Efficacy of Peracetic Acid (PAA) in Combination with a PAA Booster Against Bacterial Biofilm and Endospores

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INTRODUCTION

Peracetic acid (PAA) is one of the most commonly used sanitizers/disinfectants in the food industry due to its efficacy, low cost, lack of toxic residues, and easy application. Nevertheless, when used alone, undesirably high levels of PAA may be needed to achieve activity against difficult-to-control microbial targets such as biofilm and endospores — which may have an impact on equipment compatibility, health and safety. Therefore, a formulation containing a mixture of organic acids, chelants, surfactants, and a biodispersant was developed and evaluated for its ability to enhance PAA performance against bacterial biofilm and endospores.

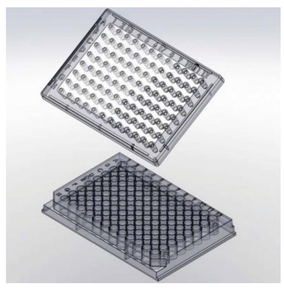
METHODS

The Minimum Biofilm Eradication Concentration (MBEC) Assay (ASTM E2799-17) was used to screen against biofilm formed by *Pseudomonas aeruginosa* ATCC 15442. Various concentrations of PAA +/– booster (1:10) in 400 ppm hard water were tested with a 10-minute contact time.

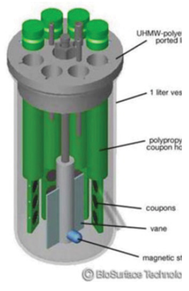
Further biofilm testing against *P. aeruginosa* was performed using the CDC Biofilm Reactor (ASTM E3161-21) with 304 stainless steel coupons and the Single Tube Method (ASTM E2871-21). Coupons were exposed to various concentrations of PAA +/– booster (1:10) for 10 minutes.

Imaging studies were performed by the Montana State University Center for Biofilm Engineering (CBE). CDC coupons containing *P. aeruginosa* biofilm were exposed to hard water (control) or PAA plus booster for 10 minutes with shear. After 1–3 exposures, the coupons were stained with LIVE/DEAD™ BacLight™ Bacterial Viability Kit stain and imaged using a Leica TCS-SP5 Confocal Scanning Laser Microscope.

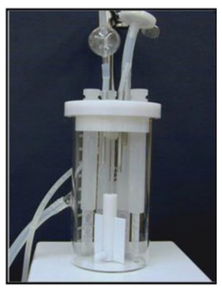
Sporicidal activity against *Bacillus subtilis* ATCC 6051 and *Clostridium sporogenes* ATCC 3584 was determined using a modified version of EN 13697. Carriers were inoculated with 0.02 ml of spore prep, dried for 1 hour (36°C / 40% RH), and then exposed to 2 ml of PAA +/– booster (1:10) in hard water for 10 minutes.



