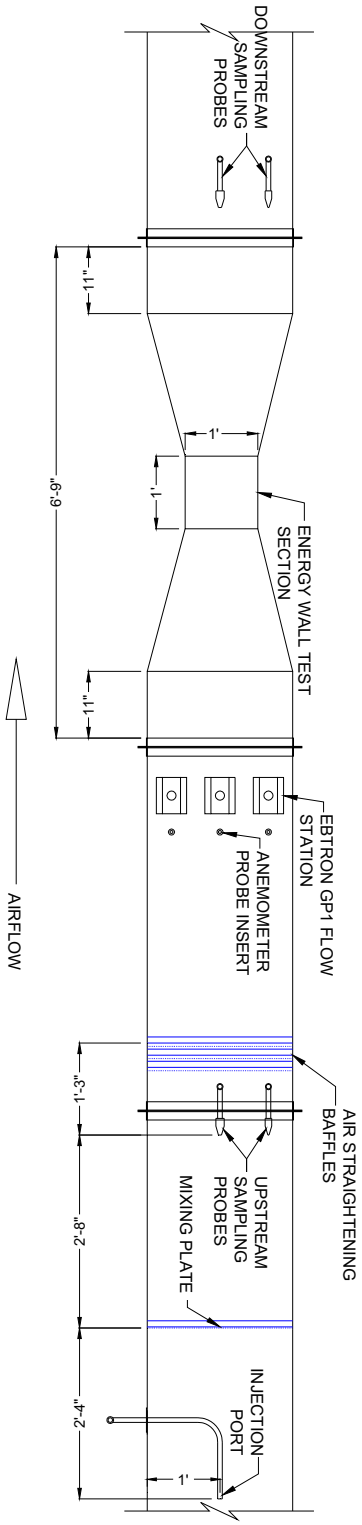


Figure 19: Schematic of the sectional layout for the portion of the rig housing the Energy Wall insert section and sampling equipment upstream and downstream of the insert section.

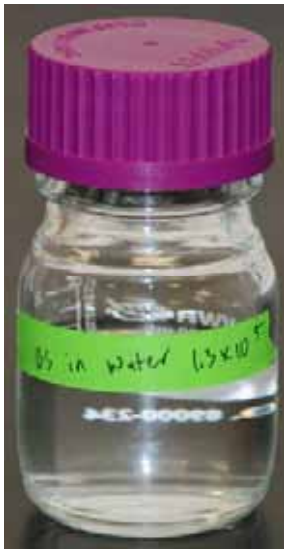
Procedure:

Step 1: Install the Energy Wall in the insert section as described in Figure 19 b.

Step 2: Fill the six-jet Collison nebulizer jar shown in Figure 20 with 40 ml of aqueous B*acillus subtilis* spore suspension at a concentration of approximately 1×10^5 spore/ml.

a.

b.



c.

Figure 20: Collison nebulizer (Note: ensure that tip of the nozzle is immersed no more than 3/8 in. below the surface of the solution being nebulized); b. Nebulizer connected to a TSI 3074B filtered air supply regulated to 16 psi (for removing oil or other liquid droplets, moisture and fine particles >0.1 µm from incoming air) and a relay-controlled solenoid valve to control introduction of aerosolized bacillus subtilis spores into the injection section of the rig; c. prepared solution for use with nebulizer.

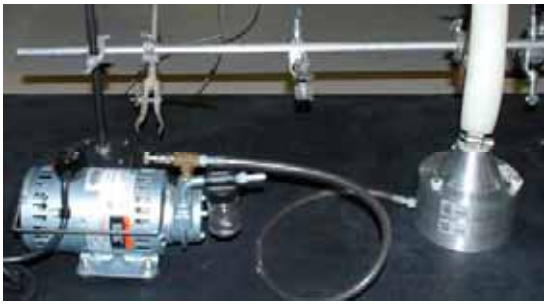
Step 3: Establish a rig air flow rate of 125 fpm as read by the downstream Ebtron flow station with the Energy Wall installed in the rig.

Step 4: Place fresh TSA agar plates in the upstream and downstream Anderson two-stage viable samplers shown in Figures 21 and 22.

Figure 21: Two stage Anderson sampler assembly (pump and sampling lines not shown).

Step 5: Power the nebulizer on with an air pressure of 16 psi. Allow the nebulizer to run for 2 minutes to stabilize.

Step 6: Simultaneously turn on the upstream and downstream sampler pumps operating at a flow rate of 28.3 L/min (1 ACFM). (Note: a BIOS Defender 520-H primary flow standard is used to check the flow rate of the pumps at frequent intervals). Allow both pumps to run for five minutes.



a.

b.

Figure 22: a. Downstream sampling pump assemblage; b. upstream sampling pump assembly.

Step 7: Turn both sampling pumps and the nebulizer off.

Step 8: Remove the TSA plates from the viable samplers as shown in Figure 23, replace the lids, and properly label the plates for incubation. Place the plates in the incubator as shown in Figure 24 at 35°C for 18-24 hours before colony enumeration.

Figure 23: A typical plate immediately after removal from the viable samplers. The indents are caused by the air pressure forcing the microbes into the holes of the sampler stages.

Figure 24: Plates removed from viable samplers and placed in incubator.

Step 9: Clean the viable sampler stages with 91% isopropanol and allow to dry. Install fresh TSA agar plates as shown in Figure 25 in both samplers.

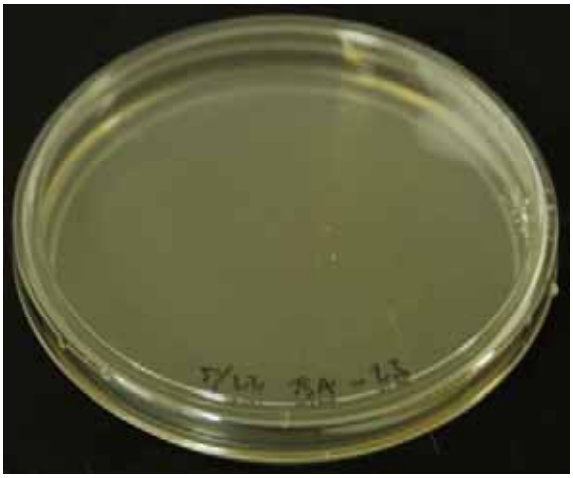


Figure 25: A fresh TSA agar plate ready for insertion in the viable samplers.

Step 10: Repeat steps 5-9 as needed to generate sufficient data for an appropriate assessment of repeatability and for subsequent analysis of a corrected capture efficiency accounting for any efficiency that can be attributed to the rig itself.

Step 11: Discard the spore suspension in the nebulizer, rinse the nebulizer jar thoroughly with reagent grade water, and refill the jar with 40 ml of reagent grade water for a control test as shown in Figure 26.

a.

b.

Figure 26: a. Reagent grade water used in these tests; b. water generated in the Department of Civil and Environmental Engineering in the environmental lab in Room 9-C Sackett Building.

Step 12: Power the nebulizer on with an air pressure of 16 psi. Allow the nebulizer to run for 2 minutes to stabilize.

Step 13: Simultaneously turn on the upstream and downstream sampler pumps operating at a flow rate of 28.3 L/min (1 ACFM). Allow both pumps to run for five minutes.

Step 14: Turn off both sampling pumps and the nebulizer.

Step 15: Remove the TSA plates from the viable samplers, replace the lids, and properly label the plates as "Control with Energy Wall" for incubation. Place the plates in the incubator at 35°C for 18-24 hours before colony enumeration.

Step 16: Turn off the blower and remove the Energy Wall from the rig and then reinsert the empty section back in the rig.

