REPORT
Evaluation pool and spa products for the ability to control Pseudomonas aeruginosa biofilm formation in the CDC biofilm reactor
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Submitted to:
Harold Evans
President
Orenda Technologies
McKinney, TX
Tel: 866-763-4269
surgeon@poolsurgeon.com

Submitted by:
Darla Goeres, Ph.D.
Assistant Research Professor
Standardized Biofilm Methods Laboratory
Center for Biofilm Engineering, MSU
Tel: 406-994-2440
darla_g@biofilm.montana.edu

Summary
The Standardized Biofilm Methods Laboratory at the Center for Biofilm Engineering has been performing biofilm efficacy tests for Orenda Technologies since June 2008. The goal the current efficacy test was to screen four Orenda formulations for their ability to control *Pseudomonas aeruginosa* biofilm formation in a CDC biofilm reactor operated according to ASTM method E2562-12. Testing demonstrated treating a CDC reactor treated with PureSPA (applied at 1 qt per 10,000 gallons) + SC1000 (applied at 1 qt per 10,000 gallons) + PR10,000 (applied at 1 qt per 10,000 gallons) resulted in a 4.8 log reduction of *Pseudomonas aeruginosa* biofilm. The reactor treated with PR10,000 (applied at 1 qt per 10,000 gallons) resulted in a 2.1 log reduction of biofilm bacteria. Reactors treated with either CV600 (applied at 1 qt per 10,000 gallons) + PR10,000 (applied at 1.16 oz per 10,000 gallons) or CV600 (applied at 1 qt per 10,000 gallons) + SC1000 (applied at 1 qt per 10,000 gallons) did not reduce biofilm growth when compared to the untreated control reactor, although visually the biofilm did not look as healthy and coupon coverage was not as consistent for the biofilm treated with the CV600 + PR10,000. The images collected of the biofilm treated with the PR 10,000 only and the PureSPA +SC1000 + PR10,000 were strikingly different than the biofilm in the untreated control reactor.

Methods
A. Grow a *Pseudomonas aeruginosa* ATCC 15442 biofilm according to ASTM Method E2562-12 on glass coupons in parallel reactors.

B. Operate one reactor according to the ASTM method as the control. To the remaining four reactors add the following treatments to both the batch and continuous flow nutrients as specified below:
1. CV600 (applied at 1 qt per 10,000 gallons) + PR10000 (applied at 1.16 oz per 10,000 gallons)
2. CV600 (applied at 1 qt per 10,000 gallons) + SC1000 (applied at 1 qt per 10,000 gallons)
3. PR10000 (applied at 1 qt per 10,000 gallons)
4. PureSPA (applied at 1 qt per 10,000 gallons) + SC1000 (applied at 1 qt per 10,000 gallons) + PR10000 (applied at 1 qt per 10,000 gallons)

C. At the end of 48 hours of growth, sample three coupons from each reactor for viable cells according to ASTM Method E2562-12.

D. Stain a fourth coupon from each reactor with BacLight Live/Dead stain and image using the confocal microscope.

Results

The test results are summarized in Table 1. Reactors treated with either CV 600 + PR 10,000 or CV 600 + SC 1,000 had no significant reduction in biofilm growth when compared to biofilm growth in the untreated control reactor operated according to ASTM Method E2562-12. The reactor treated with PR 10,000 had a 2 log reduction in biofilm and the reactor treated with Pure SPA + SC 1,000 + PR 10,000 experienced the greatest reduction in biofilm at 4.88 logs. The variability associated with the PR10,000 and PureSPA+SC1,000+PR10,000 was 1.1 and 2.9 logs, respectively.

Table 1. Log reduction in *Pseudomonas aeruginosa* biofilm associated with treating the CDC reactors with different combinations of Pure Spa, CV 600, SC 1,000 and PR 10,000.

Treatment	$log_{10}CFU/cm^2$	SD	Log Reduction
Control	7.830	0.272	NA
CV 600 + PR 10,000	7.734	0.620	0.096
CV 600 + SC 1,000	8.222	0.162	-0.393
PR 10,000	5.735	1.110	2.094
Pure SPA + SC 1,000 + PR 10,000	2.952	2.857	4.878

In addition to a quantitative measures of efficacy reported in Table 1, microscopic images were taken of the biofilm growing in each of the five reactors as shown in the series of figures below.

