SARGENT STEAM

Phone 801-326-8322
Fax 801-326-8325
www.SargentSteam.com
1 • 8 0 0 • S G T • S T E A M
3131 East 3300 South - Salt Lake City, UT 84109

SURFACE DISINFECTION STUDIES ON THE SARGENT STEAM CLEANER USING *STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA*, AND *BACILLUS SUBTILIS* SPORES

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PREPARED FOR:

Joyce Mathie, President
Sargent Steam

PREPARED BY:
Richard A. Robison, Ph.D.
Associate Professor of Microbiology
Brigham Young University

I. PURPOSE

The purpose of these studies was to determine the kinetics of kill of the Sargent Steam cleaner on dried bacterial films. Two vegetative bacteria (one gram positive and one gram negative) and bacterial endospores were used as the test organisms in these evaluations.

II. MATERIALS AND METHODS

1.0 Test organisms.

1.1 Cultures of *Staphylococcus aureus*, ATCC 6538, and *Pseudomonas aeruginosa*, ATCC 15442, were grown for 24 h in Mueller Hinton broth at 37˚C.

1.2 *Bacillus subtilis*, ATCC 6051, was grown on sporulation agar at 37˚C for 4 days. Growth was rinsed from the plate with sterile water and spores were purified by repeated centrifugation at 5,000 X g for 20 min, followed by removal of the top vegetative-rich layers. When the purity reached about 90% spores, the suspension was treated with 75% ethanol for 20 min to kill any remaining vegetative cells. The spore suspension was washed, re-suspended in sterile water and stored at 4˚C until used.

2.0 Bacterial film preparation.

Fifty-microliter aliquots of *Staphylococcus aureus* and *Pseudomonas aeruginosa* cultures and the *Bacillus subtilis* spore suspension were placed in slanted separate sterile 250 ml Erlenmeyer flasks and dried overnight at 37˚C.

3.0 Steam nozzle preparation.

Sargent Steamer brushless steam nozzles were fitted with rubber filter adapters, such that the nozzle was suspended inside the test flask and the tip of the nozzle was held one cm above the dried bacterial film. The nozzles with adapters were sterilized using a steam autoclave.

4.0 Steaming procedure.

4.1 A sterile nozzle was attached and the steamer was activated for 30 sec before use, ensuring optimal steam output and temperature.

4.2 The nozzle was placed into a flask containing a dried bacterial film. *Staphylococcus aureus* and *Pseudomonas aeruginosa* films were exposed to steam for land 2 sec. Films of *Bacillus subtilis* spores were exposed for 10, 30, 60, 120, and 240 sec.

4.3 Immediately following steam treatment, 25 ml of sterile physiological saline solution (PSS) with 0.1 % Tween 80 were added to the flask.

4.4 Flasks were sonicated for five minutes using a Sonicor SC200 sonicating bath to strip bacteria from the wall of the flask.

5.0 Viability Assay.

5.1 The number of viable organisms was determined using membrane filtration.

5.2 Following sonication, the entire contents of the flasks with *Staphylococcus aureus* or *Pseudomonas aeruginosa* films were filtered using a membrane with a 0.45 ulm pore size. The flasks were rinsed with 25 ml of PSS, which was filtered using the same membrane. The funnel was rinsed with about 100 ml of sterile PSS.

5.3 Membranes were removed to Tryptic Soy Agar (TSA) plates, which were incubated at 37˚C for 24 hr.

5.4 Flasks containing *Bacillus subtilis* spores were steamed for longer periods of time. To determine the number of viable spores remaining in the flask, the

suspension was serially diluted using 9 ml PSS blanks (1:10, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$, and $1:10^6$). Since steaming adds water, the total volume of liquid in the flasks was determined using a graduated cylinder.

5.5 One ml aliquots of each serial dilution were plated using the same membrane filtration process as described above.

5.6 After incubation, the colonies on the membranes were counted and log reductions and percent kill values were calculated.

6.0 Controls

6.1 *Staphylococcus aureus* and *Pseudomonas aeruginosa*:

Positive controls were performed by exposing dried bacteria to steam for 1 min. The positive controls were assayed for viable bacteria using vacuum filtration.

Negative controls were performed by processing dried films without exposure to steam. The suspension in the flasks was diluted $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, and $1:10^8$ and 1 ml samples of the dilutions were vacuum filtered as described above.

6.2 *Bacillus subtilis* spores:

Positive controls were performed by autoclaving flasks with dried spores for 20 min at 121°C. After being autoclaved, the flasks were processed as described and the entire contents were filtered.

Negative controls were performed by processing dried films without exposure to steam. The suspension in the flasks was diluted $1:10^3$, $1:10^4$, $1:10^5$, $1:10^6$, $1:10^7$, and $1:10^8$ and 1 ml samples of the dilutions were vacuum filtered as described above.

III. RESULTS

The following table shows the raw data from the individual replicates performed.

