Inactivation of *Pseudomonas aeruginosa* in electrochemical advanced oxidation process with diamond electrodes

M. Griessler, S. Knetsch, E. Schimpf, A. Schmidhuber, B. Schrammel, W. Wesner, R. Sommer and A. K. T. Kirschner

**ABSTRACT**

The electrochemical advanced oxidation process (EAOP) with diamond electrodes may serve as an additional technology to the currently approved methods for water disinfection. Only few data exist on the microbicidal effect of the EAOP. The aim of our study was to investigate the microbicidal effect of a flow-through oxidation cell with diamond electrodes, using *Pseudomonas aeruginosa* as the test organism. Without electrical current the EAOP had no measurable effect on investigated microbiological and chemical parameters. For direct electrical current a stronger impact was observed at low flow rate than at higher flow rate. Depending on the contact time of the oxidants and the type of quenching reagent added, inactivation of *P. aeruginosa* was in the range log 1.6–3.6 at the higher flow rate and log 2.4–4.4 at the lower rate. Direct electrical current showed a stronger microbicidal effect than alternating current (maximum reduction log 4.0 and log 2.9, respectively).

The microbiological results of experiments with this EAOP prototype revealed higher standard deviations than expected, based on our experience with standard water disinfection methods. Safe use of an EAOP system requires operating parameters to be defined and used accurately, and thus specific monitoring tests must be developed.

**Key words** | diamond electrode, electrochemical advanced oxidation process, microbicidal effect, *Pseudomonas aeruginosa*, water disinfection

**INTRODUCTION**

In coming years the treatment and disinfection of water will become an even bigger issue than at present. The term ‘drinking water disinfection’ is defined in the Austrian Food Act, chapter B1, as irreversible inactivation of water-transmittable pathogenic microorganisms (*Anonymous 2007*). Methods and conditions for the disinfection procedure have to ensure at least a 4 log reduction of these pathogens (*Anonymous 2007*). Currently approved methods for disinfection of drinking water include ozonation, UV irradiation, and chlorination with hypochlorite solutions, chlorine gas or chlorine dioxide. Boiling is listed as an emergency measure.

New technologies for the disinfection of water are being developed in several sectors. Areas of application range from ionizing radiation to the mechanical removal of microorganisms by membrane technology and the use of oxidizing substances such as peracetic acid and hydrogen peroxide. For oxidation based on electrochemistry, two types of approach have to be distinguished: (i) water electrolysed separately is added to water containing microorganisms (water to be treated) and (ii) water containing microorganisms is pumped through the oxidation cell and is electrolysed there. The experiments discussed here were based on the second type of application.

Two principles may explain the microbicidal effect when a suspension of microorganisms (water to be treated) is pumped through an oxidation cell. First, there could be indirect effects either from chlorine compounds formed from chloride present in the water or from other oxidizing substances (*Tröster et al. 2002; Jeong et al. 2006*). The production of chlorine by this technique under controlled conditions is already well established (*Austrian Standards...*).

The use of electrolysis for direct disinfection of water has been discussed since the 1950s (Bergmann et al. 2002). Studies on the electrochemical behaviour of diamond thin films began in the mid-1980s (Panizza & Cerisola 2005) and continue to this day. The EAOP is defined by its high current efficiency during the electrochemical production of ‘OH radicals, which are suggested as playing a major role in the inactivation of bacteria in chloride-free electrochemical disinfection (Diao et al. 2004; Jeong et al. 2006). The use of conductive diamond as the electrode material extends the range of conventional AOP methods (Tröster et al. 2002). According to the literature, the EAOP may serve as an additional technology to methods currently used for water treatment and disinfection.

During physical methods of water treatment, such as UV disinfection or boiling, no by-products are formed in the water and no chemicals are used. For chlorination and ozonation, chemicals need to be added to the water and undesirable by-products such as halogenated hydrocarbons (chlorination), bromate (ozonation) and chlorite (decomposition of chlorine dioxide) can be generated. Although no chemicals are added during the EAOP, the process is based on the formation of powerful oxidants during the electrolysis of water. At present we lack detailed understanding of the chain of reactions and the potential for forming health-threatening by-products (Rychen et al. 2005; Bergmann & Koparal 2005). Moreover, data on the ability of the EAOP to inactivate microorganisms in terms of safe water disinfection are inadequate (Bergmann & Koparal, 2005).

The aim of our study was to investigate the efficacy of a specific prototype of oxidation cell in inactivation of a test organism, Pseudomonas aeruginosa. The prototype was intended to treat water containing a low concentration of ions, particularly a low chloride content, such that chlorine would be generated in only negligible concentrations. P. aeruginosa is an aerobic, rod-shaped, gram-negative bacterium ubiquitous in both water and soil and was chosen as test organism because of its important role in public health, particularly with regard to nosocomial infections and diseases in immunodeficient persons.

Two questions were investigated. First, does the velocity with which the test bacteria in the water pass the flow-through oxidation cell have an influence on their inactivation and on selected chemical parameters? Second, is there a difference in the microbicidal effect of alternating electrical current (AC) and direct current (DC)?