Step 17: Discard the spore suspension in the nebulizer, rinse the nebulizer jar thoroughly with reagent grade water, and refill the jar with 40 ml of aqueous *Bacillus subtilis* spore suspension at a concentration of approximately 1×10^5 spore/ml. Use the same stock suspension as used in Step 2 to minimize differences in spore concentration.

Step 18: Establish a rig flow of 31 ft/min as read by the downstream Ebtron flow station without the Energy Wall installed in the rig. Note: this deviation in flow rate from the 125 fpm used when the Energy Wall is installed in the rig is necessary because the rig cross-sectional area is 2 ft x 2 ft, and the installed Energy Wall core has a cross-section of 1 ft x 1 ft.

Step 19: Place fresh TSA agar plates in the upstream and downstream two-stage viable samplers.

Step 20: Power the nebulizer on with an air pressure of 16 psi. Allow the nebulizer to run for 2 minutes to stabilize.

Step 21: Simultaneously turn on the upstream and downstream sampler pumps operating at a flow rate of 28.3 L/min (1 ACFM). Allow both pumps to run for five minutes.

Step 22: Turn both sampling pumps and the nebulizer off.

Step 23: Remove the TSA plates from the viable samplers, replace the lids, and properly label the plates for incubation. Place the plates in the incubator at 35°C for 18-24 hours before colony enumeration.

Step 24: Clean the viable sampler stages with 91% isopropanol and allow to dry. Install fresh TSA agar plates in both samplers.

Step 25: Repeat steps 19-24 as needed to generate sufficient data for an appropriate assessment of repeatability and for subsequent analysis of a corrected capture efficiency accounting for any efficiency that can be attributed to the rig itself.

Step 26: Discard the spore suspension in the nebulizer, rinse the nebulizer jar thoroughly with reagent grade water, and refill the jar with 40 ml of reagent grade water for a control test.

Step 27: Power the nebulizer on with an air pressure of 16 psi. Allow the nebulizer to run for 2 minutes to stabilize.

Step 28: Simultaneously turn on the upstream and downstream sampler pumps operating at a flow rate of 28.3 L/min (1 ACFM). Allow both pumps to run for five minutes.

Step 29: Turn both sampling pumps and the nebulizer off.

Step 30: Remove the TSA plates from the viable samplers, replace the lids, and properly label the plates as "Control without Energy Wall" for incubation. Place the plates in the incubator at 35°C for 18-24 hours before colony enumeration in the same manner as described above for the benchscale testing.

Step 31: Turn off blower, properly shut down the test rig, and thoroughly clean the nebulizer and both samplers.

Step 32: After 18-24 hours of incubation, count the colonies on all TSA plates. Correct plate counts using the 200-hole positive hole correction table.

Step 33: Calculate corrected plate count averages for the three plates at each of the four test conditions (i.e., upstream without the Energy Wall [C_{u0}], downstream without the Energy Wall [C_{d0}], upstream with the Energy Wall [C_u], and downstream with the Energy Wall [C_d]).

Step 34: Calculate the percent reduction in viable downstream counts for tests with and without the Energy Wall:

$$\%R_{without} = \left(1 - \frac{C_{d0}}{C_{u0}}\right) \times 100$$

$$\%R_{with} = \left(1 - \frac{C_d}{C_u}\right) \times 100$$

$\%R_{without}$ describes the reduction in downstream viable spore counts resulting from the test rig and sampling chain itself. $\%R_{with}$, which includes $\%R_{without}$, describes the reduction associated with the test rig, sampling chain, and Energy Wall.

Step 35: Calculate the percent reduction in viable downstream counts provided by the Energy Wall device alone:

$$\%R_{Energy\ Wall} = \%R_{with} - \%R_{without}$$

Bacillus subtilis Without Energy Wall Core Rig Test Results

Bacillus subtilis spore suspension at a concentration of approximately 1×10^5 spore/ml is the biological challenge used for the rig tests. A total of 24 tests were conducted without the Energy Wall core inserted in the rig to characterize the penetration of the challenge agent without the Energy Wall installed in the rig. The data from these tests, run at various points over the course of several weeks are presented in Tables 16-21. A systematic error appears to be present in the data set. Initial runs after a new fill of solution was added to the Collison nebulizer jar in four instances yielded higher downstream counts than upstream counts for a negative reduction in measured viable spores after enumeration. This is a result that is not expected during operation of the rig, and will require some additional testing to evaluate whether the first run on a new fill in the Collison nebulizer would benefit from additional nebulization time prior to starting the sampling pumps. An outlier analysis was performed on the data set, and three of the four spurious data points represented extreme outliers (i.e., on the order of 3*(Q3-Q1) from the median) as shown graphically in the box plot at Figure 27. Descriptive statistics for the data set prior to removal of the outliers and after removal of the outliers are shown in Table 22 and 23, respectively. A box plot for the data set after removal of the outliers is shown in Figure 28.

Three separate runs were conducted for each of three prototype 12 in. x 12 in. Energy Wall cores (with treated core material), and the data from these runs are presented in Tables 24-26. Descriptive statistics for the grouping of all nine data points collected across the three prototypes are presented in Table 27. As shown in box plot form in Figure 29, no outliers are present.

Using the equations at Step 34 and 35 in the procedure above, the revised data set for tests without the Energy Wall, and the unadjusted data set for tests with the Energy Wall, the percent reduction in viable downstream counts for tests with and without the Energy Wall yields, $\%R_{with} = 82.1$ and $\%R_{without} = 47.0$. Thus, the percent reduction in viable downstream counts provided by the Energy Wall device alone is 82.1 – 47.0 = 35.1%. Within the context of the benchtop tests presented herein for *Bacillus subtilis* spores, which showed no significant biocidal effects of the Energy Wall material, this result suggests that the Energy Wall has significant capture capability for microorganisms. In fact, the uncorrected average of 82.1% reduction with the energy wall in place, suggests that the energy wall operates comparably to a MERV 12 filter.

Table 16: Testing without the Energy Wall installed in the rig; testing conditions: flow rate ~ 125ft/min, temperature 78°F, relative humidity 45 - 53%, sampling time 5 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
1	06/17/2009	Upstream	0	5/26 TSA-59	06/18/2009	9	9.2	121.6	-4.44
	06/17/2009	Upstream	1	5/26 TSA-28	06/18/2009	86	112.4		
	06/17/2009	Downstream	0	5/26 TSA-48	06/18/2009	0	0.0	127.0	
	06/17/2009	Downstream	1	5/26 TSA-47	06/18/2009	94	127.0		
2	06/17/2009	Upstream	0	5/26 TSA-65	06/18/2009	2	2.0	161.7	19.60
	06/17/2009	Upstream	1	5/26 TSA-25	06/18/2009	110	159.7		
	06/17/2009	Downstream	0	5/26 TSA-36	06/18/2009	3	3.0	130.0	
	06/17/2009	Downstream	1	5/26 TSA-34	06/18/2009	94	127.0		
3	06/17/2009	Upstream	0	5/26 TSA-54	06/18/2009	20	21.1	199.4	41.22
	06/17/2009	Upstream	1	5/26 TSA-49	06/18/2009	118	178.3		
	06/17/2009	Downstream	0	5/26 TSA-63	06/18/2009	13	13.4	117.2	
	06/17/2009	Downstream	1	5/26 TSA-64	06/18/2009	81	103.8		
AVERAGE	06/17/2009	Upstream	0	N/A	06/18/2009	N/A	10.8	160.9	22.48
	06/17/2009	Upstream	1	N/A	06/18/2009	N/A	150.1		
	06/17/2009	Downstream	0	N/A	06/18/2009	N/A	5.5	124.7	
	06/17/2009	Downstream	1	N/A	06/18/2009	N/A	119.3		

