Efficacy of Peracetic Acid (PAA) in Combination with a PAA Booster for Clean-In-Place Applications

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STERILEX® Innovative Solutions for Microbial Control

INTRODUCTION

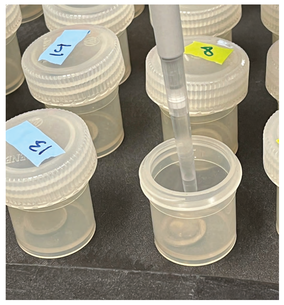
Peracetic acid (PAA) is a commonly used disinfectant/sanitizer for clean-in-place (CIP) applications in the food and beverage industries; however, undesirably high levels of PAA are often needed to control persistent microbial problems such as biofilms and bacterial endospores. A PAA booster containing organic acids, chelants and surfactants was developed specifically for CIP applications. The objective of this study was to evaluate the antimicrobial efficacy of this booster in combination with PAA against biofilm and endospores.

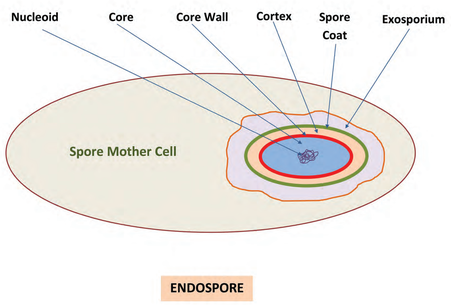
METHODS

The CDC biofilm reactor (ASTM E3161-21) and the Single Tube Method (ASTM E2871-21) were used to screen PAA alone and in combination with the PAA booster against biofilms of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Listeria monocytogenes*. The biofilms were grown on 304 stainless steel carriers as outlined below. Following biofilm development, the carriers were treated with the test substances, prepared in 400 ppm hard water, for 10 min at 35°C followed by neutralization with 2x D/E (with 0.2% catalase). After a series of vortexes and sonications the neutralized samples were filter and spread plated on R2A (*Pseudomonas*), TSA (*Staphylococcus*) or BHI (*Listeria*) to determine the bacterial count.

	Pseudomonas	*Staphylococcus*	*Listeria*
Strain	ATCC 15442	ATCC 6538	ATCC 49594
Inoculum	300 mg/L TSB 24 h at 36°C	30 g/L TSB 24 h at 35°C	37 g/L BHI 25 h at 35°C
Batch Phase	300 mg/L TSB 24 h at 21°C 125 rpm	3 g/L TSB 24 h at 35°C 60 rpm	3.7 g/L BHI 26 h at 35°C 60 rpm
Continuous Phase	100 mg/L TSB 24 h at 21°C 125 rpm 11.8 ml/min	1 g/L TSB 24 h at 35°C 60 rpm 11.8 ml/min	3.7 g/L BHI 26 h at 35°C 60 rpm 6 ml/min

Sporicidal activity was determined by using a modified EN 13697 method. A 50 µl aliquot of spore suspension (*Bacillus spizizenii* ATCC 19659 or *Clostridium sporogenes* ATCC 3584) mixed with an organic soil (fetal bovine serum or milk) was applied to 2 cm diameter 304 stainless steel carriers. After drying, 250 µl of treatment (prepared in 400 ppm hard water and heated to 35°C) was applied to the carriers for 10 min. Following treatment, the carriers were neutralized in 10 ml of 2x D/E (with 0.2% catalase), vortexed and spread plated on TSA (*B. spizizenii*) or blood agar (*C. sporogenes*) to determine the bacterial count.