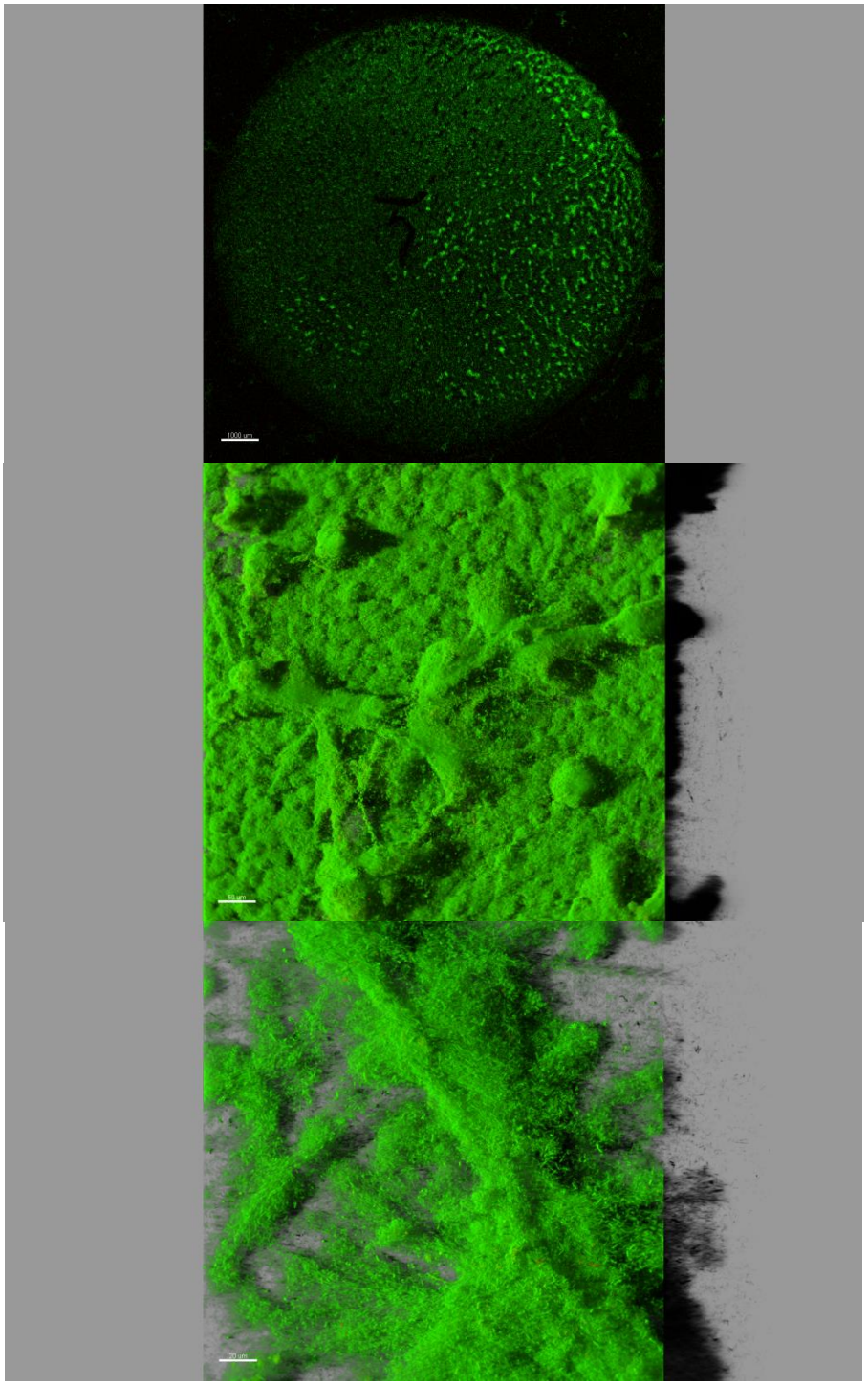

Figure 1. Confocol images taken of biofilm growing in the untreated control reactor. The top image is a 1.25x magnification, the bottom image is a 25x magnification, and the bottom image is a 63x magnification of the biofilm growing on the coupons.

