Microbicidal efficacy of an advanced oxidation process using ozone/hydrogen peroxide in water treatment


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Abstract The combined application of ozone and hydrogen peroxide represents a kind of advanced oxidation for water treatment. The radicals that are generated during the process are used for the degradation of organic pollutants from groundwater and industrial effluents. The aim of our study was to evaluate the possible microbicidal, and particularly virucidal, efficacy of such a process, since no substantial data were available. The investigations were performed at a pilot plant installed for the elimination of perchloroethylene from polluted groundwater (reduction efficacy for perchloroethylene from 26 µg/L to 5 µg/L). To enable a reliable evaluation of the microbicidal effect, a set of alternate test organisms was used. As model viruses we chose bacteriophages MS2 (F+ specific, single-stranded RNA), ΦX174 (single-stranded DNA) and PRD-1 (coated, double-stranded DNA). Furthermore, spores of Bacillus subtilis were included as possible surrogates for protozoa and Escherichia coli as representative for traditional indicator bacteria used in water analysis. The microbicidal efficiency was compared to the inactivation by means of ozone under two standard conditions (20°C): (a) 0.4 mg/L residual after 4 min and (b) 0.1 mg/L residual after 10 min. Surprisingly, a good microbicidal effect of the ozone/hydrogen peroxide process was found. This was somewhat unexpected, because we had assumed that the disinfection potential of ozone would have been interfered with by the presence of hydrogen peroxide. Escherichia coli and the three test viruses revealed a reduction of about 6-log. In contrast, spores of Bacillus subtilis showed after the total process a reduction of 0.4-log. These results matched the effect of the ozone treatment (a) with a residual of 0.4 mg/L after 4 min contact time (20°C). The test condition (b) with a residual of 0.1 mg/L ozone after a contact time of 10 min at 20°C gave a higher reduction of the B. subtilis spores (1.5-log). The presented study revealed a satisfying microbicidal efficacy of the ozone/hydrogen peroxide process with respect to vegetative bacteria and viruses (bacteriophages). However, it has to be emphasised that intense mixing and sufficient contact time have to be optimised and tested for each individual installation.

Keywords Advanced oxidation; bacteriophages; bacterial spores; hydrogen peroxide; ozone; water disinfection

Introduction

Advanced oxidation technologies have proven to be very effective in treating a wide variety of organic contaminants, such as perchloroethylene, trichloroethylene, methyl tert-butyl ether, and many others, in contaminated groundwaters and industrial effluents. These technologies utilise powerful oxidising intermediates (mainly OH radicals) to oxidise organic pollutants, leading not only to their destruction, but also, given sufficient conditions, to their complete mineralisation. The OH radicals can be generated, for example, by the combined application of ozone/hydrogen peroxide, ultraviolet radiation/ozone, ultraviolet radiation/hydrogen peroxide, ozone/electron beam (Gehringer et al., 1992; Legrini et al., 1993; Acero et al., 2001). However, so far no substantial data on the inactivation of microorganisms due to advanced oxidation processes in water are available.

It is well known that the routinely used faecal bacterial indicators, e.g. Escherichia coli, are too sensitive to disinfectants compared to viral and protozoan pathogens and are,
therefore, not appropriate to evaluate disinfection processes. Thus, there is a need for the introduction of alternative indicators to test the performance of water treatment efficiencies (Huertas et al., 2003). Bacteriophages have been proposed as viral indicators, in particular coliphage MS2, which is similar in size and shape to human enteric viruses (Anon., 1991; ECSMTP, 1999). Spores of Bacillus subtilis have been successfully established for the evaluation of UV plants for water disinfection and have also been found to be a valuable surrogate for testing the ozone inactivation of Giardia lamblia cysts and Cryptosporidium parvum oocysts (Sommer and Cabaj, 1993; Facile et al., 2000).

The present study was performed at a pilot plant installed for the elimination of perchloroethylene from polluted groundwater. The treatment process was optimised to reduce perchloroethylene concentration from about 28 µg/L to about 5 µg/L. The aim of our study was to evaluate the bactericidal and virucidal efficacy of the ozone/hydrogen peroxide process in the pilot plant by means of three bacteriophages, spores of B. subtilis and E. coli. Since advanced oxidation technologies do not belong to the recognised disinfection processes, we performed inactivation experiments with ozone, which represents an internationally accepted disinfection procedure under well-controlled batch conditions as a comparison standard.

