

How to sanitize and prevent biofilms during circulation cleaning in place (CIP) of equipment

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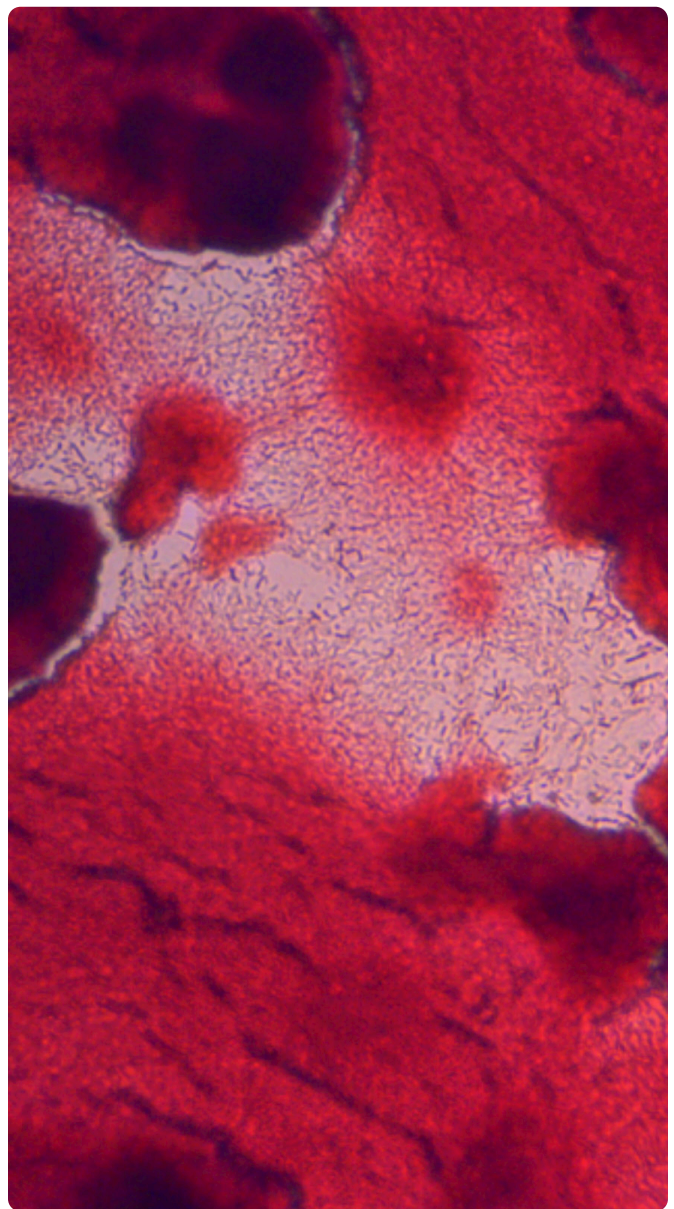
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Biofilms are known to be a major source of food contamination, causing millions of dollars in product recalls each year¹. These organic contaminants can form on any type of surface, even in conditions that normally prevent bacterial growth. Much progress has been made in recent years to eradicate biofilms; notably, peracetic acid- and surfactant-based technology has proven to be effective. One product that has been marketed is BIODESTROY®, a foaming disinfectant that kills biofilms that can form on surfaces. The product's foaming property allows the desired contact time to be achieved.

However, biofilms can also grow in pipes, especially in the dairy, brewery and juice industries. Since direct contact with the surface is impossible to maintain during pipe cleaning, equipment must be cleaned in place (CIP) with the circulation method. During this type of cleaning, the cleaning and/or disinfectant solution is pumped through the pipes for a specific period of time (at least 10 minutes). Because the pressure of the liquid must be adjusted, a foaming solution cannot be used due to the pressure fluctuation it can create in the CIP system. The setup required for circulation CIP system is also costlier. The typical system contains three solution tanks: one for the alkaline cleaner, one for the acidic cleaner, and one for the "clean" rinse water. Customers need a biofilm treatment product that can be integrated into their existing CIP system without requiring major modifications. This poses a significant challenge. In response, we developed a product that can be used for this specific type of cleaning.

Previous studies and scientific literature have shown that peracetic acid is effective against biofilm formation; it penetrates the exopolysaccharide structure and kills the microorganisms within the biofilm. Through our research, we were able to further improve the effectiveness of peracetic acid by adding adjuvants. Combining BIOTIZER™ with an acid such as ENVIRO-ACID™ increases the product's effectiveness against biofilms. This means that BIOTIZER™ can be added to the acid mixture of the CIP system and achieve the same or better sanitizing effectiveness than peracetic acid alone, but without the strong vinegar smell or corrosive effect.



¹Liangting Shao, Yang Dong, Xiaojing Chen, Xinglian Xu et Huhu Wang. Modeling the elimination of mature biofilms formed by *Staphylococcus aureus* and *Salmonella* spp. using combined ultrasound and disinfectants, *Ultrasonics Sonochemistry*, Volume 69, 2020.

Test with acid

Given the highly acidic nature of peracetic acid (pH~2), we did not expect to create a synergistic effect by adding another acid, since acids alone have no effect on biofilms. The results described in Table 1 below, however, show that there is a significant synergistic effect that allows the concentration of peracetic acid to be reduced by approximately 200 ppm. Table 1 shows the results when peracetic acid alone is used against biofilms (tests carried out according to the ASTM E2871 method). A concentration of at least 800 ppm of peracetic acid is required to achieve a 5-log reduction, which is the minimum needed to confirm efficacy.

