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

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STERILEX® Innovative Solutions for Microbial Control

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 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 was performed against *P. aeruginosa* and a mixed-species biofilm containing *P. aeruginosa* and *Listeria monocytogenes* ATCC 19111 using the CDC Biofilm Reactor (ASTM E3161-21) and Single Tube Method (ASTM E2871-21). The mixed species biofilm was prepared as previously described with some minor modifications (Moorman, E.A. PhD thesis – NC State Food Sci). The biofilms were formed on 304 stainless steel coupons which were later exposed to various concentrations of PAA +/– booster (1:10) in hard water for 10 minutes. The presence of *Listeria* in the mixed species biofilm was confirmed by colony morphology on TSA, selective media and a fluorescent Gram stain (Live BacLight™ Bacterial Gram Stain Kit).

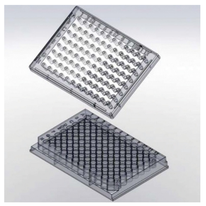
Sporicidal activity against *Bacillus subtilis* ATCC 6051 and *Clostridium sporogenes* ATCC 3584 was determined using a modified version of EN 13697. Carriers were inoculated with 0.02 ml of spore prep, dried for 1 hour (36°C/40% RH), and then exposed to 2 ml of PAA +/– booster (1:10) in hard water for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

Following treatments, residual antimicrobial activity was eliminated by neutralization with 2x D/E containing 0.2% catalase. Bacterial counts were determined using serial dilution and plating on TSA. For *Clostridium*, TSA with sheep's blood was used followed by anaerobic incubation.

RESULTS

Table 1 – MBEC data for PAA vs PAA + Booster

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 – Confocal image of *P. aeruginosa* biofilm formed on borosilicate CDC carrier and treated with hard water

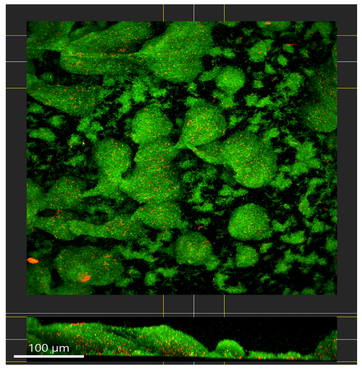


Image was provided by Lindsay Miller at the Montana State University Center for Biofilm Engineering.

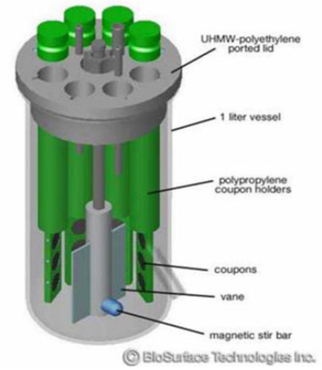
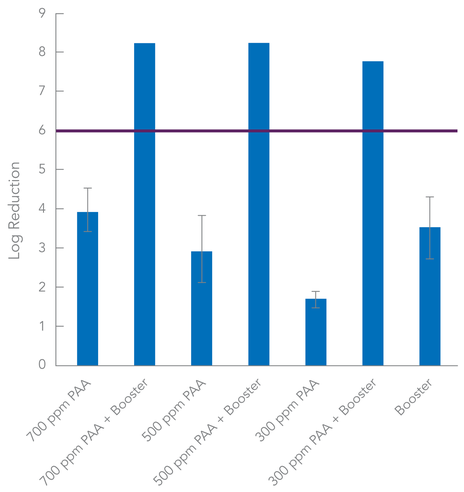
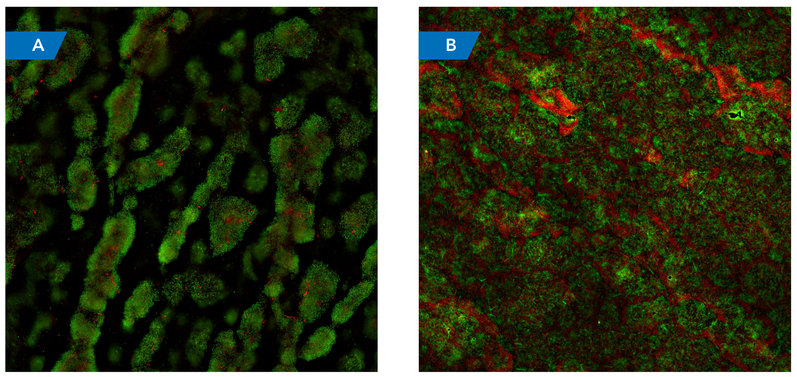