Materials and methods
Pilot-plant (ozone/hydrogen peroxide process) investigations
The investigations were performed at a pilot plant installed for the elimination of perchloroethylene from polluted groundwater. The plant consisted of a dosing station for ozone and hydrogen peroxide, followed by a static mixer and a reaction vessel. The reaction vessel was subdivided into six sectors to enable sufficient mixing. Each sector was equipped with a sampling valve that made it possible to measure the efficiency of the treatment process over the contact time (Figure 1).

The test conditions were the following: water flow – 30,000 L/h; ozone dosage – 2.5 mg/L; hydrogen peroxide dosage – 1.5 mg/L. The retention and contact times are given in Table 1. The water temperature was 11.0°C and the pH 7.6.

The test organisms were pumped from a permanently mixed storage tank into the inflow of the well water in order to obtain a concentration of about 10⁶ of each organism per volume analysed. A static mixer enabled a homogeneous distribution. At the sites indicated by numbers (Figure 1), samples were taken into sterile glass flasks containing sodium thiosul-
phate and catalase to quench the residuals of ozone and hydrogen peroxide. Two series of tests were performed with five test organisms each on two different days, giving for each test organism seven samples which were analysed in duplicate. For control purposes, the concentrations of perchloroethylene were measured in the raw and treated waters of both test series.

### Ozone inactivation experiments

A batch reactor with continually declining residual ozone was used. The ozone batch reactor consisted of a 1.5 L glass vessel equipped with a magnetic stirrer and a cooling system. Ozone was produced by an ozone-generator using pure oxygen (Sander GmbH, Germany). Two test conditions were proven: (a) residual ozone concentration 0.4 mg/L after a contact time of 4 min and (b) 0.1 mg/L residual ozone after a contact time of 10 min. The initial ozone dosages required for these test conditions were determined empirically (condition 1 – dosage about 2.5 mg/L; condition 2 – dosage about 3.1 mg/L). The ozone concentration was measured by the spectrophotometric indigo trisulphonate method (Bader and Hoigné, 1981).

The vessel of the batch reactor was filled with 500 mL of tap water and preozonated to the desired ozone concentration by bubbling ozone into the water with a glass diffuser. An aliquot of the stock solution of the respective test organism was added to give an initial concentration of $10^6$ organisms/mL. The content was continuously stirred during the whole experiment. The temperature of the water was 20–22°C and the pH 7.6. Samples were taken for the enumeration of the microorganisms (bacteriophages $\Phi$X174 and MS2, *E. coli* and spores of *B. subtilis*) at specified intervals into sterile vessels containing sodium thiosulphate and analysed in duplicate. All experiments were done at least twice.

### Test organisms

**Bacteriophages.** (a) MS2 (F+ specific, single-stranded RNA virus, Leviviridae): propagation and enumeration according to ISO 10705-1; host bacterium *Salmonella typhimurium* WG 49; (b) $\Phi$X174 (single DNA virus, Microviridae): propagation and enumeration according to ISO 10705-2, host bacterium *E. coli* WG 5; (c) PRD-1 (coated, double-stranded DNA virus, Tectiviridae): propagation and enumeration according to the method of the University of South Florida (USF); host bacterium *Salmonella typhimurium* ATCC 18585.

Before use the phage stock solutions were filtered through a 0.2 µm membrane (Millex-GV, Millipore).

**Bacteria.** (a) *Escherichia coli* ATCC 11229: cultured on Columbia agar (CM 331, Oxoid) for 24 h at 37°C. Cells were carefully removed from the agar layer and suspended in sterile 0.85% saline. The enumeration as colony-forming units (CFU) was performed by membrane filtration and violet red bile agar (CM 107, Oxoid; 37°C/48 h); (b) Spores of *Bacillus subtilis* ATCC 6633: spores were produced in liquid enrichment medium, heated at 80°C for 10 min, washed by centrifugation, and suspended in sterile distilled water (Sommer and
Cabaj, 1993). The enumeration as CFU was performed by pour plating with plate count agar (CM 325, Oxoid; 37°C/48 h).

