Phase 2 Final Report

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

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Summary: The goal of this test was to determine the efficacy of a Unique Solutions, Inc. antimicrobial chemistry and a competing product 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 by the project sponsor. The effects of the antimicrobial treatment were assessed by exposing biofilm-containing sample coupons to each treatment. Effects of each antimicrobial treatment were determined by comparison of the live cell density on the treated coupons compared to control coupons which received no antimicrobial treatment. A detailed report is shown below.

Background

Unique Solutions sponsored a test of their spa cleaning product (Ahh-Some) and a competing antimicrobial chemistry (Natural Chemistry Spa Purge) to determine each product's efficacy in biofilm killing and/or removal. The target market for these compounds is in recreational water systems (pools/spas). The CDC biofilm reactor was used to grow mature *Pseudomonas aeruginosa* biofilms under high shear conditions. After a mature biofilm had formed, coupons were removed from the reactor and placed into test tubes containing the treatments. Each treatment was tested on triplicate coupons for contact times specified below.

Method

1. A *Pseudomonas aeruginosa* biofilm was grown according to ASTM Method E2562-07 on polycarbonate coupons in a CDC reactor for 48 hours.

2. At the end of the 48 hour growth phase, coupons were removed and exposed in triplicate to treatments and exposure times as shown in Table 1. Exposure took place in 30 mL test tubes on a shaker table or in a water bath (for 35C tests). After the exposure time had elapsed, the biofilm was quantified (as specified below). The amount of each product used was based on a scale-down calculation from a 300 gallon (1200L) hot tub spa.

- Ahh-Some was added at a concentration of 0.0025 mL Ahh-Some per 30 mL test tube. Exposure was done at room temperature (20C).
- Natural Chemistry Spa Purge was added at a concentration of 0.025 mL Spa Purge per 30 mL test tube. Exposure was done at 35C, as per label instructions.
- The amount of Natural Chemistry Spa Purge used was approximately 10 times as much as the amount of Ahh-Some used.

Table 1. Test Matrix

Exposure Solution	**Time**	**Temperature**
Buffer	0 min	20C
Buffer	24 hours	20C
Buffer	24 hours	35C
Ahh-Some	10 min	20C
Ahh-Some	1 hour	20C
Ahh-Some	24 hours	20C
Spa Purge	1 hour	35C
Spa Purge	24 hours	35C

3. After the specified exposure time, sodium thiosulfate neutralizer was added to the test tubes to neutralize any oxidizing reactions. Coupons were then removed from the test tubes and biofilm was quantified. This procedure was:

> 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.

4. Calculate the mean log reduction in viable cells for each treatment by subtracting the treated coupon mean log density from the appropriate control coupon mean log density for each treatment.

Results

Results of testing are shown in tabular form in Table 2 and graphically in Figure 1 below.

Table 2. Results of Testing. Average log density of biofilm with standard deviation, log reduction from control, and percent kill.

Treatment/Time/Temp	Avg. Log Density [Log10(CFU/cm^2)]	SD Log Density [Log10(CFU/cm^2)]	Log Reduction	% Kill
Control/ time 0	8.21	0.16	na	na
Control/24 hr/20C	8.76	0.05	na	na
Control/24 hr/35C	8.86	0.06	na	na
Ahh-Some/10 min/20C	3.86	0.47	4.36	99.996
Ahh-Some/1 hr/20C	4.29	0.25	3.93	99.988
Ahh-Some/24 hr/20C	4.28	0.05	4.48	99.997
Spa Purge/1 hr/35C	8.23	0.83	0.64	76.866
Spa Purge/24 hr/35C	8.23	0.02	0.63	76.757