Figure 2 – Biofilm kill data for PAA vs PAA + Booster against *P. aeruginosa* biofilm



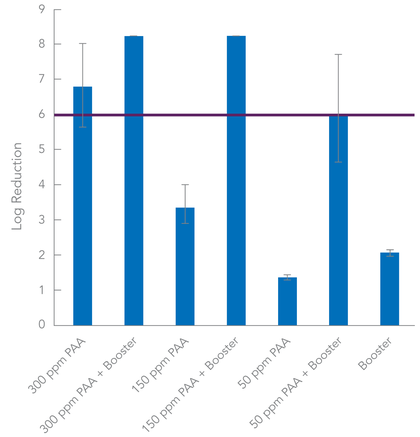
The combination of PAA and booster achieved > 6-log kill at 300 ppm PAA compared to PAA alone which required > 700 ppm. The booster alone achieved a 3- to 4-log kill.

Figure 3 – Epifluorescence images of a mixed species biofilm containing *P. aeruginosa* and *L. monocytogenes* on borosilicate CDC carriers.



The LIVE/DEAD™ BacLight™ stain (Image A) shows the morphology of the mixed species biofilm. The large mushroom-shaped plumes typical of a *P. aeruginosa* biofilm were absent. The LIVE BacLight™ Bacterial Gram Stain Kit stain (Image B) demonstrates the co-biofilm of *P. aeruginosa* (green) and *L. monocytogenes* (red).

Figure 4 – Biofilm kill data for PAA vs PAA + Booster against a mixed species biofilm of *P. aeruginosa* and *L. monocytogenes*



The combination of PAA and booster achieved 6-log kill at 50 ppm PAA compared to PAA alone which required 300 ppm. The booster alone achieved a 2-log kill.

Table 2 – Sporicidal activity of PAA vs PAA + Booster against *B. subtilis*

	Mean Log Reduction Values		
	400 ppm PAA	500 ppm PAA	600 ppm PAA
No Booster	1.82 (± 0.32)	3.33 (± 0.20)	4.79 (± 0.21)
With Booster	3.12 (± 0.47)	5.51 (± 0.26)	5.78 (± 0.00)
Δ	1.30	2.18	0.99

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

Table 3 – Sporicidal activity of PAA vs PAA + Booster against *C. sporogenes*

	Mean Log Reduction Values		
	50 ppm PAA	100 ppm PAA	200 ppm PAA
No Booster	0.09 (± 0.08)	1.04 (± 0.07)	5.08 (± 1.06)
With Booster	0.50 (± 0.05)	4.70 (± 0.37)	5.96 (± 0.00)
Δ	0.41	3.66	0.88

Against *C. sporogenes* endospores, the booster increased the sporicidal activity of PAA as much as 3- to 4-log reduction values compared to PAA alone.

DISCUSSION/CONCLUSIONS

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

Biofilm kill studies were performed on both single species (*Pseudomonas*) and mixed species (*Pseudomonas* and *Listeria*) biofilms. When used in combination with the booster, the level of PAA needed to achieve a 6-log reduction was significantly reduced compared to PAA alone. The booster alone was able to achieve log reduction values ranging from 2-4.

In contrast to biofilm, the booster alone showed little-to-no sporicidal activity against *B. subtilis* (data not shown). Nevertheless, 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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