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
Control (Water)	06/17/2009	Upstream	0	5/26 TSA-62	06/18/2009	0	0.0	0.0	N/A
	06/17/2009	Upstream	1	5/26 TSA-61	06/18/2009	0	0.0		
	06/17/2009	Downstream	0	5/26 TSA-33	06/18/2009	0	0.0	3.0	
	06/17/2009	Downstream	1	5/26 TSA-58	06/18/2009	3	3.0		

[1] actual counts on plate will be 200 or less

Table 17: Testing without the Energy Wall installed in the rig; testing conditions: flow rate ~ 125ft/min, temperature 77°F, relative humidity 58 - 61%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
1	06/18/2009	Upstream	0	N/A[2]	N/A[2]	N/A[2]	6.6	169.2	28.46
	06/18/2009	Upstream	1	N/A[2]	N/A[2]	N/A[2]	162.6		
	06/18/2009	Downstream	0	N/A[2]	N/A[2]	N/A[2]	3.1	121.1	
	06/18/2009	Downstream	1	N/A[2]	N/A[2]	N/A[2]	118.0		
2	06/18/2009	Upstream	0	N/A[2]	N/A[2]	N/A[2]	22.1	260.5	52.45
	06/18/2009	Upstream	1	N/A[2]	N/A[2]	N/A[2]	238.4		
	06/18/2009	Downstream	0	N/A[2]	N/A[2]	N/A[2]	5.1	123.9	
	06/18/2009	Downstream	1	N/A[2]	N/A[2]	N/A[2]	118.8		
3	06/18/2009	Upstream	0	N/A[2]	N/A[2]	N/A[2]	34.8	254.8	61.17
	06/18/2009	Upstream	1	N/A[2]	N/A[2]	N/A[2]	220.1		
	06/18/2009	Downstream	0	N/A[2]	N/A[2]	N/A[2]	9.3	99.0	
	06/18/2009	Downstream	1	N/A[2]	N/A[2]	N/A[2]	89.7		
AVERAGE	06/18/2009	Upstream	0	N/A[2]	N/A[2]	N/A[2]	21.2	228.2	49.76
	06/18/2009	Upstream	1	N/A[2]	N/A[2]	N/A[2]	207.0		
	06/18/2009	Downstream	0	N/A[2]	N/A[2]	N/A[2]	5.8	114.6	
	06/18/2009	Downstream	1	N/A[2]	N/A[2]	N/A[2]	108.8		

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
Control (Water)	06/18/2009	Upstream	0	6/17 TSA-30	06/19/2009	0	0.0	1.0	N/A
	06/18/2009	Upstream	1	6/17 TSA-31	06/19/2009	1	1.0		
	06/18/2009	Downstream	0	6/17 TSA-32	06/19/2009	0	0.0	2.0	
	06/18/2009	Downstream	1	6/17 TSA-33	06/19/2009	2	2.0		

[1] actual counts on plate will be 200 or less

[2] there was a control issue with the nebulizer during these tests that went unnoticed. Reported count values represent the average of the values from Test #1 and Test #3

Table 18: Testing without the Energy Wall installed in the rig; testing conditions: flow rate ~ 125ft/min, temperature 81°F, relative humidity 45 - 47%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Count[1]	Corrected Counts	Total Counts	% Reduction
1	06/29/2009	Upstream	0	6/17 TSA-54	06/30/2009	4	4.0	216.8	46.91
	06/29/2009	Upstream	1	6/17 TSA-55	06/30/2009	131	212.8		
	06/29/2009	Downstream	0	6/17 TSA-56	06/30/2009	6	6.1	115.1	
	06/29/2009	Downstream	1	6/17 TSA-57	06/30/2009	84	109.0		
2	06/29/2009	Upstream	0	6/17 TSA-58	06/30/2009	38	42.2	359.2	67.23
	06/29/2009	Upstream	1	6/17 TSA-59	06/30/2009	159	317.0		
	06/29/2009	Downstream	0	6/17 TSA-60	06/30/2009	7	7.1	117.7	
	06/29/2009	Downstream	1	6/17 TSA-61	06/30/2009	85	110.6		
3	06/29/2009	Upstream	0	6/17 TSA-62	06/30/2009	43	48.4	310.2	73.98
	06/29/2009	Upstream	1	6/17 TSA-63	06/30/2009	146	261.8		
	06/29/2009	Downstream	0	6/17 TSA-64	06/30/2009	5	5.1	80.7	
	06/29/2009	Downstream	1	6/17 TSA-65	06/30/2009	63	75.6		
AVERAGE	06/29/2009	Upstream	0	N/A	06/30/2009	N/A	31.5	295.4	64.62
	06/29/2009	Upstream	1	N/A	06/30/2009	N/A	263.9		
	06/29/2009	Downstream	0	N/A	06/30/2009	N/A	6.1	104.5	
	06/29/2009	Downstream	1	N/A	06/30/2009	N/A	98.4		

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
Control (Water)	06/29/2009	Upstream	0	6/17 TSA-66	06/30/2009	0	0.0	9.2	N/A
	06/29/2009	Upstream	1	6/17 TSA-67	06/30/2009	9	9.2		
	06/29/2009	Downstream	0	6/17 TSA-68	06/30/2009	1	1.0	6.1	
	06/29/2009	Downstream	1	6/17 TSA-69	06/30/2009	5	5.1		

[1] actual counts on plate will be 200 or less

Table 19: Testing without the Energy Wall installed in the rig; testing conditions: flow rate ~ 125ft/min, temperature 82°F, relative humidity 47%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
1[3]	*07/10/2009*	*Upstream*	*0*	*N/A*[2]	*07/13/2009*	*1*	*1.0*	*16.6*	*-539.76*
	07/10/2009	*Upstream*	*1*	*N/A*[2]	*07/13/2009*	*15*	*15.6*		
	07/10/2009	*Downstream*	*0*	*N/A*[2]	*07/13/2009*	*4*	*4.0*	*106.2*	
	07/10/2009	*Downstream*	*1*	*N/A*[2]	*07/13/2009*	*80*	*102.2*		
2	07/10/2009	Upstream	0	N/A[2]	07/13/2009	6	6.1	106.6	55.72
	07/10/2009	Upstream	1	N/A[2]	07/13/2009	79	100.5		
	07/10/2009	Downstream	0	N/A[2]	07/13/2009	0	0.0	47.2	
	07/10/2009	Downstream	1	N/A[2]	07/13/2009	42	47.2		
3	07/10/2009	Upstream	0	N/A[2]	07/13/2009	2	2.0	26.4	21.21
	07/10/2009	Upstream	1	N/A[2]	07/13/2009	23	24.4		
	07/10/2009	Downstream	0	N/A[2]	07/13/2009	2	2.0	20.8	
	07/10/2009	Downstream	1	N/A[2]	07/13/2009	18	18.8		
4	07/10/2009	Upstream	0	N/A[2]	07/13/2009	2	2.0	36.9	15.45
	07/10/2009	Upstream	1	N/A[2]	07/13/2009	32	34.9		
	07/10/2009	Downstream	0	N/A[2]	07/13/2009	1	1.0	31.2	
	07/10/2009	Downstream	1	N/A[2]	07/13/2009	28	30.2		
5	07/10/2009	Upstream	0	N/A[2]	07/13/2009	1	1.0	18.8	11.70
	07/10/2009	Upstream	1	N/A[2]	07/13/2009	17	17.8		
	07/10/2009	Downstream	0	N/A[2]	07/13/2009	1	1.0	16.6	
	07/10/2009	Downstream	1	N/A[2]	07/13/2009	15	15.6		
AVERAGE	07/14/2009	Upstream	0	N/A[2]	07/15/2009	N/A	2.4	41.1	-8.13
	07/14/2009	Upstream	1	N/A[2]	07/15/2009	N/A	38.6		
	07/14/2009	Downstream	0	N/A[2]	07/15/2009	N/A	1.6	44.4	
	07/14/2009	Downstream	1	N/A[2]	07/15/2009	N/A	42.8		