The concentrations of the test organisms were transformed to log_{10} and the reductions log \((N/N_0)\) were calculated from the concentrations before \((log-N_0)\) and after treatment \((log-N)\).

**Results and discussion**

In the ozone/hydrogen peroxide pilot plant, initial dosages of 2.5 mg/L ozone and 1.5 mg/L hydrogen peroxide were applied. The decreases of residuals of the two chemical substances at specific sampling sites are given in Table 2. At the end of the process no hydrogen peroxide and only traces of ozone were detectable.

The inactivation experiments showed, surprisingly, the beneficial microbicidal effect of the ozone/hydrogen peroxide pilot plant (Table 3). This was unexpected, because it had been assumed that the disinfection potential of ozone would be interfered with by the presence of hydrogen peroxide. Moreover, hydrogen peroxide, as such, did not possess a significant virucidal potential and, therefore, could not contribute to the disinfection. In contrast to ozone, hydrogen peroxide had only a weak microbicidal activity in water disinfection. The low virucidal effect of hydrogen peroxide was shown in a recent study, in which we found that the numbers of bacteriophages \(\Phi X174\) did not change after a treatment with 50 mg/L hydrogen peroxide and a contact time of 3 h (Sommer et al., in preparation).

At the end of the ozone/hydrogen peroxide process, \(E. coli\) and the three test viruses revealed a reduction of 6-log. This reduction was reached with phage PRD-1 after the first section, with \(E. coli\) and phage \(\Phi X174\) after the second section and with phage MS 2 after the third section. After the total process, spores of \(B. subtilis\) showed a reduction of 0.4-log. The results of the two independent test series were in good accordance; this can be seen from the small standard deviations of the microbiological results as well as from the very similar chemical degradation of the perchloroethylene concentration (Table 3).

The controlled batch experiments with ozone as a single disinfectant by means of two standard conditions (a) residual ozone concentration 0.4 mg/L after a contact time of 4 min

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Ozone/hydrogen peroxide pilot plant: residuals of ozone and hydrogen peroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual Mean (± SD) of both test series at sampling site</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Ozone (mg/L)</td>
<td>0.52 (0.12)</td>
</tr>
<tr>
<td>Hydrogen peroxide (mg/L)</td>
<td>1.5 (0.0)</td>
</tr>
</tbody>
</table>

* Detection limits – ozone 0.01 mg/L; hydrogen peroxide 0.5 mg/L

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Ozone/hydrogen peroxide pilot plant – inactivation results (The concentration of perchloroethylene decreased from 26.0 (\mu)g/L to 5.0 (\mu)g/L in test 1 and from 28.0 (\mu)g/L to 5.4 (\mu)g/L in test 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample site</td>
<td>Log concentrations of test microorganisms (mean CFU/PFU ± SD)</td>
</tr>
<tr>
<td></td>
<td>(E. coli)</td>
</tr>
<tr>
<td></td>
<td>(CFU/L)</td>
</tr>
<tr>
<td>1</td>
<td>6.59 (0.12)</td>
</tr>
<tr>
<td>2</td>
<td>0.46 (0.16)</td>
</tr>
<tr>
<td>3</td>
<td>&lt;DL(^1)</td>
</tr>
<tr>
<td>4</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>5</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>6</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>7</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Total reduction(^2)</td>
<td>–6.59(^3)</td>
</tr>
</tbody>
</table>

\(^1\)<DL = below detection limit (<20 CFU/PFU per plate); \(^2\)log \((N/N_0)\); \(^3\)log \(N\) below detection limit
and (b) 0.1 mg/L after a contact time of 10 min, are presented in Table 4. Both conditions led to a 5-log and 6-log reduction of \textit{E. coli} and the bacteriophages MS2 and \textit{\Phi}X174, respectively (below the limit of determination). Differences in the inactivation efficacy were found with the spores of \textit{B. subtilis}; condition (a) gave a spore reduction of 0.4-log, whereas under condition (b) a reduction of 1.5-log was obtained. These results were in good accordance with those of Facile \textit{et al.} (2000) at a temperature of 20°C and pH 8.2. When the findings of the hydrogen/peroxide pilot plant with the ozone inactivation are compared, the microbicidal effect matched very well the ozone standard condition (a) with an ozone residual of 0.4 mg/L after 4 min contact time (20°C).

