The efficacy of long-lasting residual drinking water disinfectants based on hydrogen peroxide and silver


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Abstract: The aim of the present work was to evaluate the disinfectant capacity and the possible fields of application of a combined silver and hydrogen peroxide (HP) water disinfectant. The findings demonstrated the high bactericidal action of silver (on E. coli) and its relatively ineffective virucidal effect (on MS-2 phage).

HP was found to have a small bactericidal effect and a mild virucidal one. When combined, silver and HP usually exhibited a synergistic action on the viability of E. coli and on the luminescence of recombinant luminescent E. coli. In some instances, the combined bactericidal effects were 1000-fold higher than the sum of the separate ones. No increased virucidal action was observed. The biocidal action of the combination generally increased with increasing temperature and pH, and decreased in secondary and tertiary effluents. The physiological effects and mechanisms of toxicity of HP, silver and their combinations, were assessed by monitoring the induction of stress promoters upon exposure to the active agents, and by assessing the sensitivity of E. coli mutated in major stress responses to HP, silver and their combinations.

The results showed that HP induced a wide array of stress responses, that both silver and HP induced promoters regulated by the heat shock response, and that the dnaK promoter (regulated by the heat shock response) was synergistically induced. The mutant sensitivity tests showed that bacteria deficient in the ability to activate central cellular stress responses (SOS, heat shock, stationary phase, oxidative) were hypersensitive to both HP and silver. These results imply that cellular proteins, and possibly the DNA, are the cellular moieties chiefly affected. The above findings suggest that the potentiated effect of HP and silver is a metabolically dependant/related process that stems from a combination and/or accumulation of physiological effects exerted by the active ingredients.

The physico-chemical properties of the combined disinfectant, and its disinfection capacity, points to its potential application as a long-term secondary residual disinfectant for water of relatively high quality.

Keywords: Disinfection; silver; hydrogen peroxide; synergistic effect; bioluminescence; stress genes; viruses

Introduction

The aim of this research was to study the disinfectant capacity and to evaluate the applicability of a new, combined hydrogen peroxide (HP) and silver formulation suggested as an alternative water disinfectant (commercial name Steril or Sanosil). The study consisted of two parallel investigational routes. The first assessed the separate and combined biocidal capabilities of the active ingredients (HP and silver) under varying conditions. Among the variables were active ingredient concentrations, experimental pH and temperature, type of target organism and the composition of the water. The obtained results provided the basic efficacy data and set the framework for the “second” investigational route aimed at determining the physiological effects and attempting to elucidate the possible mechanisms of toxicity of the combined disinfectant.

The physiological effects exerted by HP and silver were studied by monitoring changes in in vivo luminescence of challenged luminescent E. coli bacteria, as the drop in in vivo luminescence may reflect the extent of the toxic effects to which the bacteria are exposed (Ulitzur, 1991; Meighen, 1993; Pedahzur et al., 1997) and by monitoring the induction of cellular stress promoters upon exposure to HP and silver in bacteria harboring fusions of the...
structural *lux* genes to promoters of different stress genes (Van Dyk et al., 1994; Belkin et al., 1996). In addition, the sensitivity of bacteria mutated in the central cellular stress responses to HP and silver was evaluated. The mutant sensitivity tests complimented the stress promoter induction tests and provided additional information regarding the involvement of different cellular stress responses in the long-term response to HP and silver.

**Materials and method**

Most of the materials, methods and experimental procedures applied in this study (viability studies and luminescence tests) have been described elsewhere (Pedahzur et al., 1995; Pedahzur et al., 1997; Pedahzur, 1997; Barnea, 1998). Additional experimental procedures will be described.

**Phage assays** Phage type, cultivation, enumeration, and experimental procedures are described elsewhere (Nasser et al., 1993; Barnea, 1998).

**Tertiary effluents** Tertiary effluents were obtained from “Shafdan”, the Dan region reclamation program. The typical effluent quality was: BOD<0.5 mg/l, COD=6.6 mg/l, TOC=5.4 mg/l, pH=8.2 and NTU=1.3.

