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WATER & ENERGY SUSTAINABLE TECHNOLOGY

Product performance against *Staphylococcus aureus* 6538, *Escherichia coli* 25922, and *Legionella pneumophila* 33156 on treated surfaces

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Company: Fenix Group Brands

Test Organism: *Escherichia coli* 25922, *Staphylococcus aureus* 6538, *Legionella pneumophila* 33156

Test Materials: Tin coupons with antimicrobial paint coatings

Contact Times: 1, 4, and 24 hours (24 and 48 hours for *L. pneumophila*)

MATERIALS AND METHODS

Escherichia coli and *Staphylococcus aureus*:

1. A culture of the test bacterial species was prepared on the day before testing by inoculating one colony of the organism into 100 ml of tryptic soy broth (TSB) with incubation overnight at 37°C.

2. On the test date, the bacterial cells were washed by pelleting the cells via centrifugation. The supernatant was discarded and the pellet was re-suspended in 0.01 M phosphate-buffered saline (PBS). Three washing steps were performed in total.

3. The bacterial cell suspension was then diluted to obtain a density of ~2.0 x 10^8 colony-forming units (CFU) per ml.

4. All control and test surfaces measuring 2" x 2" were evaluated individually. Stainless steel type 304 control surfaces were processed concurrently with the paint-coated surfaces.

5. All test carriers were cleaned with 70% ethanol to prepare them for the experiments.

6. After the ethanol had dried, control and test carriers were inoculated with 0.050 ml of the test cell suspension (containing ~1.0 x 10^7 CFU) and the suspension was spread over the surface.

7. Parafilm cover slips were placed over the inoculum and the carriers were placed in sealed tupperware chambers with moist paper towels and incubated at room temperature (~21°C) to prevent drying.

8. Triplicate samples from the control carriers were collected immediately upon inoculation to determine the baseline microorganism titer at time (t) = 0 hours. The carriers and cover slips were swabbed to recover the test organism and the swabs placed into separate 2-ml volumes of Dey-Engley (D/E) neutralizing broth. The samples were then vortexed for 30 seconds and the swabs were discarded.

9. The samples were then 10-fold serially diluted and inoculated onto selective agar plates using the spread plate method (eosin methylene blue agar for *E. coli*; mannitol salt agar for *S. aureus*).

10. The plates were incubated for 18 to 24 hours at 37°C and then the colonies were enumerated.

11. All other control and test carriers were held at room temperature for the 1-, 4-, and 24-hour contact times (~21°C at a relative humidity of 20% to 50%). At each contact time, three replicates for each carrier type (i.e., control and treated carriers) were sampled in the manner described previously and assayed on selective agar plates as before.

12. After the incubation period, colonies were counted and the levels of CFU per carrier determined. The data are reported as the logarithmic reduction using the formula $-\log10 (N_t / N_0)$, where N_0 is the concentration of the surviving test organism at time = 0 hours and N_t is the concentration of the test organism in the sample collected at time = $_t$ (i.e., 1, 4, or 24 hours). Percent reduction is also calculated.

Legionella pneumophila:

1. A culture of *L. pneumophila* was prepared three days before testing by inoculating several colonies of the organism onto a buffered charcoal yeast agar (BCYE) plate using a sterile cotton swab. The swab was used to spread the inoculum over the entire surface of the plate, creating a complete lawn of bacterial growth after incubation at 37°C for 72 hours in a candle jar (microaerophilic environment - reduced oxygen level, increased carbon dioxide level).

2. On the test date, the bacterial cells were scraped from the surface of the plate and re-suspended in 0.01 M phosphate-buffered saline (PBS).

3. The bacterial cell suspension was then diluted to obtain a density of ~2.0 x 10^7 colony-forming units (CFU) per ml.

4. All control and test surfaces measuring 2" x 2" were evaluated individually. Stainless steel type 304 control surfaces were processed concurrently with the paint-coated surfaces.

5. All test carriers were cleaned with 70% ethanol to prepare them for the experiments.

6. After the ethanol had dried, control and test carriers were inoculated with 0.050 ml of the test cell suspension (containing ~1.0 x 10^6 CFU) and the suspension was spread over the surface.