Table 1: Antibacterial Efficacy of Peracetic Acid Alone Based on Concentration

Peracetic Acid	Log Reduction
400 ppm (20 min)	< 3.30
600 ppm (20 min)	3.43
800 ppm (20 min)	5.12
1000 ppm (20 min)	> 5.29
1200 ppm (20 min)	Total kill 6.78

According to ASTM E2871 method

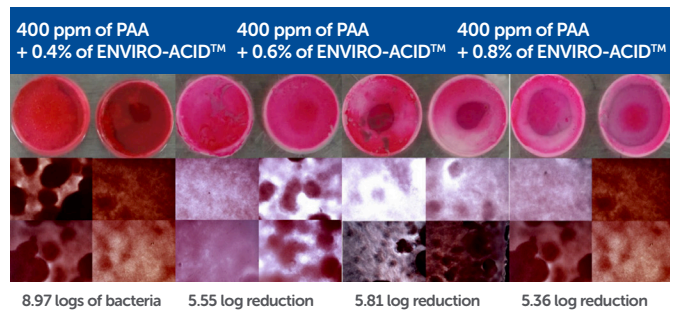
When we added nitric acid, such as from the product ENVIRO-ACID™, we were able to reduce the concentration of peracetic acid by as much as 400 ppm. Figure 1, however, shows that adding acid improved antibacterial efficacy, but it also decreased ENVIRO-ACID™'s potency to detach the biofilm from the surface.

Table 2: Variation in the Amount of ENVIRO-ACID™ Mixed with 400 ppm of Peracetic Acid (PAA)

Concentration in ENVIRO-ACID™	Log Reduction
0.2%	4.11
0.4%	4.77
0.6%	Total kill

According to ASTM E2871 method

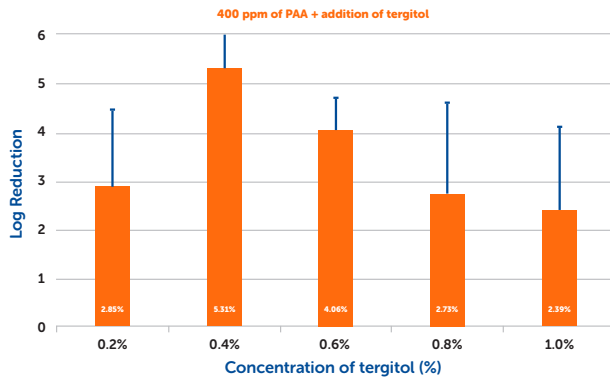
Figure 1: Efficacy in Detaching Biofilm when ENVIRO-ACID™ is Added



Test with surfactant

Given the demonstrated efficacy of the peracetic acid and surfactant contained in BIODESTROY®, adding a non-foaming surfactant to the formula was a valid hypothesis. While the intention of adding this surfactant was not to improve the product's bactericidal efficacy, Graph 1 shows that the surfactant concentration is very important; this is because the surfactant can have a synergistic effect with the peracetic acid. That said, a maximum concentration should not be exceeded to avoid any antagonistic effects.

Graph 1: Surfactant's Impact on Product Efficacy against Biofilms



The combined results of these tests indicate that by mixing 400 ppm of peracetic acid, 0.4% of ENVIRO-ACID™ (compared to 0.6% without surfactant) and 0.4% of surfactant (anionic or nonionic), a bacterial count can be performed showing more than a 5-log reduction and a biofilm detachment efficiency of more than 90%. These advances show that a technological solution for the treatment of biofilms in pipes is indeed possible.

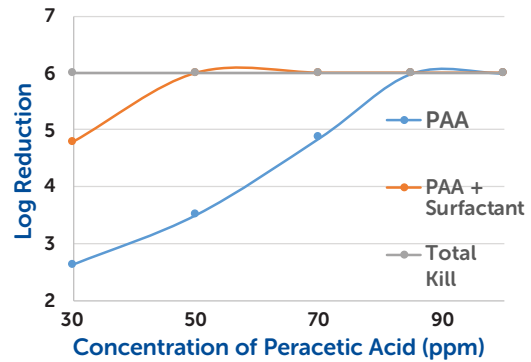
As mentioned in the introduction, CIP systems are complex and costly. The solution developed here, namely a mixture of peracetic acid, acid and surfactant, would constitute an add-on product, which may require an additional dosing system. Since the acid is already used in existing CIP systems, the use of this product is feasible. As a result, a product containing a mixture of peracetic acid and surfactant – and that can be combined with the acid already present in the system – offers the ideal solution. The product BIOTIZER™ has been developed to meet this exact need.

Sanitizing solution

Just like in the biofilm tests, the effectiveness of combining BIOTIZER™ with the acid acting as a sanitizer confirms the synergistic effect. When peracetic acid is used alone, a minimum concentration of 85 ppm is needed to obtain a 6-log reduction on *E. coli*, *Listeria*, *Salmonella*, *Pseudomonas* and *Enterococcus*. The peracetic

acid & surfactant mixture requires only 50 ppm of peracetic acid – a reduction of almost 40%. These results are illustrated in the Graph 2 below.

Graph 2: Efficacy of Peracetic Acid-Based Products against Bacteria



Conclusion

These studies show that there is a highly interesting synergy between peracetic acid, ENVIRO-ACID™ and surfactants. This synergistic mixture not only significantly reduces the amount of oxidizing agent (peracetic acid) required during treatments, it also fills the need for a viable solution for treating biofilms and sanitizing pipes in several food industry sectors. This innovative approach offers other benefits as well. Combining the acid and the sanitizer shortens both overall treatment time and water consumption. In addition to preventing and eliminating biofilms, BIOTIZER™ significantly reduces the consumption of peracetic acid. This in turn reduces the risk of corrosion, increases production time and cuts down on the strong vinegar smell.

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