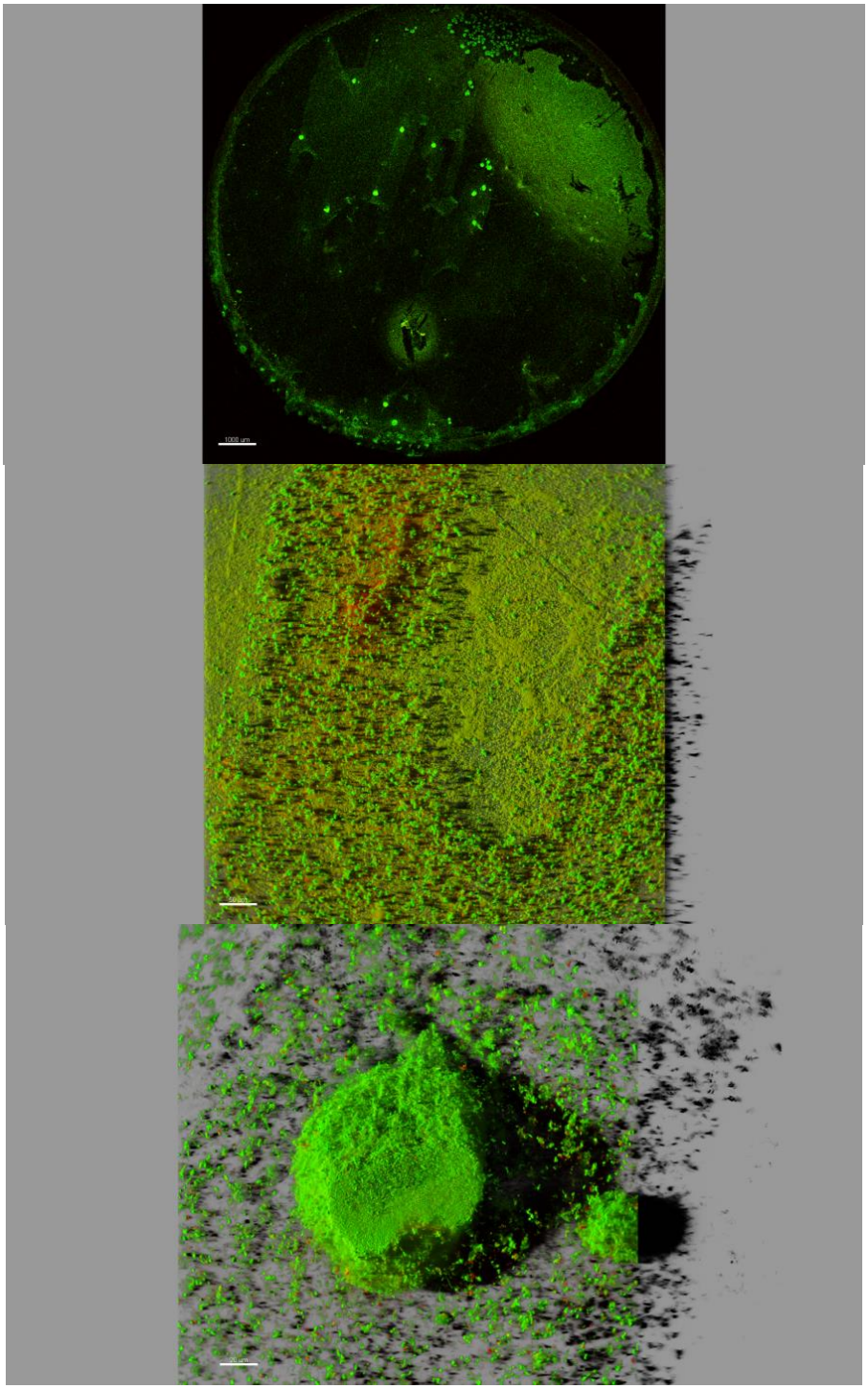

Figure 2. Confocol images taken of the biofilm growing in the reactor treated with CV600 (applied at 1 qt per 10,000 gallons) + PR10,000 (applied at 1.16 oz per 10,000 gallons). Top image is a 1.25x magnification, middle image is a 25x magnification and bottom image is a 63x magnification of the biofilm.