7. Parafilm cover slips were placed over the inoculum and the carriers were placed in sealed tupperware chambers with moist paper towels and incubated at room temperature (~21°C) to prevent drying.

8. Triplicate samples from the control carriers were collected immediately upon inoculation to determine the baseline microorganism titer at time (t) = 0 hours. The carriers and cover slips were swabbed to recover the test organism and the swabs placed into separate 2-ml volumes of Dey-Engley (D/E) neutralizing broth. The samples were then vortexed for 30 seconds and the swabs were discarded.

9. The samples were then 10-fold serially diluted and inoculated onto selective BYCE agar plates using the spread plate method.

10. The plates were incubated for 72 hours at 37°C in candle jars and then the colonies were enumerated.

11. All other control and test carriers were held at room temperature for the 24- and 48-hour contact times (20.31°C at a relative humidity of 47.2%). At each contact time, three replicates for each carrier type (i.e., control and treated carriers) were sampled in the manner described previously and assayed on selective agar plates as before.

12. After the incubation period, colonies were counted and the levels of CFU per carrier determined. The data are reported as the logarithmic reduction using the formula $-\log10 (N_t / N_0)$, where N_0 is the concentration of the surviving *L. pneumophila* at time = 0 hours and N_t is the concentration of the *L. pneumophila* in the sample collected at time = $_t$ (i.e., 24 or 48 hours).

Percent reduction is also calculated.

RESULTS

Study 1. Reduction of *Escherichia coli* (ATCC #25922) on tin coupons with antimicrobial paint coatings. Stainless steel coupons were also included as controls. Experiment was conducted under moist conditions (no drying; t = 0 collected immediately upon inoculation).

Treatment	Replicate	0 hours		1 hour		4 hours		24 hours	
		Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD
Control Stainless Steel	A	0.68	0.56 ± 0.14	0.53	0.51 ± 0.05	0.82	0.58 ± 0.21	0.94	1.11 ± 0.15
	B	0.60		0.46		0.49		1.17	
	C	0.40		0.55		0.43		1.21	
Black Coating	A	ND		0.55	0.69 ± 0.20	0.88	0.88 ± 0.09	1.87	† 2.10 ± 0.24
	B			0.91		0.97		2.09	
	C			0.60		0.79		2.34	
Silver Coating	A	ND		0.56	0.69 ± 0.21	0.93	0.84 ± 0.19	1.83	† 1.73 ± 0.25
	B			0.93		0.62		1.91	
	C			0.58		0.96		1.44	

* Initial inoculum of ~2.58 x 10^7 CFU in 0.050 ml.
† Reduction statistically significant ($P \leq 0.05$) in comparison to the reduction observed in the controls at the same time exposure point.
SD = standard deviation
ND = not determined

Study 2. Reduction of *Staphylococcus aureus* (ATCC #6538) on tin coupons with antimicrobial paint coatings. Stainless steel coupons were also included as controls. Experiment was conducted under moist conditions (no drying; t = 0 collected immediately upon inoculation).

Treatment	Replicate	0 hours		1 hour		4 hours		24 hours	
		Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD
Control Stainless Steel	A	0.17	0.15 ± 0.03	0.39	0.40 ± 0.03	0.25	0.26 ± 0.11	0.20	0.18 ± 0.09
	B	0.11		0.43		0.37		0.26	
	C	0.17		0.38		0.16		0.09	
Black Coating	A	ND		0.15	0.15 ± 0.04	0.04	0.20 ± 0.16	0.39	† 0.35 ± 0.05
	B			0.11		0.20		0.37	
	C			0.19		0.36		0.30	
Silver Coating	A	ND		0.03	0.13 ± 0.10	0.12	0.17 ± 0.05	0.55	0.37 ± 0.16
	B			0.23		0.21		0.32	
	C			0.14		0.17		0.24	

* Initial inoculum of ~2.88 x 10^7 CFU in 0.050 ml.
† Reduction statistically significant ($P \leq 0.05$) in comparison to the reduction observed in the controls at the same time exposure point.
SD = standard deviation
ND = not determined

Study 3. Reduction of *Legionella pneumophila* (ATCC #33156) on tin coupons with antimicrobial paint coatings. Stainless steel coupons were also included as controls. Experiment was conducted under moist conditions (no drying; $t = 0$ collected immediately upon inoculation).