Staphylococcus aureus		***Counts***	#Org./Flask	S/S_0	Log Reduction	% Kill
0 second	1	1.05E+07	2.63E+08	NA	NA	NA
	2	1.20E+07	3.00E+08	NA	NA	NA
	3	9.90E+06	2.48E+08	NA	NA	NA
	4	1.29E+07	3.23E+08	NA	NA	NA
	5	5.60E+06	1.40E+08	NA	NA	NA
	6	6.90E+06	1.73E+08	NA	NA	NA
1 second	1	TNC	>2.50E+05	ND	ND	ND
	2	TNC	>2.50E+05	ND	ND	ND
	3	TNC	>2.50E+05	ND	ND	ND
	4	TNC	>2.50E+05	ND	ND	ND
	5	TNC	>2.50E+05	ND	ND	ND
	6	TNC	>2.50E+05	ND	ND	ND
2 second	1	0	0	0	> -8.41	100
	2	0	0	0	> -8.48	100
	3	5	5	2.02E-08	= -7.69	99.99999798
	4	0	0	0	> -8.51	100
	5	3	3	2.14E-08	= -7.67	99.99999786
	6	0	0	0	> -8.24	100

Pseudomonas aeruginosa		***Counts***	#Org./Flask	S/S_0	Log Reduction	% Kill
0 second	1	4.40E+04	1.10E+06	NA	NA	NA
	2	1.44E+05	3.60E+06	NA	NA	NA
	3	3.30E+04	8.25E+05	NA	NA	NA
	4	5.70E+04	1.43E+06	NA	NA	NA
1 second	1	0	0	0	> -6.04	100
	2	0	0	0	> -6.56	100
	3	0	0	0	> -5.92	100
	4	4	0	2.81E-06	= -5.55	99.9997193
2 second	1	0	0	0	> -6.04	100
	2	0	0	0	> -6.56	100
	3	0	0	0	> -5.92	100
	4	0	0	0	> -6.15	100
Bacillus subtilis		***Counts***	#Org./Flask	S/S_0	Log Reduction	% Kill
0 second	1	1.69E+05	4.23E+06	NA	NA	NA
	2	1.51E+05	3.78E+06	NA	NA	NA
	3	1.52E+05	3.80E+06	NA	NA	NA
	4	1.67E+05	4.18E+06	NA	NA	NA
10 second	1	7.10E+04	2.13E+06	5.04E-01	-2.97E-01	49.59
	2	5.90E+04	1.77E+06	4.69E-01	-3.29E-01	53.11
	3	3.70E+04	1.22E+06	3.21E-01	-4.93E-01	67.87
	4	7.90E+04	2.45E+06	5.87E-01	-2.32E-01	41.34
30 second	1	2.08E+04	6.66E+05	1.58E-01	-8.03E-01	84.25
	2	1.15E+04	3.68E+05	9.75E-02	-1.01E+00	90.25
	3	1.79E+04	5.91E+05	1.55E-01	-8.08E-01	84.46
	4	1.77E+04	5.84E+05	1.40E-01	-8.54E-01	86.01
60 second	1	1.85E+04	5.55E+05	1.31E-01	-8.82E-01	86.86
	2	1.33E+04	4.12E+05	1.09E-01	-9.62E-01	89.08
	3	1.18E+04	4.01E+05	1.06E-01	-9.76E-01	89.44
	4	1.24E+04	4.22E+05	1.01E-01	-9.96E-01	89.90
120 second	1	8.70E+03	3.48E+05	8.24E-02	-1.08E+00	91.76
	2	7.70E+03	3.08E+05	8.16E-02	-1.09E+00	91.84
	3	1.21E+03	4.36E+04	1.15E-02	-1.94E+00	98.85
	4	6.20E+03	2.29E+05	5.49E-02	-1.26E+00	94.51
240 second	1	1.90E+03	8.55E+04	2.02E-02	-1.69E+00	97.98
	2	1.60E+02	6.08E+03	1.61E-03	-2.793013	99.84
	3	1.30E+02	4.94E+03	1.30E-03	-2.886057	99.87
	4	4.80E+02	1.92E+04	4.60E-03	-2.337355	99.54

IV. DISCUSSION

The active steam produced by the Sargent Steam cleaner exhibits extremely quick kill on both gram positive and gram negative vegetative bacteria. The steam killed over 8 logs of *Staphylococcus aureus* within 2 sec. It was also able to effect more than a 6 log kill of *Pseudomonas aeruginosa* within 1 sec. Because of the extremely rapid kill rate, kinetic kill curves could not be generated for these organisms.

Bacterial endospores are the most heat-resistant forms of life on the planet. The Sargent Steam cleaner was evaluated for its ability to inactivate spores of *Bacillus subtilis*. The exposure time was in minutes instead of seconds. Attached is a regression analysis of this data. It shows that the steamer was able to inactivate spores in a time-dependent manner. It is likely that this kill levels off after 4 min, due to the heat-resistant nature of these spores. Steam under pressure is usually required to effect complete kill of these organisms.

In summary, the Sargent Steam Cleaner was able to effect the following average log reductions:

Staphylococcus aureus: >8.17 within 2 sec.

Pseudomonas aeurginosa: > 6.02 within 1 sec, and >6.17 within 2 sec.

Bacillus subtilis spores: 2.43 in 4 min.

This represents extremely rapid kill which cannot be duplicated by chemical disinfectants.

Test Dates: September 3 - October 9, 2001

By:

Richard A. Robison, Ph.D.
Associate Professor
791 WIDB
Brigham Young University
Provo, Utah 84602

Kinetics of Sargent Steam Cleaner Kill on B. subtilis Spores