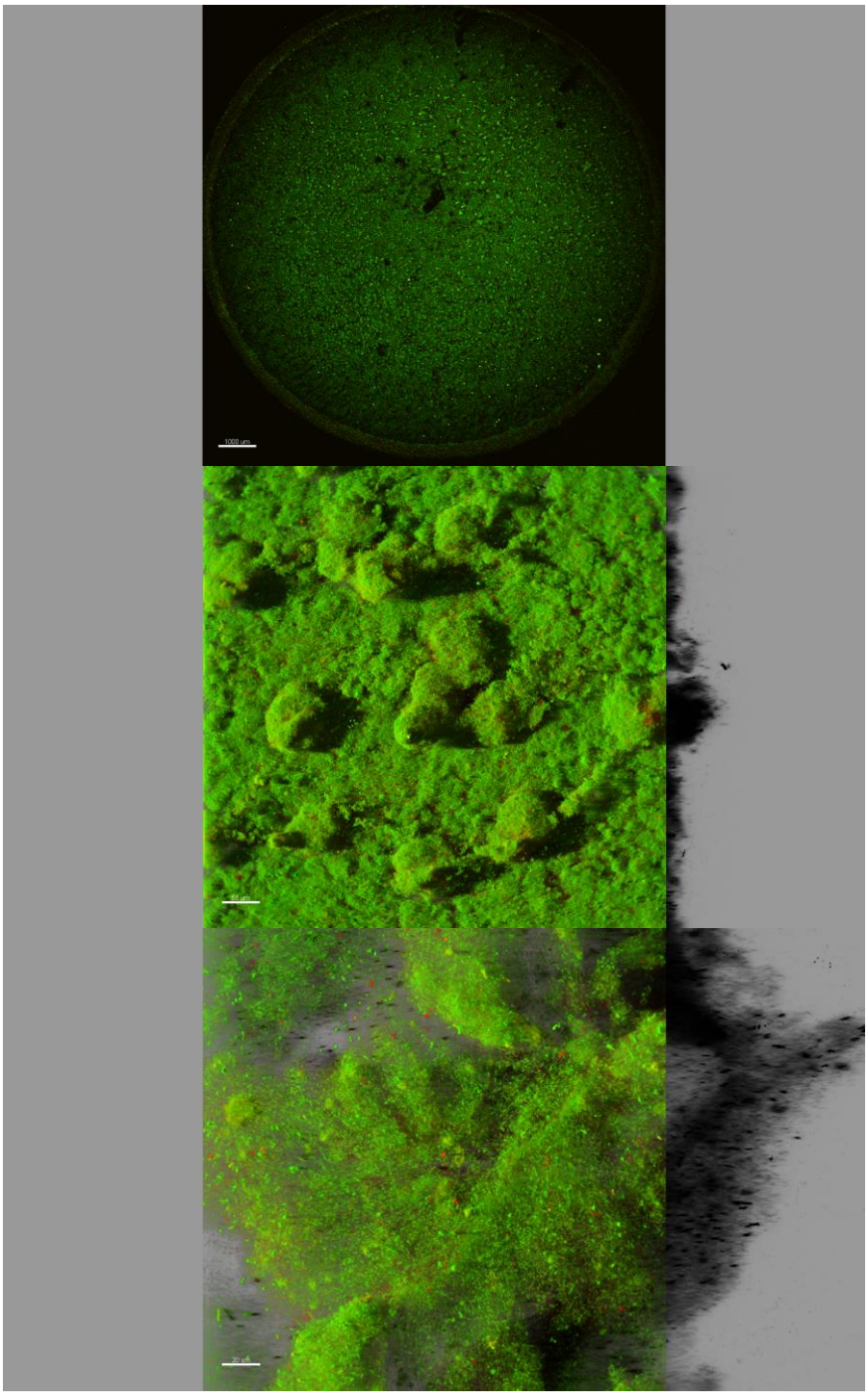

Figure 3. Confocol images taken of the biofilm growing in the reactor treated with CV600 (applied at 1 qt per 10,000 gallons) + SC1000 (applied at 1 qt per 10,000 gallons). Top image is a 1.25x magnification, middle image is a 25x magnification and bottom image is a 63x magnification of the biofilm.

