

Comparative Efficacy of Perasan MP-2 to Perasan MP-2C Against *Salmonella* Heidelberg

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Joseph Donabed B.S.

Enviro Tech Chemical Services, Inc.

Purpose

This study will determine if there is a difference in efficacy when using 10 ppm and 30 ppm as peracetic acid from Enviro Tech's Perasan MP-2 and MP-2C products. Perasan MP-2 and Perasan MP-2C were challenged against *Salmonella* Heidelberg, a representative organism from the Enterobacteriaceae family, in solution for 30 and 60 seconds to determine biocidal efficacy against the target organism.

Materials and Methods

Two cultures of *S. Heidelberg* ATCC 8326 were grown on Hardy Diagnostics 5% blood agar plates (Cat. No.:A600) and incubated at 32°C for 48 hours. One culture was used for the 10 ppm efficacy testing and one culture was used for the 30 ppm efficacy testing. Bacteria from each culture was separated from blood agar plates using a sterile L-shaped spreader and transferred to 300 mL sterile reverse osmosis water. Aliquots were taken from each bacterial stock solution and plated on 5% blood agar plates and these served as the controls for the 10ppm and 30ppm efficacy testing. Each of the bacterial stock solutions were then divided into two, 100 mL samples.

Perasan MP-2 Lot # 825-040814-1 was analyzed at 5.96% hydrogen peroxide and 15.23% peracetic acid. Perasan MP-2C Lot# JDNB4-45-1 was analyzed at 4.75% hydrogen peroxide and 22.79% peracetic acid. Each test material was diluted 1:100 with sterile reverse osmosis water. Using a micropipette, each bacterial test solution was dosed with an aliquot of the respective peracetic acid formulation (See [Table 1](#)). After 30 and 60 seconds of contact, a 10 mL aliquot was removed from each sample and neutralized with 37 µL of a 1% erythorbic acid solution for the 10 ppm peracetic acid solutions and 110 µL of a 1% erythorbic acid for the 30 ppm peracetic acid solutions. The neutralized solutions were then serially diluted and plated on 5% Sheep's Blood agar. The actual concentration of peracetic acid and hydrogen peroxide was measured using the Masters Company MP 9700 Peracetic Acid electronic meter (See [Table 2](#)).

Table 1 shows the amount of the peracetic acid product that was added to each test solution.

Description	Volume of 1:100 Test Solution added
Perasan MP-2 10 ppm	660 µL
Perasan MP-2C 10 ppm	1970 µL

Perasan MP-2 30 ppm	440 µL
Perasan MP-2C 30 ppm	1320 µL

Table 2 details the pH prior to dosing with peracetic acid and the pH after dosing with peracetic acid as well as the actual concentrations of peracetic acid and hydrogen peroxide in solution.

Description	Initial pH	Final pH	ppm PAA	ppm H ₂ O ₂
Control	7.87	7.87	0	0
Perasan MP-2 10 ppm	7.86	4.85	9.8	3.5
Perasan MP-2C 10 ppm	7.85	4.88	9.7	1.4
Perasan MP-2 30 ppm	7.85	4.65	27.4	10.4
Perasan MP-2C 30 ppm	7.88	4.71	27.0	4.2

Results and Discussion

The blood agar plates were incubated at 32°C for 48 hours. After incubation the plates were enumerated.

Table 3 shows the log₁₀ reduction in *Salmonella* Heidelberg colonies after treatment with 10 ppm peracetic acid from Perasan MP-2 and MP-2C.

Description	Avg. log ₁₀ (CFU/mL)	log ₁₀ Reduction (CFU/mL)	% Reduction
Control	5.42	0	0
MP-2 10 ppm 30 sec.	4.36	1.06	91.290
MP-2 10 ppm 60 sec.	0	>5.42	>99.999
MP-2C 10 ppm 30 sec.	4.27	1.15	92.921
MP-2C 10 ppm 60 sec.	0	>5.42	>99.999

Table 4 shows the log₁₀ reduction in *Salmonella* Heidelberg colonies after treatment with 30 ppm peracetic acid from Perasan MP-2 and MP-2C.

Description	Avg. log ₁₀ (CFU/mL)	log ₁₀ Reduction (CFU/mL)	% Reduction
Control	6.50	0.00	0%
MP-2 30 ppm 30 sec.	0	>6.50	>99.9999
MP-2 30 ppm 60 sec.	0	>6.50	>99.9999
MP-2C 30 ppm 30 sec.	0	>6.50	>99.9999
MP-2C 30 ppm 60 sec.	0	>6.50	>99.9999

The control for the for the samples treated with 10 ppm Perasan MP-2 and MP-2C had an average *Salmonella* Heidelberg \log_{10} of 5.42 CFU/mL. After 30 seconds of treatment with a nominal 10 ppm peracetic acid from Perasan MP-2, the average \log_{10} *Salmonella* Heidelberg colonies remaining was 4.36 CFU/mL which equates to a 1.06 CFU/mL reduction. Treatment with 10 ppm peracetic acid from Perasan MP-2 for 60 seconded yielded zero viable *Salmonella* Heidelberg colonies.

After treatment with 10 ppm of peracetic acid from Perasan MP-2C for 30 seconds the average \log_{10} *Salmonella* Heidelberg colonies remaining was 4.27 CFU/mL which equates to a 1.15 CFU/mL reduction. Treatment with 10 ppm peracetic acid from Perasan MP-2C for 60 seconds yielded zero viable *Salmonella* Heidelberg colonies.

The control for the for the samples treated with 30 ppm Perasan MP-2 and MP-2C had an average *Salmonella* Heidelberg \log_{10} of 6.50 CFU/mL. After 30 seconds of treatment with a nominal 30 ppm peracetic acid from Perasan MP-2 and Perasan MP-2C there was zero viable *Salmonella* Heidelberg colonies remaining. Therefore, this data suggests that treatment with a nominal 30 ppm from either Perasan MP-2 or Perasan MP-2C will yield the same results. After 30 seconds of treatment there was a \log_{10} reduction of >6.50 CFU/mL.

Therefore, this data suggests that the efficacy of Perasan MP-2 and Perasan MP-2C against *Salmonella* Heidelberg is virtually indistinguishable.

Conclusions

- When Perasan MP-2 and Perasan MP-2C are diluted to 10 and 30 ppm (as peracetic acid) the Perasan MP-2C contains approximately 50% less hydrogen peroxide than the Perasan MP-2.
- When planktonic *Salmonella* Heidelberg was treated for 30 seconds with a nominal 10 ppm peracetic acid from Perasan MP-2 or Perasan MP-2C there was a \log_{10} reduction of 1.06 and 1.15 CFU/mL, respectively. After 60 seconds of treatment there were zero remaining colonies.
- When planktonic *Salmonella* Heidelberg was treated with a nominal 30 ppm peracetic acid from Perasan MP-2 or Perasan MP-2C there was a \log_{10} reduction of >6.50 CFU/mL (99.9999% reduction).
- The results of this study suggest that even though the Perasan MP-2C contains lower concentrations of hydrogen peroxide, there is no decrease in efficacy against *Salmonella* Heidelberg. The active peracetic acid in the two formulas is the causative agent responsible for decreasing bacterial colonies.
- Since *Salmonella* Heidelberg is a representative organism from the Enterobacteriaceae family, the Perasan MP-2C efficacy against other organisms from this family should be similar.