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Validation Study:

PERASAN[®] Efficacy Against *Lactobacillus plantarum* in 15% Salt Brine Solutions at 40° F

Background: Meat, poultry, seafood and dairy industries often utilize brine chilling operations to cool or prepare the end product for final packaging. Brine (salt) solutions are preferred in many cases to preserve the osmotic balance between the finished product and the cooling water medium. The products subject to brine chilling may include ready-to-eat or further processed products of all kinds, shapes and ingredients. In an effort to prevent the spread of harmful organisms such as E.coli, Pseudomonas, Listeria, and Salmonella most processing companies employ some kind of antimicrobial or inhibitory treatment. Decay and shelf-life reducing organisms are also a major concern for the processor in terms of economic losses for meat and poultry producers. Lactobacillus, although typically not a human pathogen, reduces shelf-life and marketability of the final product if it is a problematic organism during food processing operations.

The goal in this evaluation is to validate the efficacy of Enviro Tech's PERASAN[®] 'A' Antimicrobial solution using *Lactobacillus plantarum* organisms under in-use conditions. PERASAN[®] 'A' is an equilibrium concentrate of 5.6% peracetic acid (PAA), 26.5% hydrogen peroxide, and minor amounts of acetic acid. The product is a very powerful oxidizer. PAA is a more powerful oxidizer than chlorine dioxide and second only to ozone at near neutral pH's (6-8). In addition, it is quite reactive to microbiological fauna, yet is selectively tolerant to many types of organic matter, fats, and oils typically associated with food processing operations such as meat, poultry, and seafood. In addition, the product is non-corrosive to most materials of construction, including aluminum and stainless steel. Since PERASAN[®] 'A' contributes no conductivity to the wastewater stream and does not persist in wastewater treatment operations, it is an ideal candidate for use as an antimicrobial treatment for chilled water or brine-based solutions.

Evaluation and Considerations: Peracetic acid (PAA) is quite unique in its broad spectrum antimicrobial use. It is well known that PAA solutions are degraded very rapidly by seawater, typically expressing a half-life of less than 3-5 minutes. However, in another paper disclosed previously by Enviro Tech, it was discovered that PERASAN[®] 'A' had surprising stability in 15% salt brine solutions at room temperature. Many antimicrobial treatments perform poorly in cold water, and it was determined to be prudent to challenge PERASAN[®] 'A' in these reduced temperature regimes against the decay-causing organism *Lactobacillus plantarum* using low concentrations (which are very economical to justify for the end-user).

Methods: 150 grams of non-iodized salt was added to 850 gms of tap water to yield a 15% w/w salt brine solution. ATCC strains of *Lactobacillus plantarum* were incubated on appropriate nutrient agars to yield colony counts that were at least 2.7×10^6 in numeration. The brine solution was pre-chilled to 38-41° F, to which were added serial dilutions of PERASAN® 'A' to yield PAA concentrations of approximately 15 ppm (activity). Subsequently, aliquats of the challenge organism were added to the brine-PAA solution, and samples of the resulting mixture were extracted and neutralized at intervals of 1, 3, 5, 10, 15 minutes. The neutralization process consisted of adding 225 ppm of pH adjusted sodium metabisulfite, which is sufficient to consume all the combined peracetic acid and hydrogen peroxide. All subsequent microbiological plating was done using the organism-specific appropriate 3-M Petrifilm plates and incubated at 35°C. Duplicate plating was the standard in all cases, and neutralization-efficacy controls were performed using non-PAA treated/neutralized challenge organisms as the confirmation of no interference of the neutralizer or brine.

Results: When challenged against 15 ppm active PERASAN® 'A' vs. *Lactobacillus plantarum* in 15% chilled brine water, the product was surprisingly effective and rapid destruction of this organism occurred. The microbiological results yielded zero growth in 3 minutes from a challenge concentration of 2.7×10^6 cfu/ml.

During all the challenge exposures the activity of the peroxyacetic acid concentration was recorded. The PAA residual was determined throughout the test challenge at each time interval using a colorimeter procedure. The test method has a peracetic acid minimum detection level of 0.03 ppm. The concentration of active PAA remained above 12 ppm in all cases.