[1] had contamination

[2] plates were only labeled for sampler and sampler stage

[3] italicized data indicates outlier data

Table 20: Testing without the Energy Wall installed in the rig; testing conditions: flow rate ~ 125ft/min, temperature 80°F, relative humidity 64 - 68%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts*	Corrected Counts	Total Counts	% Reduction
1[2]	*07/14/2009*	*Upstream*	*0*	*07/09 TSA-21*	*07/15/2009*	*0*	*0.0*	*23.3*	*-147.21*
	07/14/2009	*Upstream*	*1*	*07/09 TSA-22*	*07/15/2009*	*22*	*23.3*		
	07/14/2009	*Downstream*	*0*	*07/09 TSA-23*	*07/15/2009*	*0*	*0.0*	*57.6*	
	07/14/2009	*Downstream*	*1*	*07/09 TSA-24*	*07/15/2009*	*50*	*57.6*		
2	07/14/2009	Upstream	0	07/09 TSA-25	07/15/2009	2	2.0	24.2	74.79
	07/14/2009	Upstream	1	07/09 TSA-26	07/15/2009	21	22.2		
	07/14/2009	Downstream	0	07/09 TSA-27	07/15/2009	0	0.0	6.1	
	07/14/2009	Downstream	1	07/09 TSA-28	07/15/2009	6	6.1		
3	07/14/2009	Upstream	0	07/09 TSA-29	07/15/2009	0	0.0	7.1	43.66
	07/14/2009	Upstream	1	07/09 TSA-30	07/15/2009	7	7.1		
	07/14/2009	Downstream	0	07/09 TSA-31	07/15/2009	1	1.0	4.0	
	07/14/2009	Downstream	1	07/09 TSA-32	07/15/2009	3	3.0		
4	07/14/2009	Upstream	0	07/09 TSA-33	07/15/2009	0	0.0	5.1	80.39
	07/14/2009	Upstream	1	07/09 TSA-34	07/15/2009	5	5.1		
	07/14/2009	Downstream	0	07/09 TSA-35	07/15/2009	0	0.0	1.0	
	07/14/2009	Downstream	1	07/09 TSA-36	07/15/2009	1	1.0		
5	07/14/2009	Upstream	0	07/09 TSA-37	07/15/2009	0	0.0	4.0	100.00
	07/14/2009	Upstream	1	07/09 TSA-38	07/15/2009	4	4.0		
	07/14/2009	Downstream	0	07/09 TSA-39	07/15/2009	0	0.0	0.0	
	07/14/2009	Downstream	1	07/09 TSA-40	07/15/2009	0	0.0		
AVERAGE	07/14/2009	Upstream	0	N/A	07/15/2009	N/A	0.4	12.7	-7.85
	07/14/2009	Upstream	1	N/A	07/15/2009	N/A	12.3		
	07/14/2009	Downstream	0	N/A	07/15/2009	N/A	0.2	13.7	
	07/14/2009	Downstream	1	N/A	07/15/2009	N/A	13.5		

[1] actual counts on plate will be 200 or less

[2] talicized data indicates outlier data

Table 21: Testing without the Energy Wall installed in the rig; testing conditions: flow rate ~ 125ft/min, temperature 80°F, relative humidity 64 - 68%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
1[2]	*07/14/2009*	*Upstream*	*0*	*07/09 TSA-41*	*07/15/2009*	*5*	*5.1*	*35.3*	*-110.76*
	07/14/2009	*Upstream*	*1*	*07/09 TSA-42*	*07/15/2009*	*28*	*30.2*		
	07/14/2009	*Downstream*	*0*	*07/09 TSA-44*	*07/15/2009*	*0*	*0.0*	*74.4*	
	07/14/2009	*Downstream*	*1*	*07/09 TSA-45*	*07/15/2009*	*62*	*74.4*		
2	07/14/2009	Upstream	0	07/09 TSA-46	07/15/2009	5	5.1	62.7	38.12
	07/14/2009	Upstream	1	07/09 TSA-47	07/15/2009	50	57.6		
	07/14/2009	Downstream	0	07/09 TSA-48	07/15/2009	5	5.1	38.8	
	07/14/2009	Downstream	1	07/09 TSA-49	07/15/2009	31	33.7		
3	07/14/2009	Upstream	0	07/09 TSA-50	07/15/2009	0	0.0	44.6	50.22
	07/14/2009	Upstream	1	07/09 TSA-51	07/15/2009	40	44.6		
	07/14/2009	Downstream	0	07/09 TSA-52	07/15/2009	0	0.0	22.2	
	07/14/2009	Downstream	1	07/09 TSA-53	07/15/2009	21	22.2		
4	07/14/2009	Upstream	0	07/09 TSA-54	07/15/2009	4	4.0	44.9	70.16
	07/14/2009	Upstream	1	07/09 TSA-55	07/15/2009	37	40.9		
	07/14/2009	Downstream	0	07/09 TSA-56	07/15/2009	0	0.0	13.4	
	07/14/2009	Downstream	1	07/09 TSA-57	07/15/2009	13	13.4		
5	07/14/2009	Upstream	0	07/09 TSA-58	07/15/2009	0	0.0	18.8	39.89
	07/14/2009	Upstream	1	07/09 TSA-59	07/15/2009	18	18.8		
	07/14/2009	Downstream	0	07/09 TSA-60	07/15/2009	0	0.0	11.3	
	07/14/2009	Downstream	1	07/09 TSA-61	07/15/2009	11	11.3		
AVERAGE	07/14/2009	Upstream	0	N/A	07/15/2009	N/A	2.8	41.3	22.39
	07/14/2009	Upstream	1	N/A	07/15/2009	N/A	38.4		
	07/14/2009	Downstream	0	N/A	07/15/2009	N/A	1.0	32.0	
	07/14/2009	Downstream	1	N/A	07/15/2009	N/A	31.0		

[1] actual counts on plate will be 200 or less

[2] talicized data indicates outlier data

Table 22: Descriptive statistics without Energy Wall installed in rig including outlier data

Variable	Total Count	Mean	StDev	Variance	CoefVar	Q1	Median	Q3
% Reduction W/O_With Out	24	7.9	129.2	16685.0	1630.14	16.5	42.4	65.7

Table 23: Descriptive statistics without Energy Wall installed in rig after removal of outlier data

Variable	Total Count	Mean	StDev	Variance	CoefVar	Q1	Median	Q3
% Reduction W/O	21	47.04	25.99	675.40	55.24	24.83	46.91	68.69