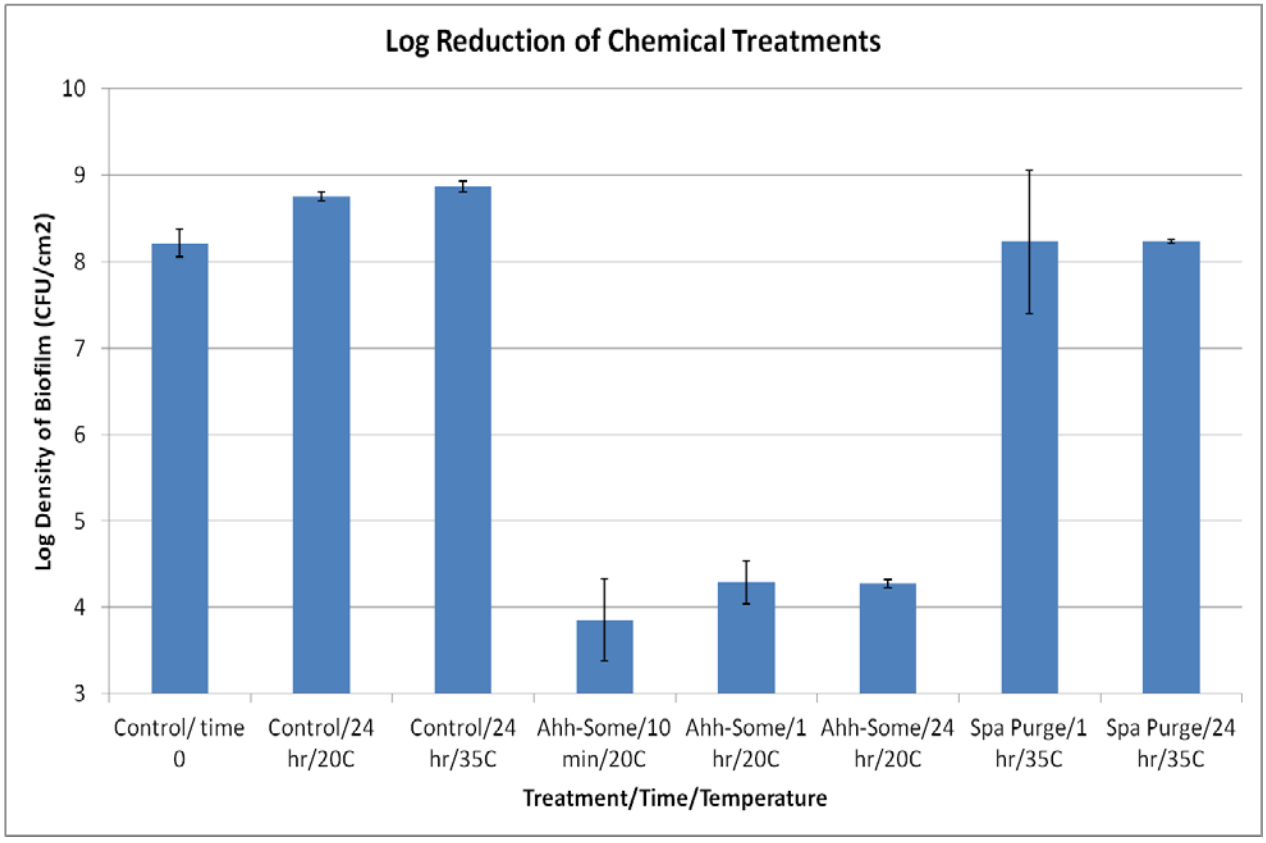

Figure 1. Results of testing. Error bars show ±1 standard deviation from the mean.

In these tests, Ahh-Some achieved an approximate 3.9-4.5 log reduction for all time periods tested. This represents a percent kill of 99.988% – 99.997% of the live organisms present. Log reduction did not significantly increase following the first 10 minutes of exposure. Natural Chemistry Spa Purge achieved a log reduction of 0.64 at 1 hour, remaining unchanged at 24 hours. This represents a percent kill of approximately 77%. These data indicate that Ahh-Some is approximately 5000 times as effective in biofilm killing as Spa Purge under the conditions tested.