MBEC Plate



CDC Biofilm Reactor



Carrier and Jar for Modified EN 13697

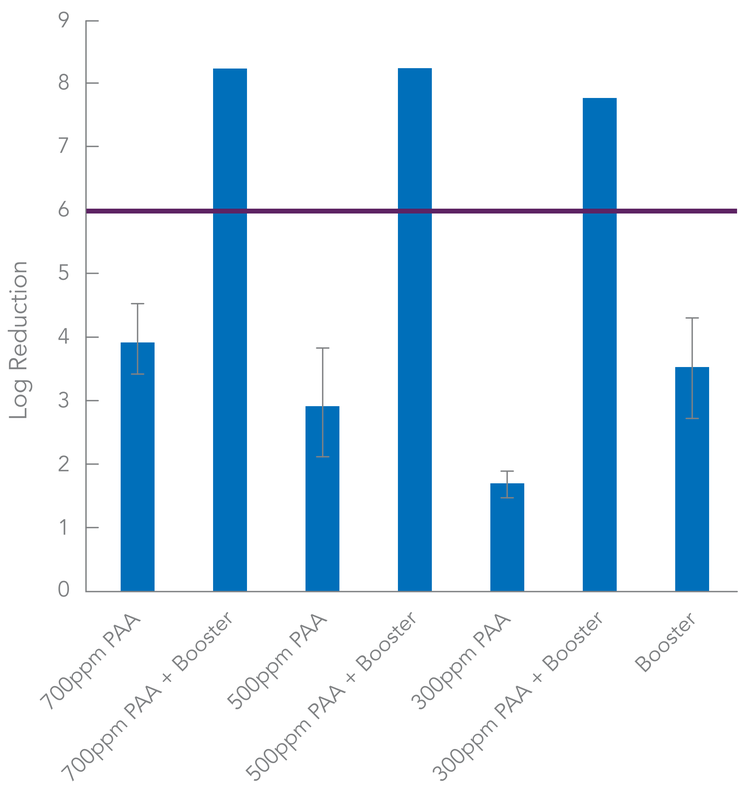
RESULTS

Table 1 – PAA vs. PAA with Booster MBEC Results

Treatment	Treatment Level Required for a 6-log Reduction
PAA Alone	> 400 ppm PAA
PAA + Booster	100 ppm PAA

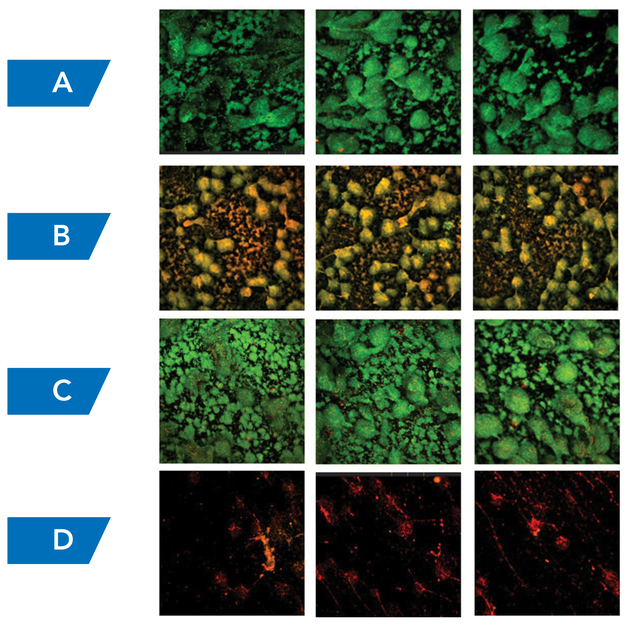
The combination of PAA and booster achieved consistent > 6-log kill at 100 ppm PAA whereas PAA alone required > 400 ppm PAA. The booster alone achieved 4-log kill.

Figure 1 – PAA vs. PAA with Booster CDC Reactor Results



The combination of PAA and booster achieved > 6-log kill at 300 ppm PAA compared to PAA alone which required > 700 ppm. The booster alone achieved a 3- to 4-log kill.

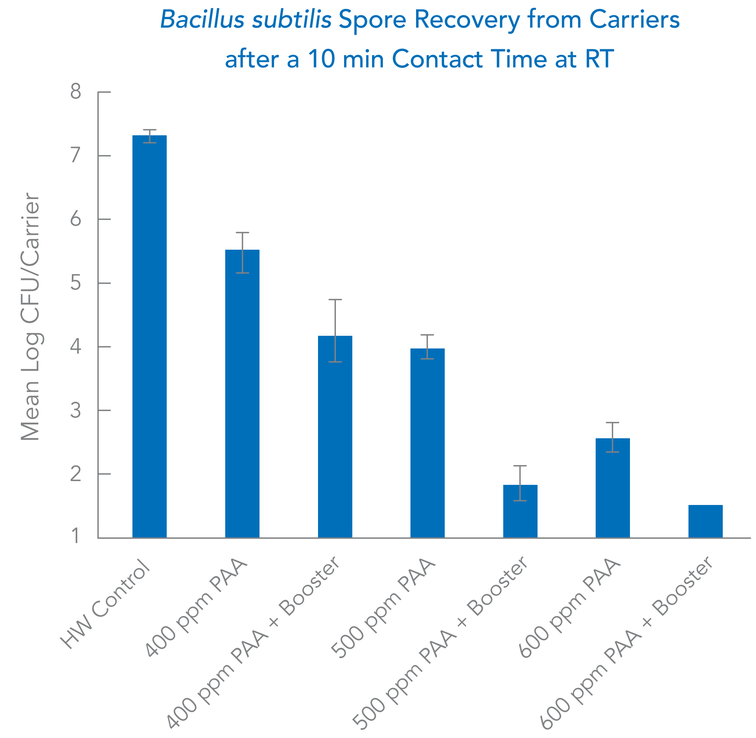
Figure 2 – *Pseudomonas aeruginosa* Biofilm on CDC Reactor Carriers