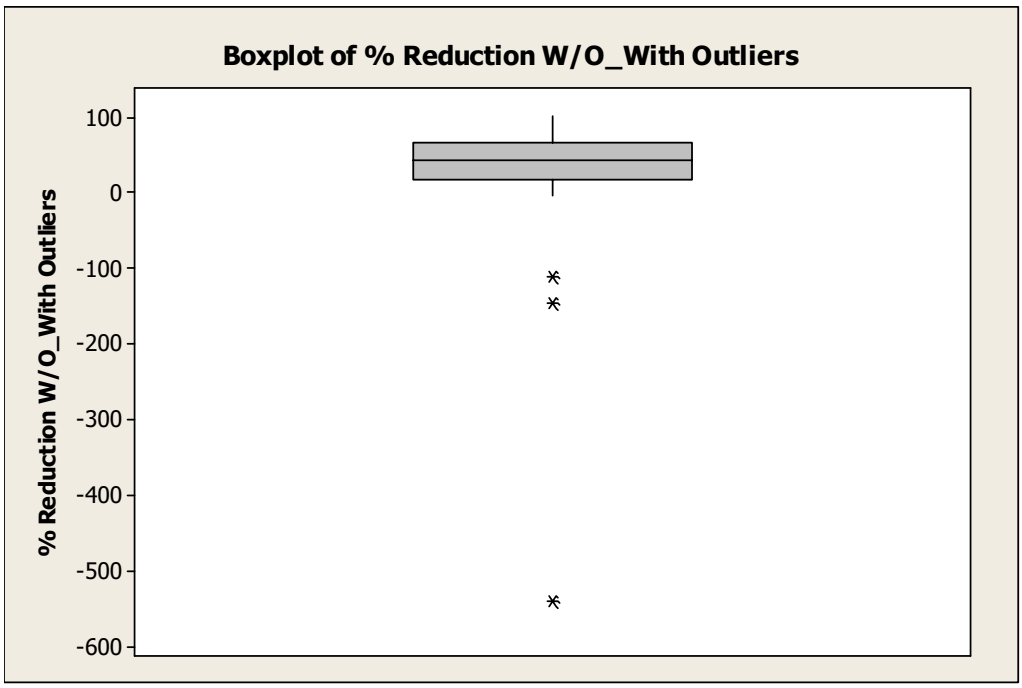


Figure 27: Box Plot of % Reduction without Energy Wall installed in rig including outlier data

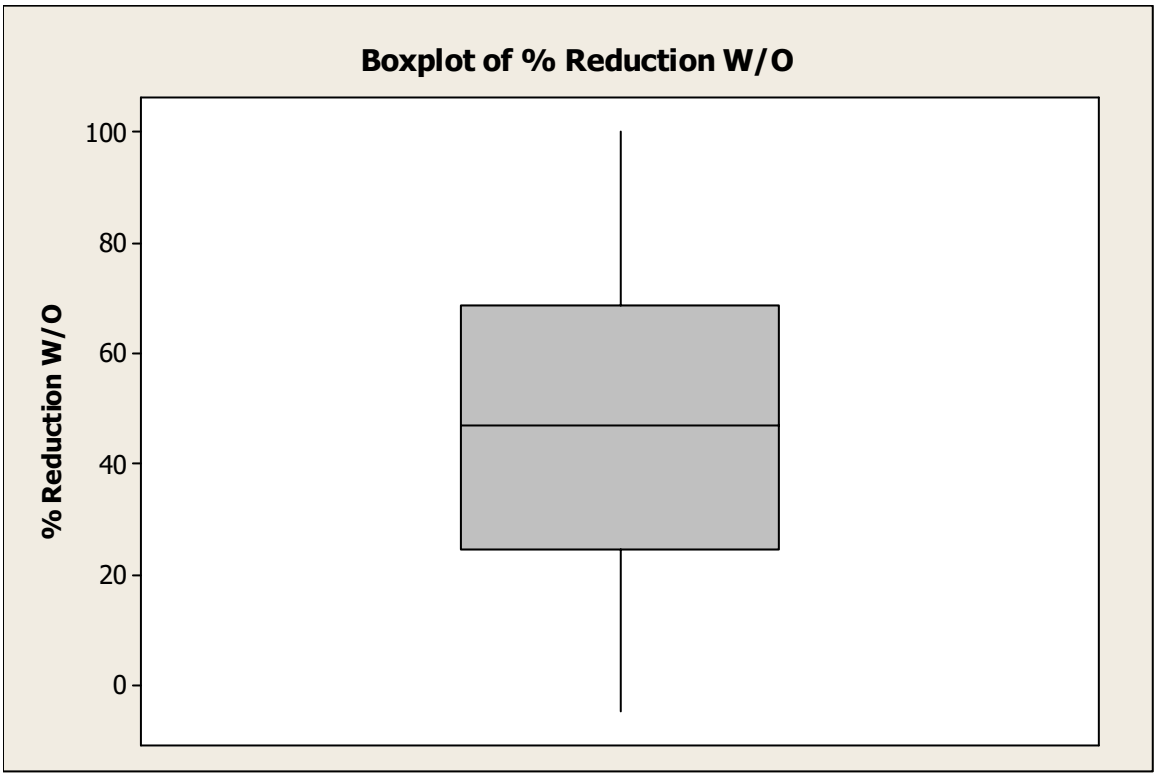


Figure 28: Box Plot of % Reduction without Energy Wall installed in rig with outlier data removed

Bacillus subtilis With Energy Wall Core Rig Test Results

Table 24: Testing with the Energy Wall installed in the rig; prototype 1 testing conditions: flow rate ~ 125ft/min, temperature 78F, relative humidity 45 - 53%, sampling time 5 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
1	06/17/2009	Upstream	0	5/22 TSA-10	06/18/2009	4	4.0	929.1	87.29
	06/17/2009	Upstream	1	5/22 TSA-50	06/18/2009	198	925.1		
	06/17/2009	Downstream	0	5/22 TSA-21	06/18/2009	9	9.2	118.1	
	06/17/2009	Downstream	1	5/22 TSA-22	06/18/2009	84	108.9		
2	06/17/2009	Upstream	0	5/22 TSA-49	06/18/2009	7	7.1	627.5	80.10
	06/17/2009	Upstream	1	5/22 TSA-15	06/18/2009	191	620.4		
	06/17/2009	Downstream	0	5/22 TSA-14	06/18/2009	7	7.1	124.9	
	06/17/2009	Downstream	1	5/22 TSA-18	06/18/2009	89	117.8		
3	06/17/2009	Upstream	0	5/22 TSA-48	06/18/2009	2	2.0	843.8	84.04
	06/17/2009	Upstream	1	5/22 TSA-16	06/18/2009	197	841.8		
	06/17/2009	Downstream	0	5/22 TSA-24	06/18/2009	0	0.0	134.7	
	06/17/2009	Downstream	1	5/22 TSA-25	06/18/2009	98	134.7		
AVERAGE	06/17/2009	Upstream	0	N/A	06/18/2009	N/A	4.4	800.1	84.27
	06/17/2009	Upstream	1	N/A	06/18/2009	N/A	795.8		
	06/17/2009	Downstream	0	N/A	06/18/2009	N/A	5.4	125.9	
	06/17/2009	Downstream	1	N/A	06/18/2009	N/A	120.5		

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
Control (Water)	06/17/2009	Upstream	0	5/26 TSA-57	06/18/2009	0	0.0	4.0	N/A
	06/17/2009	Upstream	1	5/26 TSA-37	06/18/2009	4	4.0		
	06/17/2009	Downstream	0	5/26 TSA-9	06/18/2009	0	0.0	1.0	
	06/17/2009	Downstream	1	5/26 TSA-8	06/18/2009	1	1.0		