**Results**

During the experimental work, both the commercial product (Steril) and equivalent formulations prepared in the laboratory were used. In studies that investigated the separate and combined effects, only self-prepared formulations were used. In general, as the performance of the commercial and the equivalent laboratory prepared formulations were found to be similar, the results presented here (unless otherwise noted) were obtained by applying the self-prepared formulations.

**Inactivation (efficacy) tests**

Inactivation tests were performed on two *E. coli* strains and on the MS-2 phage. The experiments were conducted in tap water, phosphate buffer and secondary and tertiary effluents. The effects of experimental pH, temperature, exposure times and agent concentrations on the activity of the disinfectant were assessed. The experiments here presented were performed in either phosphate buffer or tertiary effluents (described above).

**The efficacy of HP, silver and their combinations, on E. coli B and the MS-2 phage** (Katzenelson, 1996; Barnea, 1998). The efficacy of HP, silver and their combinations (in terms of log reduction) on *E. coli* B and MS-2 phage in phosphate buffer and in tertiary effluents, is presented in Table 1.

**Table 1** The separate and combined log reduction values of H$_2$O$_2$ and silver against *E. coli* B and MS-2 phage, in phosphate buffer and tertiary effluents, at an exposure of 120 min. The initial bacterial concentration was 1×10$^5$ CFU/ml and the MS-2 phage concentration was 1×10$^5$ PFU/ml.

<table>
<thead>
<tr>
<th></th>
<th>H$_2$O$_2$</th>
<th>Ag$^+$</th>
<th>H$_2$O$_2$+Ag$^+$</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>E. coli B</td>
<td>0.4</td>
<td>2.3</td>
<td>H$_2$O$_2$ (30 mg/l), Ag$^+$ (30 µg/l)</td>
</tr>
<tr>
<td>Buffer</td>
<td>MS-2 phage</td>
<td>2.4</td>
<td>0.2</td>
<td>H$_2$O$_2$ (100 mg/l), Ag$^+$ (100 µg/l)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>E. coli B</td>
<td>0.1</td>
<td>0.7</td>
<td>H$_2$O$_2$ (30 mg/l), Ag$^+$ (30 µg/l)</td>
</tr>
<tr>
<td>Effluents</td>
<td>MS-2 phage</td>
<td>2.8</td>
<td>0.1</td>
<td>H$_2$O$_2$ (100 mg/l), Ag$^+$ (100 µg/l)</td>
</tr>
</tbody>
</table>
The results show that the efficacy of HP against *E. coli* B was low but was more pronounced against the MS-2 phage. Silver exhibited a mild activity against *E. coli* B but was inactive against MS-2 phage. When combined, a slightly increased bactericidal effect, and a diminished virucidal one, were observed. Additional experiments revealed a substantial bactericidal effect by HP and silver combinations holding as little as 10 µg/l of silver and 10–30 mg/l of HP (Katzenelson, 1996; Barnea, 1998).

The results obtained in tertiary effluents showed a substantial decrease in the bactericidal activity of silver (the dominant active ingredient) which was probably caused by interfering complexing agents and chloride ions present in the effluents. The virucidal activity (dominated by the activity of HP) was not as affected by the interferences present in the effluents. The influence of pH and temperature on the activity of HP and silver was also examined (Katzenelson, 1996; Barnea, 1998). The results (not presented) showed that increasing the pH and temperature increased the bactericidal action of HP, silver and their combinations. On the other hand, while the virucidal activity of HP was indifferent to temperature changes, it decreased with increasing pH (probably due to its decomposition at basic pHs). The individual virucidal activity of silver, which was originally very low, was not affected by either temperature or by pH changes.