Treatment	Replicate	0 hours		24 hours		48 hours	
		Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD	Log_{10} Reduction*	Average Log_{10} Reduction ± SD
Control Stainless Steel	A	0.49	0.49 ± 0.27	0.68	0.97 ± 0.43	1.74	2.36 ± 0.56
	B	0.22		1.47		2.83	
	C	0.75		0.77		2.50	
Black Coating	A	ND		1.85	† 2.45 ± 0.52	> 5.21	† > 5.21 ± 0.00
	B			2.66		> 5.21	
	C			2.83		> 5.21	
Silver Coating	A	ND		1.21	1.84 ± 0.60	5.21	† > 5.21 ± 0.00
	B			2.41		> 5.21	
	C			1.90		> 5.21	

* Initial inoculum of ~1.63 x 10^6 CFU in 0.050 ml.
† Reduction statistically significant ($P \leq 0.05$) in comparison to the reduction observed in the controls at the same time exposure point.
SD = standard deviation
ND = not determined
> = the bacteria had fallen to below the detection limit of the assay (< 1.0 x 10^1 CFU per carrier or a 5.21-log_{10} reduction); therefore, the reduction was greater than this number (i.e., > 5.21-log_{10} reduction), but it is impossible to know by how much.

CONCLUSIONS

Small reductions were observed after each time exposure for *E. coli* in comparison to the control (uncoated) stainless steel surfaces; however, these reductions were not statistically significant until 24 hours of exposure. Statistically significant ($P \leq 0.05$) average reductions of 0.99-$\log_{10}$ and 0.62-$\log_{10}$ were observed for the black and silver paint coatings, respectively, over the reduction observed for the controls after 24 hours (1.11-$\log_{10}$). These correspond to 89.8% and 76.0% reductions, respectively. Therefore, the Fenix paint coatings were found to be only modestly effective against *E. coli*.

A statistically significant reduction in *S. aureus* was observed for the antimicrobial black paint-coated tin coupons in comparison to the control stainless steel surfaces only after 24 hours of exposure. Nevertheless, this average reduction of 0.19-$\log_{10}$ over the reduction observed in the control is quite small and corresponds to only a 35.4% reduction in the numbers of surviving *S. aureus*. No significant reductions were observed with the silver paint-coated surfaces. This suggests that the Fenix paint coatings are not very effective against *S. aureus* within this time frame.

Following 24 hours of exposure, average reductions of 2.45-$\log_{10}$ and 1.84-$\log_{10}$ were observed for *L. pneumophila* on the black and silver paint coatings, respectively. Nevertheless, only the reductions observed for the black coatings (2.45-$\log_{10}$) were statistically significant ($P \leq 0.05$) in comparison to the stainless steel control surfaces after 24 hours (average reduction of 0.97-$\log_{10}$). These correspond to 96.7% and 86.5% reductions, respectively, in comparison to the control

surfaces. After 48 hours of exposure, average reductions of > 5.21-$\log_{10}$ were observed on both the black and silver paint coatings. These reductions were statistically significant in comparison to the 2.36-$\log_{10}$ average reduction observed for the stainless steel controls after 48 hours. These correspond to a > 99.9994% reduction in comparison to the controls.

Although only minimal reductions were observed for *E. coli* and *S. aureus*, the time frame of 24 hours could potentially be quite short relative to the exposure of bacteria to coated surfaces under real-world conditions. An antimicrobial surface coating has the potential to act on bacteria over prolonged periods and facilitate an overall reduction in bacterial numbers on such surfaces over time, thus reducing the risk of human exposure. The increased efficacy of the coated surfaces against *L. pneumophila* after 48 hours of exposure would also support the idea that a longer time frame would likely be more effective.