[1] actual counts on plate will be 200 or less

Table 25: Testing with the Energy Wall installed in the rig; prototype 2 testing conditions: flow rate ~ 125ft/min, temperature 77°F, relative humidity 58 - 61%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts [1]	Corrected Counts	Total Counts	% Reduction
1	06/18/2009	Upstream	0	6/17 TSA-1	06/19/2009	3	3.0	844.8	76.47
	06/18/2009	Upstream	1	6/17 TSA-2	06/19/2009	197	841.8		
	06/18/2009	Downstream	0	6/17 TSA-3	06/19/2009	0	0.0	198.8	
	06/18/2009	Downstream	1	6/17 TSA-5	06/19/2009	126	198.8		
2	06/18/2009	Upstream	0	6/17 TSA-6	06/19/2009	1	1.0	581.2	78.48
	06/18/2009	Upstream	1	6/17 TSA-7	06/19/2009	189	580.2		
	06/18/2009	Downstream	0	6/17 TSA-8	06/19/2009	0	0.0	125.1	
	06/18/2009	Downstream	1	6/17 TSA-9	06/19/2009	93	125.1		
3	06/18/2009	Upstream	0	6/17 TSA-10	06/19/2009	2	2.0	345.0	78.90
	06/18/2009	Upstream	1	6/17 TSA-11	06/19/2009	164	343.0		
	06/18/2009	Downstream	0	6/17 TSA-12	06/19/2009	0	0.0	72.8	
	06/18/2009	Downstream	1	6/17 TSA-13	06/19/2009	61	72.8		
AVERAGE	06/18/2009	Upstream	0	N/A	06/19/2009	N/A	2.0	590.3	77.60
	06/18/2009	Upstream	1	N/A	06/19/2009	N/A	588.3		
	06/18/2009	Downstream	0	N/A	06/19/2009	N/A	0.0	132.2	
	06/18/2009	Downstream	1	N/A	06/19/2009	N/A	132.2		

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
Control (Water)	06/18/2009	Upstream	0	6/17 TSA-14	06/19/2009	0	0.0	2.0	N/A
	06/18/2009	Upstream	1	6/17 TSA-15	06/19/2009	2	2.0		
	06/18/2009	Downstream	0	6/17 TSA-16	06/19/2009	0	0.0	0.0	
	06/18/2009	Downstream	1	6/17 TSA-17	06/19/2009	0	0.0		

[1] actual counts on plate will be 200 or less

Table 26: Testing with the Energy Wall installed in the rig; prototype 3 testing conditions: flow rate ~ 125ft/min, temperature 81°F, relative humidity 45 - 47%, sampling time 3 min

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
1	06/29/2009	Upstream	0	6/17 TSA-38	06/30/2009	0	0.0	470.8	80.50
	06/29/2009	Upstream	1	6/17 TSA-39	06/30/2009	181	470.8		
	06/29/2009	Downstream	0	6/17 TSA-40	06/30/2009	1	1.0	91.8	
	06/29/2009	Downstream	1	6/17 TSA-41	06/30/2009	73	90.8		
2	06/29/2009	Upstream	0	6/17 TSA-42	06/30/2009	2	2.0	472.8	86.76
	06/29/2009	Upstream	1	6/17 TSA-43	06/30/2009	181	470.8		
	06/29/2009	Downstream	0	6/17 TSA-44	06/30/2009	1	1.0	62.6	
	06/29/2009	Downstream	1	6/17 TSA-45	06/30/2009	53	61.6		
3	06/29/2009	Upstream	0	6/17 TSA-46	06/30/2009	12	12.4	530.6	86.37
	06/29/2009	Upstream	1	6/17 TSA-47	06/30/2009	185	518.2		
	06/29/2009	Downstream	0	6/17 TSA-48	06/30/2009	1	1.0	72.3	
	06/29/2009	Downstream	1	6/17 TSA-49	06/30/2009	60	71.3		
AVERAGE	06/29/2009	Upstream	0	N/A	06/30/2009	N/A	4.8	491.4	84.62
	06/29/2009	Upstream	1	N/A	06/30/2009	N/A	486.6		
	06/29/2009	Downstream	0	N/A	06/30/2009	N/A	1.0	75.6	
	06/29/2009	Downstream	1	N/A	06/30/2009	N/A	74.6		

Test #	Date Sampled	Sampler	Sampler Stage	Plate #	Date Counted	Raw Counts[1]	Corrected Counts	Total Counts	% Reduction
Control (Water)	06/29/2009	Upstream	0	6/17 TSA-50	06/30/2009	2	2.0	8.1	N/A
	06/29/2009	Upstream	1	6/17 TSA-51	06/30/2009	6	6.1		
	06/29/2009	Downstream	0	6/17 TSA-52	06/30/2009	0	0.0	2.0	
	06/29/2009	Downstream	1	6/17 TSA-53	06/30/2009	2	2.0		

[1] actual counts on plate will be 200 or less

Table 27: Descriptive statistics and Anderson Darling normality test results with Energy Wall installed in rig

Variable	Total Count	Mean	StDev	Variance	CoefVar	Q1	Median	Q3	AD	Pvalue
% Reduction W/	9	82.10	4.07	16.54	4.95	78.69	80.50	86.57	0.421	0.250
% Reduction W/_P1	3	83.81	3.60	12.97	4.30	80.10	84.04	87.29	0.193	0.615
% Reduction W/_P2	3	77.947	1.299	1.686	1.67	76.468	78.476	78.899	0.332	0.211
% Reduction W/_P3	3	84.54	3.51	12.30	4.15	80.50	86.37	86.76	0.429	0.093

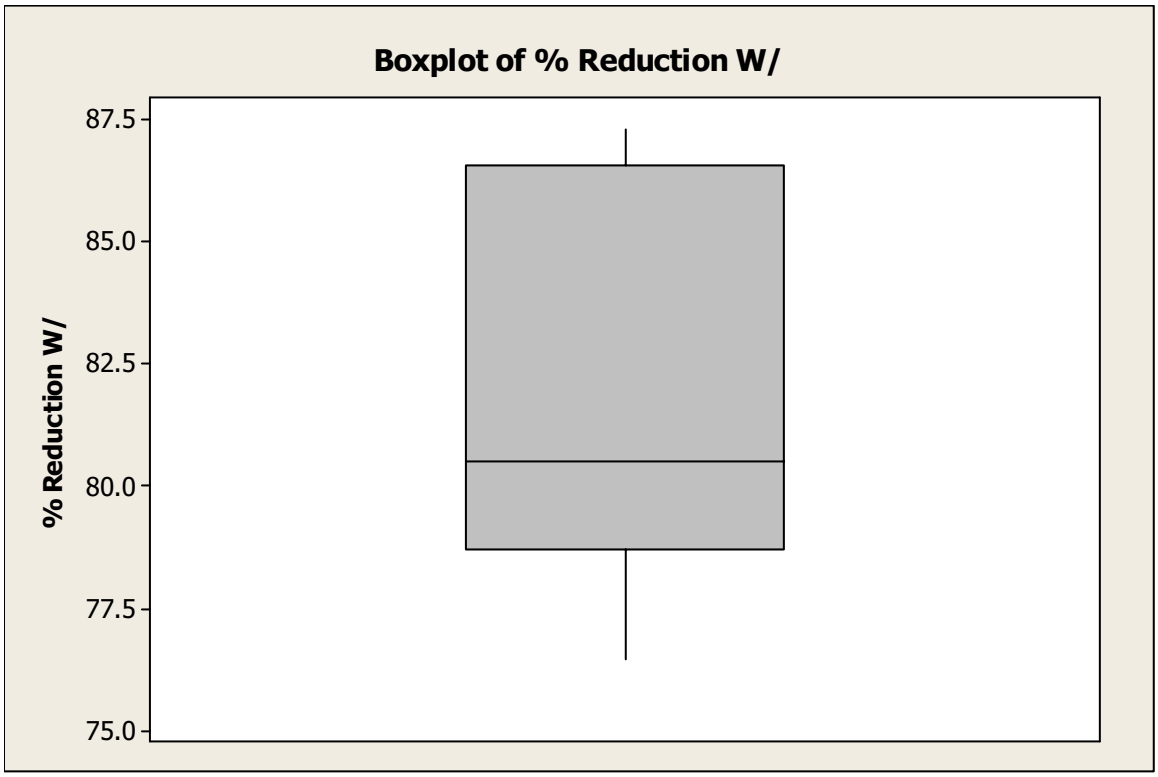


Figure 29: Box Plot of % Reduction with Energy Wall installed in rig

References

ASHRAE. 1999. ANSI/ASHRAE Standard 52.2-1999, *Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size*. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Atlanta, GA.