The increased bactericidal effect of the combination of HP and silver was verified by additional efficacy studies conducted on wild type *E. coli* K-12, known to be more resistant than *E. coli* B to environmental stresses. The efficacy of HP on *E. coli* MC 4100/pChU1 was low. Concentrations as high as 250 mg/l had no apparent effect on its viability. Silver exhibited a higher efficacy of 0.3 and 1 logs reduction at concentrations of 25 and 50 µg/l, respectively. When combined, a pronounced increase in the bactericidal effect was observed. For example, when separately administered, 100 mg/l of HP and 25 µg/l of silver caused a reduction of 0.08 and 0.31 logs, at a contact time of 60 min., respectively. When combined, a reduction of 2.3 logs was obtained, thus resulting in a synergistic effect of 1.9 logs (Pedahzur, 1997). A synergistic effect in the action of HP and silver was also discovered in the luminescence tests, in which the luminescence of recombinant *E. coli* (*E. coli* MC 4100/pChU1) was monitored (Pedahzur et al., 1997). The results of the luminescence and viability tests correlated well, reaffirming the validity of the results, and further justified the use of bacterial luminescence for toxicity evaluation (Pedahzur, 1997).

**The biocidal activity of HP and copper (Barnea, 1998).** In a complementary set of experiments the bactericidal and virucidal activities of HP (30 mg/l) and copper (ranging from 125 to 1400 µg/l) were evaluated. Representative results are presented in Table 2.

The results demonstrate the low bactericidal and virucidal effects of HP, the low bactericidal but appreciable virucidal effects of copper, and the enhanced effects of their combination, on both bacteria and viruses (unlike the combined effect of HP and silver). Comparison of the separate activities of HP with either silver or copper in phosphate buffer and tertiary effluents (Katzenelson, 1996; Barnea, 1998) revealed a substantial decrease in the separate and combined activity of both silver and copper. It is plausible that the

<table>
<thead>
<tr>
<th></th>
<th>H2O2 (30 mg/l)</th>
<th>Cu++ (250 µg/l)</th>
<th>H2O2 and Cu++</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli B</td>
<td>0.1</td>
<td>0.1</td>
<td>3.5</td>
</tr>
<tr>
<td>MS-2 phage</td>
<td>0.5</td>
<td>3.8</td>
<td>5.3</td>
</tr>
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presence of organic or inorganic ligands in the effluents diminishes the activity of these metals, and as a result, their combined efficacy with HP.

Stress gene induction and mutant sensitivity tests
The final stage of the research included an attempt to study the physiological effects and mechanisms of toxicity of the active ingredients. This was achieved by monitoring the induction of stress promoters, in bacteria harboring different stress promoters fused to the structural genes of the lux system, upon exposure to HP, silver and their combinations. In addition, the involvement of major stress responses in the response to HP and/or silver was evaluated by determining the extent of growth inhibition (in inhibition zone tests) of bacteria carrying mutations in specified stress responses upon exposure to HP and silver. The expectancy was that the exposure of bacteria lacking stress responses, which participate in the response to the active ingredients in question, would result in an increased sensitivity (a larger inhibition zone) of the challenged bacteria. Unlike the gene induction tests that reflect the short-term cellular responses, the mutant sensitivity tests reflect the long-term damages caused by the active agents, because the effects on the viability of the bacteria are assessed. The results of the promoter induction tests (partially presented in Pedahzur et al., 1997) and the mutant sensitivity tests, are presented in Table 3.

The results of the stress promoter induction tests confirmed that HP is a potent inducer of a variety of stress promoters belonging to the major cellular stress responses (Van Dyk et al., 1994a; Belkin et al., 1995). Silver induced the katG, grpE, lon and uspA promoters (belonging to the oxidative, heat shock and stationary-phase stress responses). Upon joint administration, the grpE promoter was induced additively and the dnaK gene was synergistically induced. Both genes are regulated by the heat shock response that responds to protein damages. Such an increased induction of heat shock regulated stress promoters upon exposure to a combination of toxicants was reported previously (Van Dyk et al., 1995). The mutant sensitivity tests revealed that bacteria mutated in the recA, oxyR, rpoH and rpoS genes were all hypersensitive to both HP and silver. These results correlate well with the gene induction tests. In addition, the finding that recA mutant bacteria were hypersensitive to silver suggested that the DNA is also an affected cellular moiety.

