Enviro Tech Chemicals, Inc

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Evaluation Study:

PERASAN® Efficacy on *E. coli* in water using various vegetables.

Background: Food safety has become prime importance to the food processor and general public alike. Recent illnesses and human exposure to pathogenic organisms have been highlighted by the media, and consecutive public health outbreaks have occurred which involved E. coli. This study will evaluate the efficacy of peroxyacetic acid (PAA) at moderate concentrations against the E. coli pathogenic organism in water that is used to process or wash asparagus, green onions and spinach.

PAA has become the antimicrobial product of choice by many food processors. It is peroxygen-based and degrades into harmless by-products and is convenient to use and measure in terms of residual concentrations. PAA is also one of the most effective products to use for organic food production and in all cases can be used without a potable water rinse.

Evaluation and Considerations: Peracetic acid (PAA) is quite unique in its broad spectrum use. It is well known that PAA solutions are more efficacious than many oxidizing biocides at low or depressed temperature regimes. Indeed, when PAA was first introduced to the USA about 20 years ago it was advertised as an effective "cold water disinfectant". Therefore, it was decided to challenge PAA in cold water which would simulate chilling or pre-cooling operations. Additionally, organic matter was to be intimately blended into the water matrix which would simulate actual process conditions. The die-away of PAA would be determined over time, and the E. coli would be plated for microbiological analysis at 30 and 60 seconds immediately after neutralization of the test solution with a sodium metabisulfite/sodium hydroxide solution (which maintained a pH of 7 in the final solution). The results are presented below.

Methods:

- 1) A stock solution of E. coli was incubated and determined to be 3.35×10^8 cfu/ml. After addition to the challenge solution the resultant population was 3.35×10^5 cfu/ml in all cases.
- 2) To 1000 ml of 150 ppm hard tap water (as CaCO₃) were added 2.5 grams of vegetable pieces which were representative of each of the 3 vegetables used in this study. One of each solution was made for asparagus, green onions and spinach. This solution was blended with a "Waring" blender on high speed for 5 minutes. The resulting mixture was then cooled down using an ice water bath to about 15° C.
- 3) A 25,000 ppm stock solution of Perasan® (15% PAA, 22% H₂O₂) was prepared by simple serial dilution using dionized water.

- 4) The subsequent challenge consisted of adding 1 ml each of the E. coli and the 25,000 ppm PAA stock solutions. Microbiological samples were taken prior to the PAA addition to serve as the control. Duplicate plating was the standard in all cases, and neutralization controls were performed using non-PAA treated/neutralized challenge organisms as the confirmation of no interference of the neutralizer solution.
- 5) After adding the PAA to the challenge solution, it was mixed thoroughly and aliquats were taken at the 30 and 60 second intervals, neutralized and plated using 3M Petrifilm E. colispecific microbiological plates, incubated at 35° C for 24 hours and then counted.

Results:

When challenged against E. coli, 25 ppm active PAA resulted in surprisingly effective and rapid destruction of this organism. The microbiological results in 1 minute resulted in log reductions from 4.3-4.7 in all cases. The PAA degraded only slightly over a 4-6 minute time period, indicating a small to moderate organic demand from these three vegetable variants. PAA appears to have very good to excellent efficacy against E. coli in simulated chilled water challenge testing.

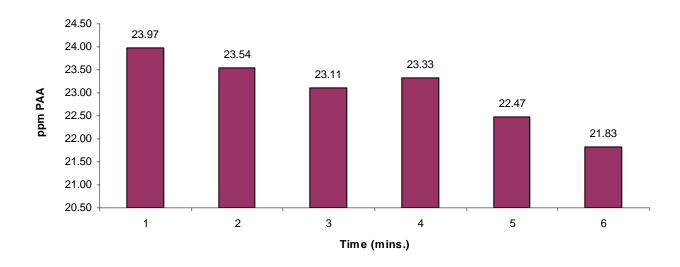
Conclusion:

PERASAN[®] appears to be a very viable option for microbiological control in chilled water for 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. This product is also approved for organic production in 7 CFR 205.605.

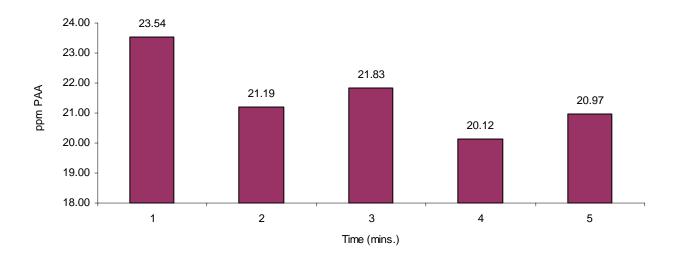
PAA DECAY ANALYSIS

0 time = 25 ppm

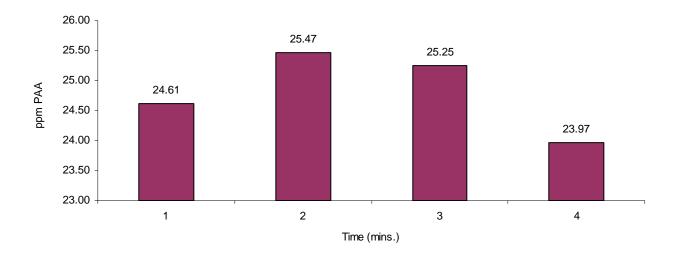
Spinach - Residual Demand Data



Green Onion - Residual Demand Data



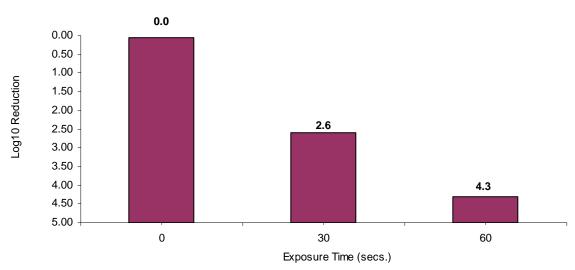
Asparagus - Residual Demand Data



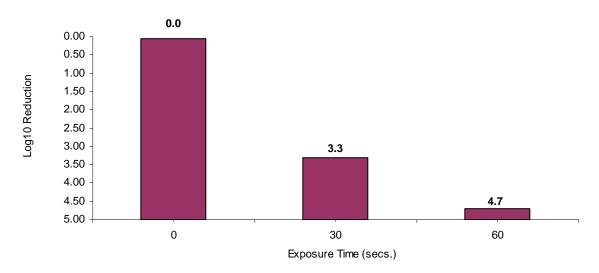
MICROBIOLOGICAL ANALYSIS

 $0 \text{ time} = 3.35 \times 10^5 \text{ cfu/ml}$

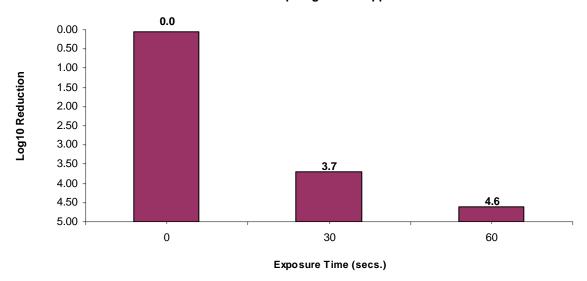
E. coli in water: Spinach vs. 25 ppm PAA



E. coli in water: Green Onion vs. 25 ppm PAA



E. coli in water: Asparagus vs. 25 ppm PAA



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