ASHRAE. 2007. ANSI/ASHRAE Standard 52.2-2007, *Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size*. American Society of Heating, Refrigerating and Air-Conditioning Engineers, Atlanta, GA.

BGI. 2002. *Collison Nebulizer - Instructions*. BGI Incorporated, Waltham, MA.

Eaton, A.D., L.S. Clesceri and A.E. Greenberg eds. 1995. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, D.C.

Macher, J.M. 1989. Positive-Hole Correction of Multiple-Jet Impactors for Collecting Viable Microorganisms. *American Industiral Hygiene Association Journal*, **50**(11):561-568.

May, K.R. 1973. The Collison Nebulizer: Description, Performance and Application. *Aerosol Science*, **4**:235-243.

Mercer, T.T., M.I. Tillery and H.V. Chow. 1968. Operating Characteristics of Some Compressed-Air Nebulizers. *American Industiral Hygiene Association Journal*, **29**(1):66-78.

Nedeljkovic-Davidovic, A. 2008. *Fluence Response Curves for Bacillus subtilis Spores Applied to UVC Lamps Testing*. Thesis for the degree of Master of Science, College of Engineering, Department of Architectural Engineering, Pennsylvania State University, University Park, PA.

RTI. 2006. *Test/QA Plan for Bioaerosol Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Air Cleaners: Revision 1*. Project 09367, Research Triangle Institute, Research Triangle Park, NC.

Thermo. 2003. *Series 10-800 Six and Two Stage Viable Samplers: Instruction Manual*. Thermo Electron Corporation, Franklin, MA.

Appendix A: Preparing Tryptic Soy Agar (TSA) Plates

Materials

Difco Tryptic Soy Agar Powder (Soybean-Casein Digest Agar) REF 236950 Lot 9020346
Reagent grade water (deionized water of 15 to 18 megaohm resistivity is obtained from a Culligan Aqua-Summa II reagent Grade Water System)
1 – 1000 ml graduated cylinder
2 - 1000 ml flasks
1 - 2000 ml flask
2 - foil squares
75 - petri dishes (VWR) 100mm diameter polystyrene plates
microspatula
disposable weighing dishes (Cole-Parmer weigh canoe with pour spout, medium, EW-01018-04)
Adam AAA 160L electronic scale (0.0001 g resolution)
Autoclave
Autoclave Tape (VWR Autoclave Indicator Tape, 36432-188)
1- Barnstead Thermolyne Cimarec magnetic stirrer/hot plate with 1 in. PTFE-coated stir bar

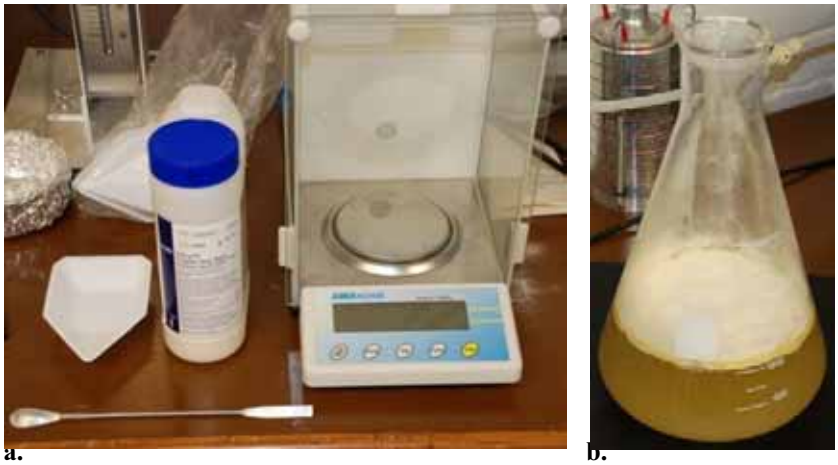
Procedure

Step1: Using a graduated cylinder, transfer 1000 ml of reagent grade water from the container in Figure A-1 into a 2000 ml flask.

Figure A-1: Reagent grade water container with blue stop valve in "off" position. Unscrew cap slightly and then turn the valve counterclockwise to dispense water.

Step2: Tare the electronic scale (Figure A-2) with the weighing dish on the scale pan prior to weighing agar powder. Use a microspatula to transfer 60 g of tryptic soy agar to the weighing dish on the electronic scale and then transfer the weighted powder to the 2000 ml flask with the

reagent grade water. Record the exact weight of tryptic soy agar used. Clean the microspatula and scale surfaces after weighing to avoid cross contamination during subsequent weighing operations. Use the sliding doors along the sides of the scale enclosure to minimize air currents while weighing.



a. b.

Figure A-2: a. Electronic scale and weighing equipment used to weigh agar powder; b. 2000 ml flask with the tryptic soy Agar.

Step 3: Using a graduated cylinder, transfer another 500 ml of reagent grade water into the 2000 ml flask containing the tryptic soy agar.

Step 4: Place a one-inch magnetic stir bar into the 2000 ml flask containing the tryptic soy agar and set it onto the magnetic stirrer/hot plate (Figure A-3). Turn the heat knob clockwise to its highest heat setting, and turn the stir setting to six. Ensure that the magnet in the flask is correctly spinning and mixing. Stir and heat the flask at these settings for approximately 15 minutes and remove the flask from the plate as the agar solution becomes clear in the flask. (Notes: if the stir setting is too high, then the stir bar will not spin correctly and just rattle in the flask. Regularly check to ensure that the solution is being stirred properly and that it does not boil for too long or it will overflow. The solution is clear enough when the magnetic stir bar is visible through the flask.).

Figure A-3: Heating and stirring the agar solution on the magnetic stirrer/hot plate.

Step 5: Once the solution has been mixed and boiled, divide the solution equally into two 1000 ml flasks. Each flask should contain about 750 ml of TSA solution. Cover each 1000 ml flask with foil and place autoclave tape onto the top of the flasks as shown in Figure A-4.

Figure A-4: Prepared solutions prior to autoclaving.

Step 6: Place the two 1000 ml flasks onto a tray in the autoclave and shut the door for the autoclave to begin its cycle (Figure A-5). Note: the rack for the autoclave is placed below the last slot available to fit the 1000 ml flasks. The rack may slide slightly to the left or right but that is normal.

Figure A-5: Autoclave the prepared solutions in the 1000 ml flasks.

Step 7: Shut the autoclave door and press the flask button on the top of the display as noted in Figure A-6. Then press the start button to start the cycle. The cycle will take about an hour to complete. The autoclave works by using steam and pressure to sterilize the solutions and their containers. The flask setting will heat the autoclave contents to 121 ºC. The autoclave will beep once its cycle is complete.

Figure A-6: Autoclave front panel controls.

Step 8: Once the autoclave cycle is complete, remove the flasks from the autoclave and place onto a workbench to cool. The solution will be ready to use once the flasks are cool enough to be handled with bare hands. Do not allow the solutions to cool to ambient temperature because they will solidify making it very difficult to prepare plates from them. A good strategy to avoid overcooling is to pull out one flask to cool down. Once that flask is ready to be plated pull out the other flask. This will prevent the flasks from cooling down too much.