Discussion
The aims of this research were to study the characteristics of a new water disinfectant comprising HP and silver, to assess its fields of application and to attempt to elucidate its possible mechanisms of toxicity.

The efficacy studies were conducted on bacteria and viruses under varying conditions (Pedahzur et al., 1995; Zuaretz, 1996; Katzenelson, 1997; Pedahzur et al., 1997; Pedahzur,
1997; Barnea, 1998) and the relative stability and capacity in maintaining the quality of emergency waters for several months (Ben Bassat, 1996) illustrated the disinfectant capacity and kinetics of the combined disinfectant, and underlined its possible application as a long acting residual disinfectant for waters of high quality.

The findings of an enhanced effect of HP and silver on the immediate metabolic state of bacteria (as reflected in the luminescence tests) and on viability, as opposed to the indifferent virucidal effect, pointed to the possibility that the enhanced physiological damages exerted by HP and silver are metabolically related dependent processes.

Furthermore, the findings that unlike HP and silver, HP and copper, which are known to produce active oxygen species (Stohs and Bagchi, 1995), exhibit an enhanced bactericidal and virucidal effect, as was reported by other investigators (Sagripanti et al., 1992; Sagripanti et al., 1993), pointed to a possible mechanistic difference in the toxicity mechanisms of silver and copper with HP. It thus seemed that the enhanced toxic effect of HP and silver might result from the combination and/or accumulation of physiological damage exerted by these agents, rather than from the formation of an active oxygen species. Additional support for this assumption was obtained from the stress-promoter induction tests and the stress-response mutant bacteria sensitivity tests.

The major findings of the stress promoter induction tests (Pedahzur, et al., 1997) were that silver induced several stress promoters, specifically ones regulated by the heat shock response. These results, that target cellular proteins as the moieties immediately effected, are in agreement with current knowledge of the metal’s known effect on -SH protein groups (Thurman and Gerba, 1989). HP was found to be a strong inducer of most of the stress promoters tested. The similarities in the induction of different stress promoters by HP and silver suggested that these agents might affect similar cellular moieties.

The finding of a synergistic induction of the dnaK promoter, which is regulated by the heat shock response (that responds to protein damage), indicated that enhanced damage to cellular proteins may be the cause of the enhanced toxic effect of HP and silver.

The mutant sensitivity tests revealed that bacteria mutated in the recA, oxyR, rpoH and rpoS genes, were all hypersensitive to HP and silver and pointed to their involvement in the response to the toxic effects exerted by these agents. These results correlated well with the stress promoter induction tests and gave additional evidence that cellular proteins, and possibly the DNA, may be the major moieties affected.

This assumption is conceivable, as both agents are considered to affect cellular proteins and DNA (Thurman and Gerba, 1989; Schrufstatter et al., 1990; Russell and Hugo, 1994). The fact that the toxic effects of silver on the DNA were not evident in the stress promoter induction tests (the recA fusion was not induced) but were evident in the mutant sensitivity tests (recA mutants were hypersensitive to silver) suggests that the toxicity of silver may appear in later stages of the bacterial metabolism.

Conclusions

Our results, which indicate that both HP and silver may induce similar stress promoters and exhibit increased toxicity towards bacteria mutated in similar stress responses, and that the enhanced effect is metabolically dependant/related may imply that the enhanced combined toxicity of HP and silver may result from the combination and/or accumulation of physiological effects exerted by the active ingredients rather than by the ability to interact in the well documented redox reactions in which active oxygen species are produced, i.e. the Haber-Weiss mediated Fenton redox reactions (Imlay et al., 1988; Stohs and Bagchi, 1995).

We finally conclude that the characteristics of the combined disinfectant, i.e. the slow and moderate bactericidal effect, and the prolonged stability and efficacy at relatively low concentrations, point to its use as a secondary long-acting residual disinfectant for good quality drinking waters.
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References