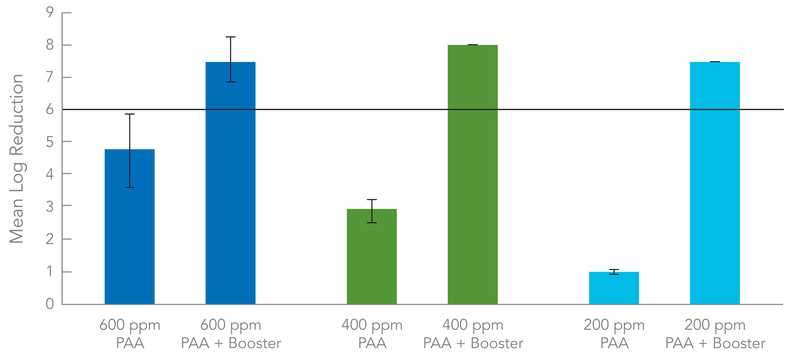




ENDOSPORE

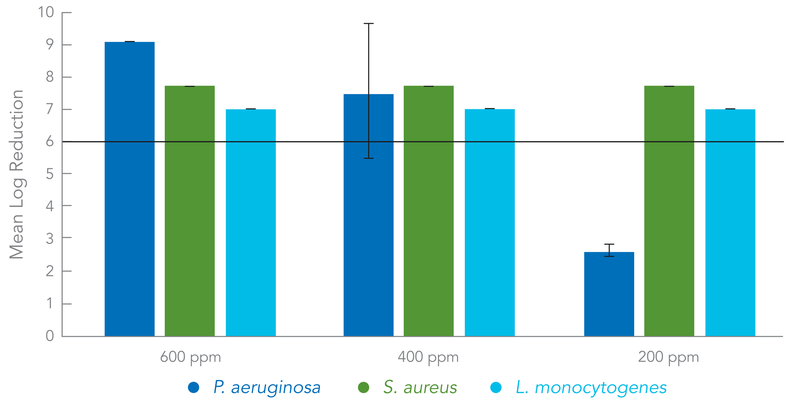
RESULTS

Figure 1 – Efficacy of PAA alone and with booster against *P. aeruginosa* biofilm



PAA at ≤ 600 ppm did not achieve a mean 6-log reduction, the standard level of kill required to pass the biofilm test. When combined with booster at 1 oz/gal, 200 ppm PAA was sufficient to achieve a 6-log reduction.

Figure 2 – Efficacy of PAA with booster against different biofilms



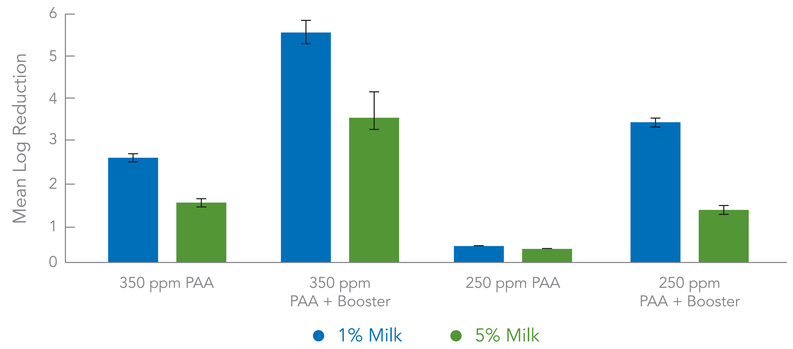
Three combinations of PAA plus booster were tested as follows: 1) 600 ppm PAA + 0.94 oz/gal booster, 2) 400 ppm PAA + 0.62 oz/gal booster, and 200 ppm PAA + 0.31 oz/gal booster. All three concentrations achieved a > 6-log reduction against *S. aureus* and *L. monocytogenes* biofilms. The two highest concentrations were also effective against *P. aeruginosa*.

Table 1 – PAA (ppm) and booster (oz/gal) concentrations needed to achieve a 3, 4 or 5-log reduction against *B. spizizenii* and *C. sporogenes* spores

	Log Reduction	Alone		Combination		% PAA Reduction
		PAA (ppm)	PAA Booster (oz/gal)	PAA (ppm)	PAA Booster (oz/gal)	
Bacillus spizizenii	3	350	> 128	200	1	43
	4	400	> 128	250	1	38
	5	450	> 128	300	1	33
Clostridium sporogenes	3	200	> 128	100	1	50
	4	250	> 128	100	1	60
	5	250	> 128	150	1	40

In the presence of hard water and 5% serum, the PAA booster had no sporicidal activity even at 128 oz/gal; however, when the booster (1 oz/gal) was combined with PAA , the level of PAA needed to achieve 3, 4, or 5-log kill was reduced by an average of 44%.

Figure 3 – Sporicidal efficacy against *B. spizizenii* in the presence of milk soil



In the presence of 1% milk the sporicidal efficacy of PAA was increased by 2–3 log values when combined with booster at 1 oz/gal. In the presence of 5% milk the efficacy increased by 2 log values at 350 ppm PAA.

DISCUSSION/CONCLUSIONS

For CIP systems (e.g., dairy) the two most challenging microbial problems are often biofilms and bacterial endospores. Peracetic acid is commonly used in CIP systems as a sanitizer/disinfectant, and while it is effective against both targets, undesirably high levels may be needed to achieve efficacy. In this study, PAA in combination with a PAA booster designed for CIP applications was tested against biofilm and endospores. The booster is formulated with a combination of organic acids, surfactants and chelants.

At a use rate of 1 oz/gal in hard water the booster **increased PAA efficacy by more than three-fold against a *P. aeruginosa* biofilm** (Figure 1). Even at a lower use level of 0.62 oz/gal, the **booster in combination with 400 ppm PAA achieved a mean 6-log reduction** against the three standard test organisms used to make biofilm kill claims in the U.S. (Figure 2).

When tested alone, the booster had no sporicidal activity against *B. spizizenii* and *C. sporogenes* endospores; however, when used with PAA at 1 oz/gal, the **booster reduced the level of PAA needed to achieve a desired level of kill by an average of 44%** (Table 1). Furthermore, the booster improved the sporicidal activity of PAA in the presence of different organic soils including milk (Figure 3).

In conclusion, the PAA CIP booster enhanced the activity of PAA against two common microbial problems in CIP systems, biofilms and bacterial endospores.

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