Appendix B: Procedure for Preparing Dichloran Glycerol (DG) Plates

Materials

To prepare DG Plates the materials) listed in Appendix A are needed in addition to those listed below (except the TSA powder and the additional petri dishes noted below)

EMD Chemicals Dichloran Glycerol Agar (DG 18 Agar) powder cat. no. 1.00465.0500, Lot VM962565
EMD Chemicals Glyceraol cat. no.GX0185-5, Lot 49044920
80- petri dishes
Fisher Thermix 120M Magnetic Stirrer

Procedure

Only variations in procedural steps for preparing DG plates from those described at Appendix A for preparing TSA plates are shown below. Otherwise, follow the procedures presented for preparing TSA plates.

Step2: Tare the electronic scale (Figure B-1) with the weighing dish on the scale pan prior to weighing agar powder. Use a microspatula to transfer 31.6 grams of dichloran glycerol agar to the weighing dish on the electronic scale and then transfer the weighted powder to the 2000 ml flask with the reagent grade water. Record the exact weight of dichloran glycerol agar used. Clean the microspatula and scale surfaces after weighing to avoid cross contamination during subsequent weighing operations. Use the sliding doors along the sides of the scale enclosure to minimize air currents while weighing.

Step 3: Turn on the magnetic stirrer to about six and let the solution thoroughly mix. Place a one-inch magnetic stir bar into the 2000 ml flask containing the dichloran glycerol agar and set it onto the magnetic stirrer/hot plate (Figure B-2). Turn the heat knob clockwise to its highest heat setting, and turn the stir setting to six. Ensure that the magnet in the flask is correctly spinning and mixing. Stir and heat the flask at these settings for approximately 15 minutes and remove the flask from the plate as the agar solution becomes clear in the flask. (Notes: if the stir setting is too high, then the stir bar will not spin correctly and just rattle in the flask. Regularly check to ensure that the solution is being stirred properly and that it does not boil for too long or it will overflow. The solution is clear enough when the magnetic stir bar is visible through the flask.)

Figure B-1: DG being weighed.

Step 4: Once the solution is boiling, remove the flask from the hot plate and place onto the Fisher magnetic stirrer as shown in Figure B-2a. Use a graduated cylinder to measure and pour 175 ml of Glycerol (Figure B-2b) into the flask. Turn on the magnetic stirrer to a setting of about 6 and let the solution thoroughly mix.

Figure B-2: a. Stirrer for use with boiling DG solutions; b. glycerol added to boiling solution.

Appendix C: Plating Tryptic Soy and Dichloran Glycerol Agar Solutions

Disposable nitrile gloves and other protective wear should be worn as appropriate at all times during plating operations (including labeling) to avoid contamination of the plates and protection of laboratory personnel. Steps 1 through 4 of this procedure should be always done inside a biological safety cabinet with the glass door for the cabinet lowered so that only the hands can fit through the opening to minimize potential contamination of the plates with unfiltered laboratory air.

Materials

VWR Cat. No. 20171-042, 10 x 1/10 ml sterile polystyrene serological pipets

Drummond P-85803 Pipet-Aid

Procedures

Step 1: Label all petri dishes with the date of preparation and culture media as shown in Figure C-1 in the following format: "Today's Date Media-Plate #" (e.g., 5/28/09 TSA-25).

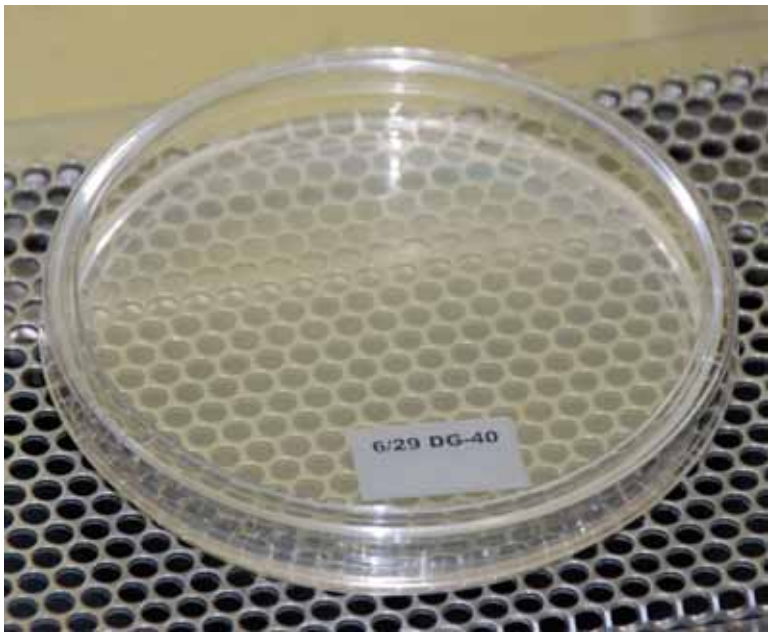


Figure C-1: Properly labeled DG plate.

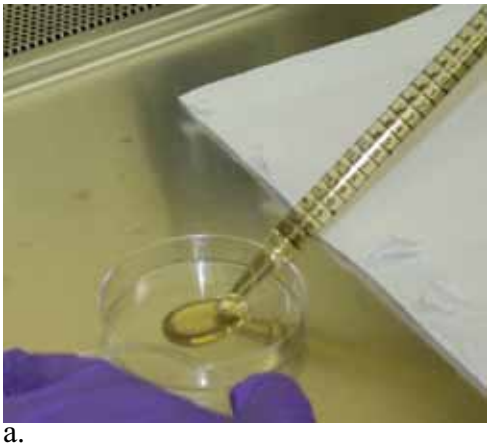
Step 2: Attach a sterile serological pipet (Figure C-2) to the Pipet-Aid handle (Figure C-3).

Figure C-2: Serological pipet.

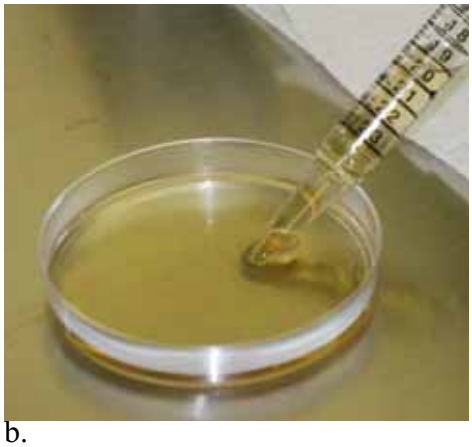
Step 3: Press the top button on the Pipet-Aid to fill the pipette with 20 ml of tryptic soy agar solution. It is easier and more accurate for many users to draw up 25 ml and leave 5 ml behind upon release. Regardless of the technique used, work quickly and accurately so that the solution does not cool down into a gel before pipetting into the plate. Also take care not to overfill the pipette because it can damage the pipette and the Pipet-Aid device. Press the bottom button to release the 20 ml of solution into a petri dish. Ensure that there are no air bubbles on the plates and that the solution is evenly distributed onto the plate at the completion of this step. Pipetting with the pipette held at an angle as shown in Figure C-4 helps to prevent bubbles from forming on the surface of the agar. If bubbles do form, it is best to draw the bubbles out of the plate with the pipette. Repeat until all petri dishes are plated.

Step 5: Once the plates solidify, organize them in stacks of three, upside-down inside of the biological safety cabinet for 24 hours as shown in Figure C-5. Then stack the plates into groups of eight and place them into petri dish bags. Tie the top of the bags and place them inside the aerosols lab refrigerator until ready to use.

Figure C-3: Pipet-Aid device in use.



a.



b.

Figure C-4: a. Pipet held at an angle while pipetting to help prevent the formation of air bubbles in the plate; b. iif bubbles do form, the pipette can be used to draw the bubbles out of the plate.

Figure C-5: Storage of plates in the biological safety cabinet prior to refrigerating.