Assessment of the Efficacy of Unique Solutions, Inc. Active Agents in Biofilm Removal
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Project Summary

The goal of this test was to determine the efficacy of a Unique Solutions, Inc. antimicrobial chemistry and several competing products in inactivating a mature *Pseudomonas aeruginosa* biofilm grown according to ASTM Method E2562-07 in the CDC biofilm reactor on polycarbonate coupons. The CDC reactor generates biofilm under high fluid shear. The biofilm was grown to maturity (48 hours) without antimicrobial present. The antimicrobial formulations were then added to test tubes at concentrations specified on the label instructions. The effects of the antimicrobial treatments were assessed by exposing biofilm-containing sample coupons to each treatment and then comparing the live cell density on the treated coupons to control coupons which received no antimicrobial treatment.

Methods

A pure culture *Pseudomonas aeruginosa* biofilm was grown according to ASTM Method E2562-07 on polycarbonate coupons in a CDC reactor for 48 hours. At the end of the 48 hour growth phase, three control coupons were removed from the reactor and exposed to 30 mL buffer solution on a shaker table for 10 minutes, and then the biofilm was quantified (as specified below). The 4 antimicrobial products tested are shown in Table 1. Each product was applied according to the label instructions. The amount of product used was scaled down from an 80 gallon bathtub to a 30 mL test tube – a 1:10,000 dilution. Three biofilm-containing coupons were exposed to each antimicrobial solution in test tubes for a 10 minute contact time.

Table 1.

Product	**Manufacturer**	**Volume or Mass of Full Strength Product in 30 mL**
Ahh-some	Unique Solutions	0.0015 ml
SeaKlear	Halosource	0.006 ml
Swirl Away	Swirl Away	0.015 ml
SpaClean	AquaFinesse	0.0082 g

After 10 minutes exposure, sodium thiosulfate neutralizer was added to the test tubes to neutralize any oxidizing reactions. Coupons were then removed from the test tubes and the biofilm will be quantified according to the following procedure:

> Remove coupon from the test tube and place into sterile buffer containing sodium thiosulfate (neutralizing compound). Sonicate the biofilm from the coupon surface into the dilution buffer to remove and disaggregate the sample, then serially dilute and plate for viable cells.

The mean log reduction in viable cells was then calculated for each treatment by subtracting the treated coupon mean log density from the control coupon mean log density for each treatment.

Results

The mean log density of viable organisms enumerated from coupons following treatment is shown in Table 1. Also shown is the standard deviation of the measurement and the log reduction that each treatment resulted in relative to the control coupons. This information is also shown graphically in Figures 1 and 2. The 4 treatments tested ranged in efficacy from a 1.5 log reduction for Ahh-some to 0.12 LR for SwirlAway, the least effective treatment in biofilm viable cell reduction.

Table 1. Log density of viable organisms for all treatments.

Treatment	Avg. Log Density (CFU/cm^2)	Standard Deviation of Log Density (CFU/cm^2)	Log Reduction
Ahh-some	7.12	0.35	1.51
SeaKlear	8.48	0.16	0.14
SwirlAway	8.51	0.03	0.12
AquaFinesse SpaClean	8.49	0.05	0.14
Control - No Treatment	8.63	0.03	N/A

The 1.51 log reduction measured in Ahh-some represents a 96.88% reduction in live organisms measured on the coupon surface.