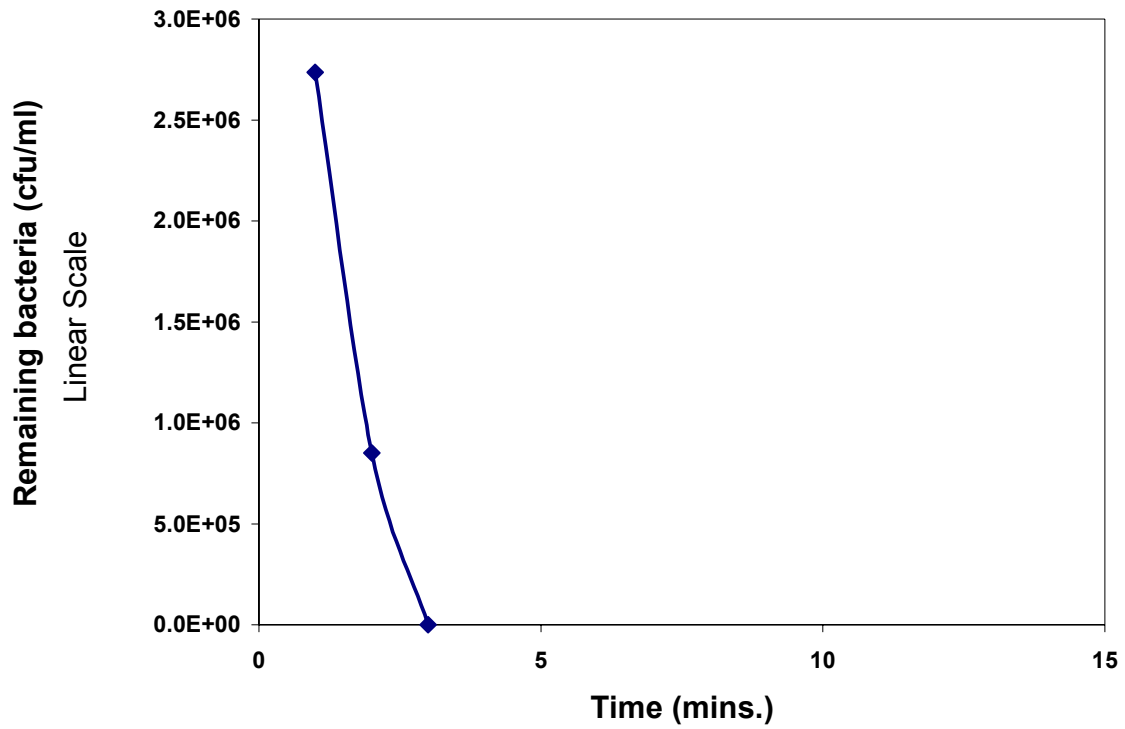
Conclusion: PERASAN® 'A' appears to be a very viable option for chilling and brine-cooling food contact situations. The product is approved by the FDA in 21 CFR 173.315 and 21 CFR 173.370 (among other citations) for use in *direct* contact situations for fruits, vegetables, meat, poultry and seafood applications that do not require a water rinse. PERASAN® 'A' has outstanding efficacy against *Lactobacillus plantarum* at low concentrations, and has 100% kill ability within 3 minutes under the conditions tested.

It was noted that typical drop-count titration test kits were highly inaccurate in the chilled 15% brine challenge system. Test strip testing proved to be accurate within each concentration color class, and it was determined that for routine field analysis the test strips were sufficiently accurate. Enviro Tech's 0-160 ppm test strips were utilized in this laboratory evaluation. The authors cannot comment of the accuracy of other brands of test strips.

Note: The results reported herein are part of intellectual property (patented) uses that have been or are being developed by Enviro Tech, Inc. Modesto, CA. This public disclosure does not waive any rights or issue conveyance of intellectual property from Enviro Tech to any person or user of Enviro Tech's peroxyacetic acid-based applications described herein without expressed written consent of Enviro Tech, Inc.

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**Efficacy of PAA (15 ppm) against *Lactobacillus plantarum*
in 15% saltwater @ 40°F**



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