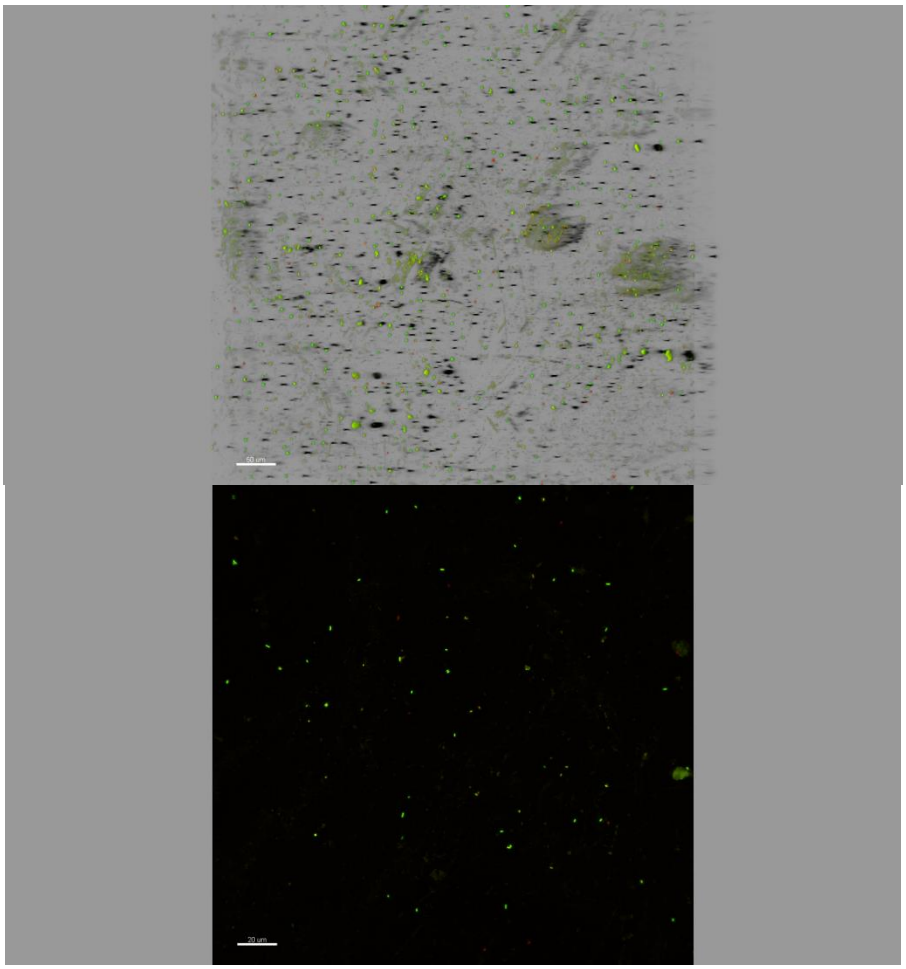

Figure 4. Confocol images taken of the biofilm growing in the reactor treated with PR10,000 (applied at 1 qt per 10,000 gallons). Top image is a 25x magnification and bottom image is a 63x magnification of the biofilm. The 1.25x magnification did not provide any useful information, as the image was mostly black.