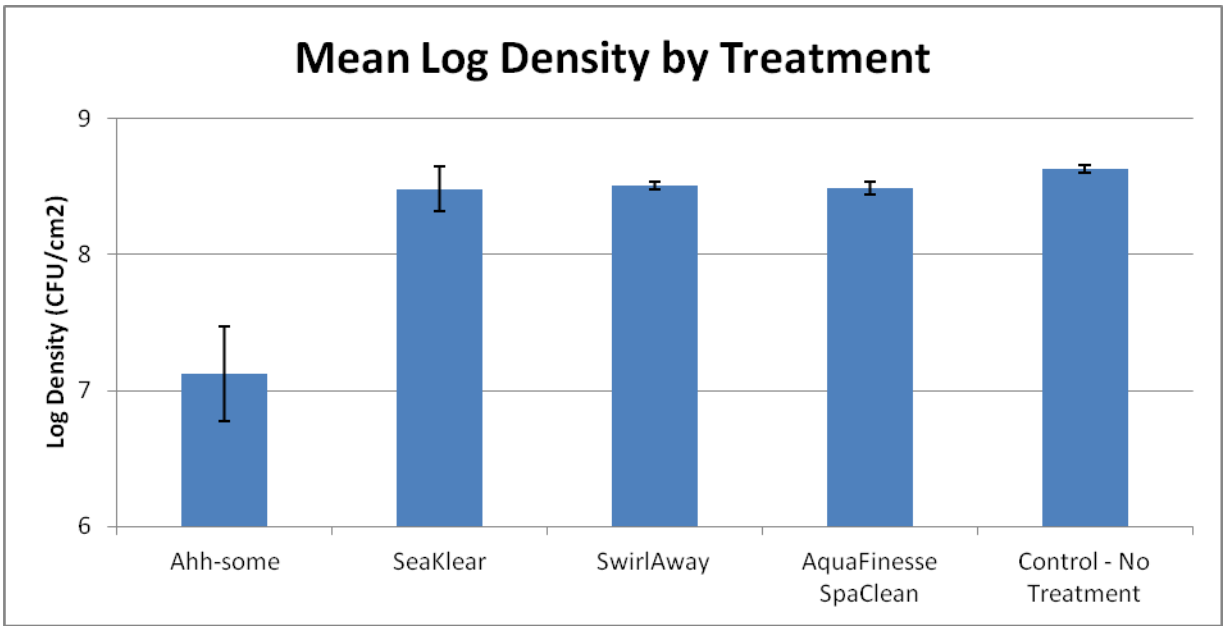

Figure 1. Mean log density of viable cells by treatment.

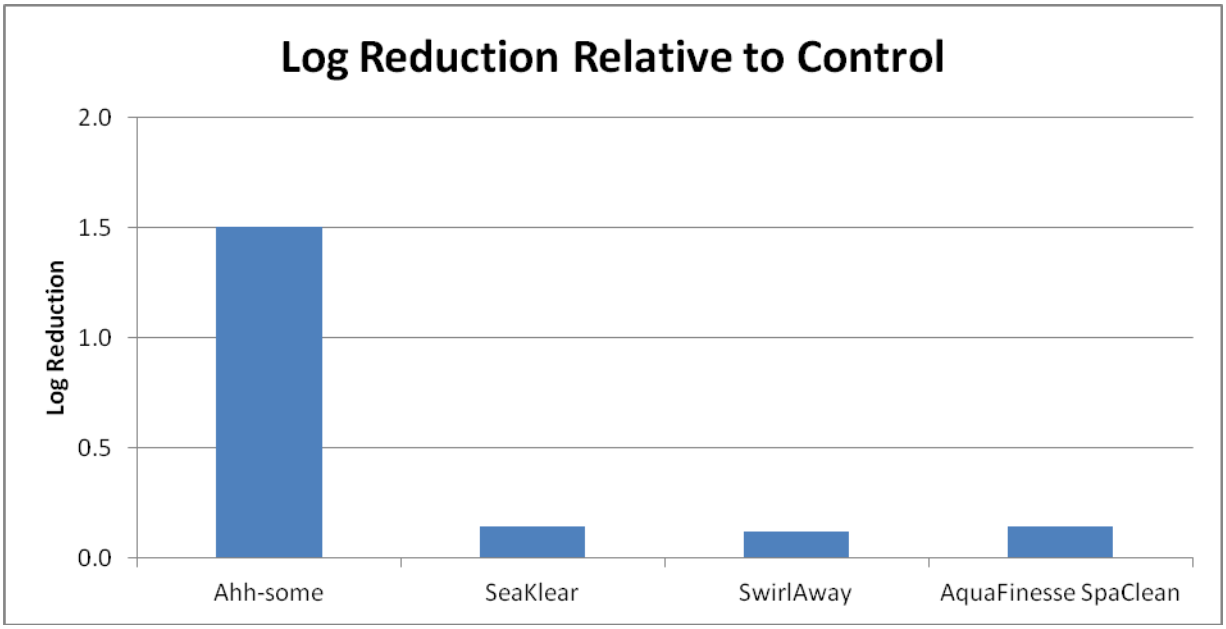

Figure 2. Mean log reduction of viable cells for each treatment relative to the untreated control.

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