The presented study revealed a good microbicidal efficacy of the ozone/hydrogen peroxide process with respect to vegetative bacteria and viruses (bacteriophages). A 4-log reduction as demanded for viruses by USEPA (USEPA, 1989) was reached. However, it has to be emphasised that an intense mixing and a sufficient contact time have to be optimised and tested. This can be seen from the large discrepancies between the hydraulic retention time and the contact times of the reactor in the pilot plant tested (Table 1).

### Conclusions

The microbicidal efficiency of an advanced oxidation process was evaluated by means of a set of test organisms (\textit{E. coli} as a traditional indicator with three bacteriophages and spores of \textit{B. subtilis} as alternate indicators). The investigated pilot plant was optimised to degrade perchloroethylene in a contaminated groundwater. Reductions of 6-log with bacteriophages and \textit{E. coli} as well as 0.4 log with \textit{B. subtilis} spores were obtained. We found that this disinfection capacity corresponded to the results of an ozone inactivation in controlled batch experiments (residual 0.4 mg/L after 4 min contact time, 20°C). Spores of \textit{Bacillus subtilis} proved to be an appropriate indicator (a) to evaluate and optimise disinfection processes, (b) to compare different inactivation mechanisms, as well as (c) to test the hydraulic properties of a treatment plant.

### Table 4

Results of the ozone batch inactivation experiments under the two test conditions: (a) 0.4 mg/L residual ozone concentration after a contact time of 4 min and (b) 0.1 mg/L residual ozone concentration after a contact time of 10 min. Initial concentration of the test organisms (log \(N_o\)) and the reduction log(\(N/N_o\)) obtained are given as the means (standard deviations) of at least two test series.

#### (a) Standard condition – ozone residual 0.4 mg/L after 4 min (20°C)

<table>
<thead>
<tr>
<th>Initial O₃ mg/L</th>
<th>0 min</th>
<th>1.3 min</th>
<th>2.7 min</th>
<th>4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>2.17</td>
<td>0.93</td>
<td>0.59</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Test organism:
- \(\Phi\)X174 (log PFU/mL)
- MS2 (log PFU/mL)
- \textit{E. coli} (log CFU/mL)
- \textit{B. subtilis} spores (log CFU/mL)

Initial:
- \(6.79 \pm 0.06\) for \(\Phi\)X174
- \(4.90 \pm 0.27\) for MS2
- \(5.99 \pm 0.28\) for \textit{E. coli}
- \(6.15 \pm 0.11\) for \textit{B. subtilis} spores

After 4 min:
- \(-6.79^*\) for \(\Phi\)X174
- \(-4.90^*\) for MS2
- \(-5.99^*\) for \textit{E. coli}
- \(-0.27 \pm 0.10\) for \textit{B. subtilis} spores

#### (b) Standard condition – ozone residual 0.1 mg/L after 10 min (20°C)

<table>
<thead>
<tr>
<th>Initial O₃ mg/L</th>
<th>0 min</th>
<th>1.3 min</th>
<th>2.7 min</th>
<th>4 min</th>
<th>7 min</th>
<th>10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.10</td>
<td>2.75</td>
<td>1.47</td>
<td>1.02</td>
<td>0.75</td>
<td>0.35</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Test organism:
- \(\Phi\)X174 (log PFU/mL)
- MS2 (log PFU/mL)
- \textit{E. coli} (log CFU/mL)
- \textit{B. subtilis} spores (log CFU/mL)

Initial:
- \(6.83 \pm 0.06\) for \(\Phi\)X174
- \(5.00 \pm 0.05\) for MS2
- \(6.05 \pm 0.01\) for \textit{E. coli}
- \(6.15 \pm 0.06\) for \textit{B. subtilis} spores

After 10 min:
- \(-6.83^*\) for \(\Phi\)X174
- \(-5.00^*\) for MS2
- \(-6.05^*\) for \textit{E. coli}
- \(-1.44 \pm 0.07\) for \textit{B. subtilis} spores

* log \(N\) below detection limits (<20 CFU/PFU per plate)
References