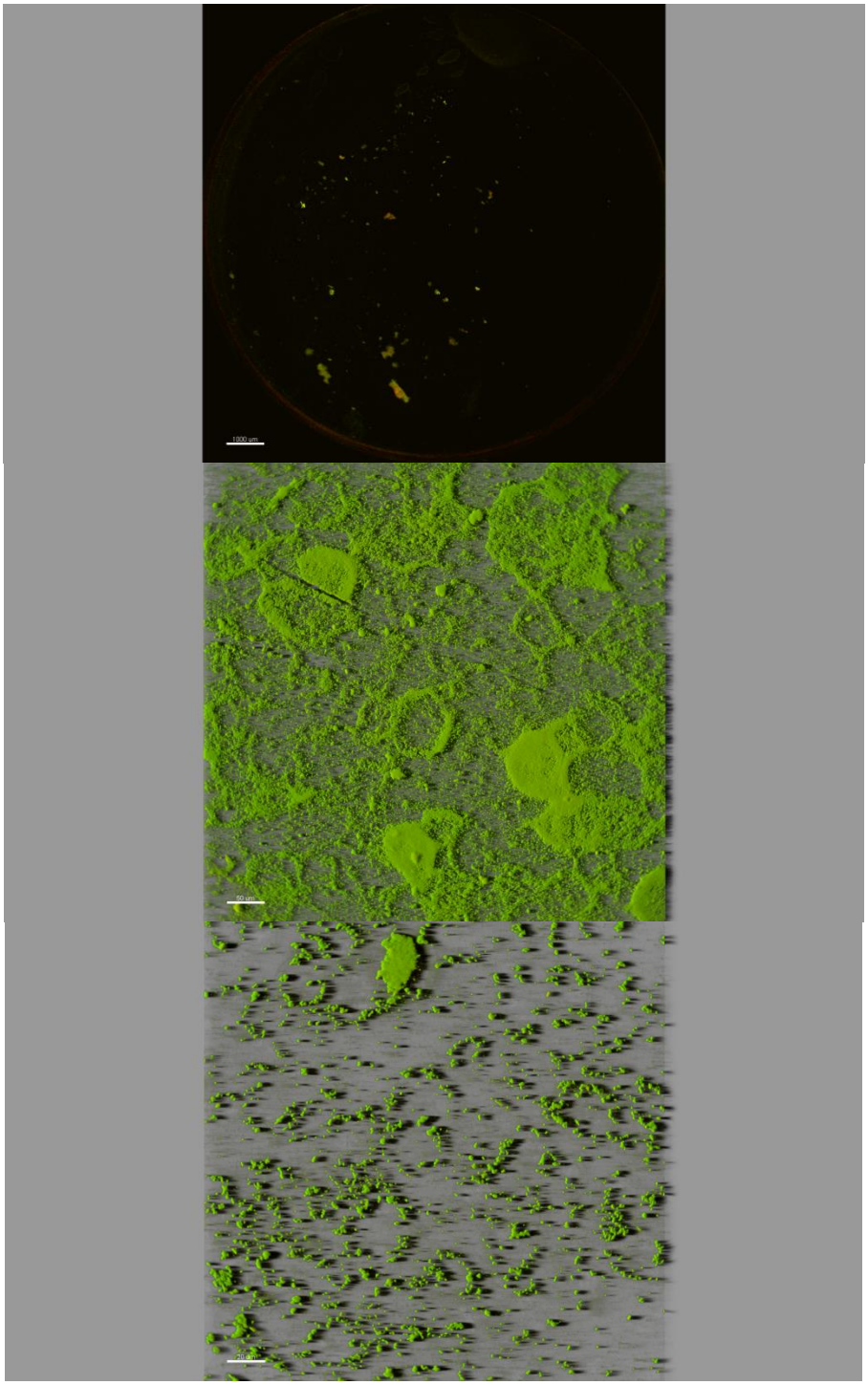

Figure 5. Confocol images taken of the biofilm growing in the reactor treated with PureSPA (applied at 1 qt per 10,000 gallons) + SC1000 (applied at 1 qt per 10,000 gallons) + PR10,000 (applied at 1 qt per 10,000 gallons). Top image is a 1.25x magnification, middle image is a 25x magnification and bottom image is a 63x magnification of the biofilm.

Discussion

Viable plate counts provide a quantitative measure of the efficacy associated with adding various combinations of CV600, SC1000, PR10,000 and PureSPA to a CDC biofilm reactor operated according to ASTM Method E2562-12. The reactors that contained PR10,000 by itself or PR10,000 added in combination with SC1,000 and PureSPA resulted in the greatest log decrease in biofilm growth. Biofilm growth in these two reactors was heterogeneous, though, as is suggested by the large standard deviations associated with the measured log density in each reactor and the qualitative images taken of a coupon from each of these reactors. The technician who conducted the study noted the bulk liquid in each of these two reactors was very clear, especially when compared the bulk liquid in the untreated control reactor and the reactors treated with either CV600 + SC1,000 or CV600+ PR10,000, all three of which were visually very turbid.

The microscopic images collected as part of this project provide useful information in understanding the quantitative results. The images of the biofilm from the reactors that contained PR10,000 or PR10,000+ SC1,000 + PureSPA show a biofilm that is notably different from the untreated control with regards to surface coverage and biofilm topography. In comparison, the reactor that contained CV600 + SC1,000 is visually very similar to the biofilm grown in the untreated control reactor. The qualitative images support the quantitative results. The image of the biofilm treated with CV600 + PR10,00 0 is intriguing, because although the quantitative results suggest this treatment combination had no efficacy, the images show a biofilm with less topography and surface coverage when compared to the images from the untreated control reactors, suggesting some impact on the biofilm.

Because this was a screening project, these results are based upon data from one experiment. It is good practice to repeat the above test to have more confidence in the results, at least for the chemistries that demonstrated some efficacy.