METHODS

Oxidation cell and experimental set-up

A prototype bench-scale unit with an oxidation cell containing a bed of packed diamonds was used for the experiments. Water flows were in the range 1–2 L h⁻¹. A current of 80–118 mA at 230–300 V was used, giving a current density of 140–210 mA cm⁻² (based on a cross section of 0.56 cm⁻²). The experimental set-up consisted of a sterile glass bottle containing a continuously stirred test suspension of P. aeruginosa (NCTC 10662, HPA, UK) at approximately 10⁶ CFU mL⁻¹, a peristaltic pump (Europump PA-ST1 basic, IKA, Germany), the oxidation cell (ECHEMA, Austria), a drain bottle and a laboratory power supply providing DC (Consort E820, Labortechnik, Germany). A separate laboratory power supply (ECHEMA, Austria) was used for tests with AC because the E820 supply could not deliver AC. In this case the current was measured using an amperemeter (350 E, Voltcraft, Germany). The frequency of the current was 50 Hz. A schematic representation of the experimental set-up is shown in Figure 1.

The oxidation cell (Figure 2) contained a bed of dipolar boron-doped diamonds (BDD). Each of these diamonds serves as an electrode, providing an especially large surface area. The diamond bed was enclosed in a polyethylene shell. The contact electrodes consisted of titanium-iridium-oxide, representing a semiconducting surface.

Experimental procedures

Before the test runs, the test suspension of P. aeruginosa was pumped through the system, without applying electrical current, to clean the pipes and the oxidation cell. To investigate whether the flow rate of the test suspension being pumped through the cell had an influence on the inactivation of the test organism and on selected chemical parameters, tests were run at two different velocities using DC. To test whether there was a difference in the microbicidal effect of AC and DC, a custom-made power supply providing both types of current was used combined with an amperemeter. This power supply did not permit adjustment of voltage or amperage. In order to obtain the same amperage for the AC/DC experiments as for those investigating the effect of flow rate it was necessary...
to use a test suspension with higher ion concentration. This was obtained by mixing tap water with ultrapure water produced in a reverse osmosis system (ELGA Option 7, Veolia, France).

For the microbiological analyses samples were collected in sterile glass bottles. Control samples without applying electrical current were taken prior to the experiments and after finishing. The action of oxidants in the samples was quenched by the addition of 0.013% (w/v) sodium thiosulfate (1.06516, MERCK, Germany), 0.05% (v/v) catalase (60634, FLUKA, Switzerland), or a combination of both. To test the effect of contact time, the quenching reagents were added accordingly. All experiments were performed in at least three independent test series.

**Microbiological analysis**

Before each test series of disinfection experiments, the bacterium was streaked onto Columbia agar plates (CM 331B, Oxoid, UK) and incubated at 36 ± 2°C for 44 ± 4 h. For preparing the stock suspension, colonies were suspended in peptone saline (64544, BIORAD, UK) and the optical density was adjusted to 1 at 620 nm in a spectrophotometer (Lambda40 UV/VIS, PerkinElmer, USA). The stock suspension contained approximately $1 \times 10^8$ CFU mL$^{-1}$ and was diluted to approximately $1 \times 10^6$ CFU mL$^{-1}$ for the experiments.

The concentration of *P. aeruginosa* in samples collected during the experiments was determined in triplicate with a cultivation method on selective agar (CM 559B, OXOID, UK; incubation 36 ± 2°C for 44 ± 4 h). The samples were processed by membrane filtration or by surface plating. The determined concentrations were expressed as log 10. The reduction in bacterial counts was calculated as log ($N/N_0$), where $N_0$ represented the concentration in untreated water and $N$ the concentration in treated water.

**Chemical analysis**

The composition of the test water and the nature of the oxidants formed during electrolysis were characterized by investigation of selected chemical parameters. Since no specific methods for electrochemically generated oxidants...
exist, standard methods for disinfectants were used. The concentrations of active chlorine, total chlorine, ozone and other oxidants generated were determined using N,N-diethyl-p-phenylenediamine (DPD) colorimetric methods (Lovibond, Tintometer, Germany). DPD 1 (N,N-diethyl-p-phenylenediamine) was used to measure active chlorine and chlorine dioxide. DPD 1 combined with DPD 3 (potassium iodide) as well as DPD 4 (equal to a combination of DPD 1 and DPD 3) served for measurement of total chlorine and ozone. The addition of glycine was used to destroy the ultimately formed ozone. Hydrogen peroxide was measured using a colorimetric test kit (Nr. 1.10011.0001, MERCK). All tests were performed according to the manufacturers’ instructions. Ion chromatography (DX-120, Dionex, USA) according to EN ISO 10304-1 and EN ISO 14911 was used for chemical analyses of selected anions and cations respectively. The electrical conductivity of the water was measured with a conductivity meter (Cond 315i, WTW, Germany).

