

# Activity of a pH Neutral Super-Oxidized Solution Against Bacteria Selected for Sodium Hypochlorite Resistance

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## OBJECTIVE

The objective of this research was to compare Microcyn®'s Super-Oxidized Solution (SOS), acidified bleach (NaOCl, pH 7), and hypochlorous acid (HOCl) in terms of efficacy against bacterial isolates with demonstrated resistance to domestic bleach solutions.

## BACKGROUND

Due to common use of chlorine compounds for water and surface disinfection, it is easy to isolate bacteria demonstrating resistance to these compounds. This study's purpose was to examine the activity of a pH neutral super-oxidized solution (SOS), Microcyn®, against bacteria selected for high resistance to domestic bleach containing sodium hypochlorite (NaOCl).

## MATERIALS & METHODS

NaOCl-resistant bacteria were isolated from pool water, recycled grey water, treated waste-water, and river soil. Ten bacterial isolates, identified by sequence analysis and demonstrating resistance to 5 min exposures of 40% domestic bleach (NaOCl at 24,000 ppm free-chlorine [FC] by the DPD-titration method), were used to test the activity of the SOS, which contained 75-80 ppm FC as determined by DPD-titration. Cell suspensions adjusted to 10<sup>8</sup> colony forming units (cfu)/ml in diH<sub>2</sub>O were prepared from 24 hr growth on tryptic soy agar (TSA). 1 ml of a suspension was added to 5 ml SOS or di H<sub>2</sub>O (control for quantitative dilutions). Timed SOS exposures of 30 sec, 1 min, and 2 min were neutralized by adding 5 ml tryptic soy broth. 100 µl of each neutralized-SOS-cell mixture and appropriate quantitative control dilutions were spread-plated onto TSA; then incubated at 37°C. Additional comparisons were made using 1) acidified bleach (pH = 7) at 94 ppm FC and 2) hypochlorous acid (HOCl) standard at 75 ppm FC, using the same protocol. Plates were examined and cfu's enumerated after seventy-two (72) hrs. Log<sub>10</sub> reductions of the SOS-treatments were calculated against the control cfu counts obtained for each isolate.

## RESULTS

At 30 sec of SOS-exposure, 3 of 9 isolates tested had 5 log<sub>10</sub> reductions, 1 isolate had a 4 log<sub>10</sub> reduction; the remaining 5 isolates showed no detectable reduction. After 1 min of exposure, 4 of 10 isolates had reductions of 5 logs; the other 6 isolates had reductions of 4 logs. After 2 min SOS-exposure, no growth was detectable for any of the 10 isolates. In contrast, following 2 min exposure to acidified bleach, 5 of 9 isolates showed no detectable reduction, 1 isolate had a 4 log<sub>10</sub> reduction, 2 isolates had a reduction of 5 logs, and 1 had a 6 log<sub>10</sub> reduction. Following 2 min exposure with hypochlorous acid standard, 7 of 10 isolates showed no detectable reduction of growth, 1 isolate had a 4 log<sub>10</sub> reduction, 1 isolate was reduced by 5 logs, and 1 isolate had no detectable growth. See Table 1.

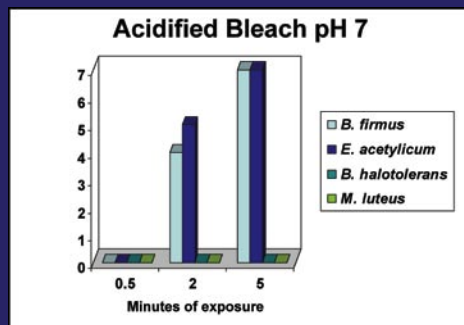
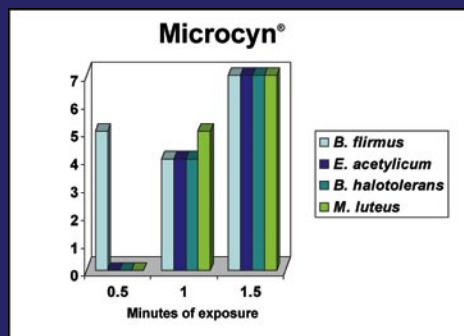
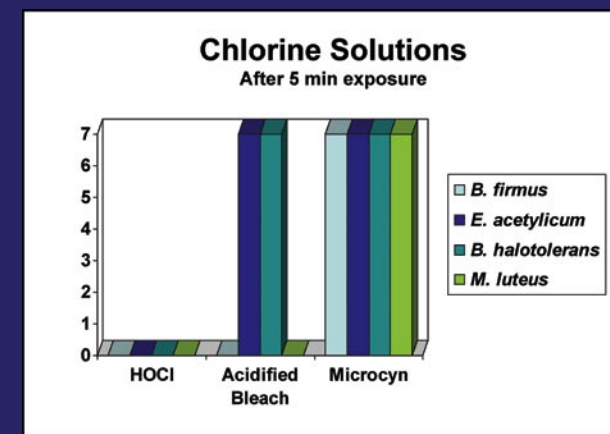


Table 1: Microcyn® Efficacy vs. Bleach-Resistant Bacteria After 2 min. Exposure.

Organism	Reduction in Organisms (at 72 hrs)		
	Microcyn	Acidified Bleach (pH 7)	HOCl
<i>Bacillus firmus</i>	100%	Undetectable	Undetectable
<i>Bacillus azotoformans</i>	100%	10 <sup>6</sup>	10 <sup>4</sup>
<i>Exiguobacterium acetylicum</i>	100%	10 <sup>4</sup>	Undetectable
<i>Microbacterium sp</i>	100%	NA	Undetectable
<i>Brevibacterium halotolerans</i>	100%	10 <sup>3</sup>	Undetectable
<i>Bacillus firmus</i>	100%	Undetectable	100%
<i>Bacillus thuringiensis</i>	100%	Undetectable	Undetectable
<i>Micrococcus luteus</i>	100%	Undetectable	Undetectable
<i>Brevibacterium halotolerans</i>	100%	Undetectable	Undetectable
<i>Bacillus fastidiosus</i>	100%	10 <sup>5</sup>	10 <sup>4</sup>



## CONCLUSION

These results demonstrate the unique nature of this pH neutral SOS. It is not simply pH neutral dilute bleach; it is a much more powerful solution of oxidants that are able to eliminate even hypochlorite-resistant bacteria. It also demonstrated greater efficacy than pure hypochlorous acid.