Confocal images of *Pseudomonas aeruginosa* biofilm after a 10-minute treatment at 20°C with A) Hard water 1X, B) PAA with Booster 1X, C) Hard water 3X, and D) PAA with Booster 3X. After one treatment of PAA in combination with the booster, the biofilm showed significant kill but appeared to be intact. After three treatments of PAA in combination with booster, the images indicated significant biofilm destruction.

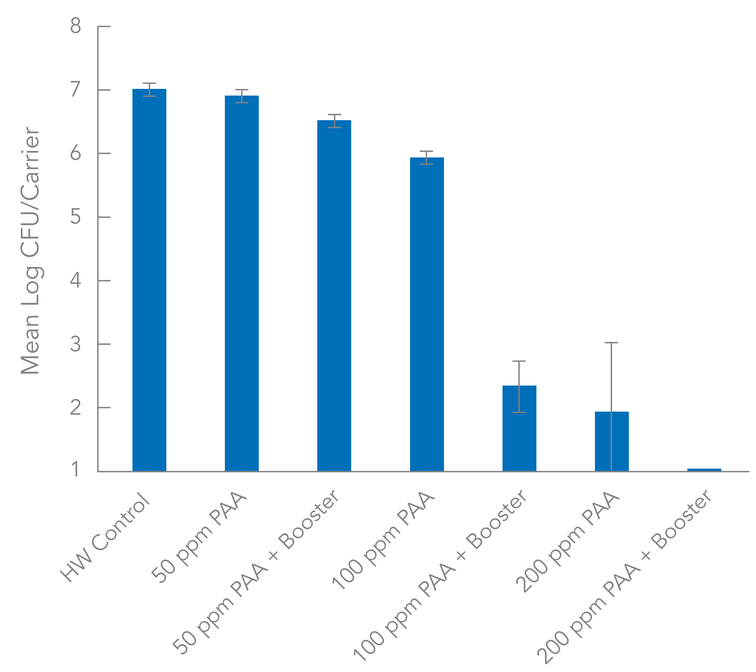
Images were obtained from *Lindsay Miller at the Montana State University CBE.*

Figure 3 – PAA vs. PAA with Booster Sporicidal Results for *Bacillus subtilis*



Against *Bacillus subtilis* endospores, the booster increased the sporicidal activity of PAA as much as 1- to 2-log reduction values compared to PAA alone.

Figure 4 – PAA vs. PAA with Booster Sporicidal Results for *Clostridium sporogenes*



Against *Clostridium sporogenes* endospores, the booster increased the sporicidal activity of PAA as much as 1- to 3-log reduction values compared to PAA alone.

DISCUSSION / CONCLUSIONS

A booster product containing a mixture of organic acids, chelants, surfactants, and a biodispersant was evaluated for its ability to enhance PAA performance against biofilm and endospores. In all testing performed, the booster was applied at a rate of 1:10 regardless of the PAA concentration.

In both the MBEC and Single Tube Assays, much lower levels of PAA were needed to achieve 6-log reductions in biofilm cell counts compared to PAA alone. The booster alone achieved some level of log reduction (approx. 3–4) but was unable to generate 6-log reductions without PAA. Confocal imaging was used to visualize the performance of PAA plus booster against biofilm. After one treatment, significant kill was observed, but the biofilm appeared to remain intact. After three treatments significant destruction of the biofilm became evident.

Endospores are highly resistant structures that form under stressful conditions. The booster alone showed little-to-no sporicidal activity against *B. subtilis* (data not shown), but it did enhance the performance of PAA by 1- to 2-log reduction values depending on the PAA concentration. Against *C. sporogenes* spores, a > 3-log reduction increase was observed at one concentration of PAA.

In conclusion, the results indicate that the booster can increase the performance of PAA against bacterial biofilm and endospores.

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