RESULTS AND DISCUSSION

Beds of diamond electrodes such as were used in this investigation are intended for the treatment of water with low conductivity because the distance between the individual electrodes is very small. For water with high conductivity, thin film electrodes providing sufficient distance between the electrodes are usually chosen. In the test series to investigate whether the flow rate has an influence on the inactivation of the test organism and on selected chemical parameters, the conductivity of the test water (ultrapure water) was always below 25 µS cm\(^{-1}\). In the test series to investigate whether there is a difference in the microbicidal effect of AC and DC, water of higher conductivity was used (mixture of tap water and ultrapure water, maximum conductivity 361 µS cm\(^{-1}\)). In both test suspensions the chloride concentration was below 8 mg L\(^{-1}\).

For ultrapure water the mean concentrations of chloride and sodium were 5.6 ± 2 and 3.5 ± 1 mg L\(^{-1}\), respectively (n = 4). The concentrations of the other measured ions (mg L\(^{-1}\)) were below the detection limit of the respective method: fluoride (0.1), nitrate (0.1), sulphate (0.1), potassium (0.5), magnesium (0.5) and calcium (2.0).

For ultrapure water mixed with tap water the mean concentrations of chloride, nitrate, sulphate, sodium, magnesium and calcium were 7.0 ± 1, 2.7 ± 1, 7.8 ± 5, 23.2 ± 1, 6.2 ± 4 and 26.3 ± 4 mg L\(^{-1}\), respectively (n = 3). The concentrations of fluoride and potassium were below the detection limit of the respective method (0.1 and 0.5 mg L\(^{-1}\)).

### Effect of the EAOP on the inactivation of *P. aeruginosa*

Without current flow the EAOP had no measurable effect on the investigated chemical and microbiological parameters. The chosen contact times for the formed oxidants were 1, 15 and 30 min. The time of 30 min was selected for two reasons: this is the standard time for chlorination of drinking water, and Kerwick *et al.* (2005) state that longer contact times will not enhance the efficacy. The contact time of 15 min represents the time for chlorine dioxide treatment according to the Austrian Food Act (Anonymous 2007); the contact time of 1 min was chosen to investigate a possible direct effect of the oxidation cell.

### Effect of different flow rates

Using DC, the effect on the chemical and microbiological parameters was greater at the lower flow rate of the test.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Log inactivation of <em>P. aeruginosa</em> in relation to flow rate, contact time of oxidants and quenching reagents when using DC (mean of four test series, samples analysed in triplicate). Test water: ultrapure water, average current density: 210 mA cm(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher flow rate = 2.18 ± 0.14 L h(^{-1}), flow-through time approximately 0.6 s</td>
<td></td>
</tr>
<tr>
<td>Contact time (min)</td>
<td>Thiosulfate added</td>
</tr>
<tr>
<td>1</td>
<td>2.09 ± 0.34</td>
</tr>
<tr>
<td>10</td>
<td>2.74 ± 0.45</td>
</tr>
<tr>
<td>30</td>
<td>3.56 ± 0.91</td>
</tr>
</tbody>
</table>

| Lower flow rate = 1.38 ± 0.12 L h\(^{-1}\), flow-through time approximately 1.0 s |
| Contact time (min) | Thiosulfate added | Thiosulfate and catalase added | Catalase added |
| 1 | 2.79 ± 0.31 | 2.40 ± 0.62 | 2.55 ± 0.52 |
| 10 | 3.61 ± 0.48 | 2.67 ± 0.50 | 2.72 ± 0.46 |
| 30 | 4.36 ± 1.07 | 3.47 ± 0.77 | 2.87 ± 0.50 |
suspension than at the higher flow rate. The microbiological results are given in Table 1. Depending on the contact time of the oxidants and the type of quenching reagent added, inactivation of \textit{P. aeruginosa} was in the range log 1.6–3.6 at the higher flow rate and log 2.4–4.4 at the lower rate. This finding resembles those obtained by Jeong \textit{et al.} (2006, 2009) for \textit{Escherichia coli} in chloride-free phosphate buffer at a current density of 33–100 mA cm\textsuperscript{-2}.

**Effect of contact time**

The inactivation of \textit{P. aeruginosa} increased with the contact time of the oxidants (Table 1). This can be explained by an indirect effect of the oxidation cell. Oxidants were produced during passage through the oxidation cell and continued to inactivate microorganisms until they were stopped by the addition of the quenching reagents. Samples to which only catalase was added showed reduced bacterial inactivation compared with sodium thiosulfate-inactivated samples. It is widely accepted that the presence of hydrogen peroxide indicates formation of 'OH radicals on BDD electrodes (Michaud \textit{et al.} 2003); therefore it may be assumed that oxidants quenched with catalase (e.g. hydrogen peroxide) are more effective than those quenched by adding sodium thiosulfate (e.g. chlorine, ozone).

**Effect of type of current**

DC showed a stronger microbicidal effect than AC (Figure 3). Depending on the contact time and reagents used, DC resulted in reductions between log 0.9 and log 4.0, AC between log 0.4 and log 2.9.

These findings are consistent with data in the literature. Park \textit{et al.} (2004) studied the inactivation of \textit{Vibrio parahaemolyticus} in sea water (high chloride concentration) using AC and DC and concluded that with AC, because the polarity alternates periodically, the water undergoes less electrolysis compared with DC, making AC less efficient for electrochemical treatment.

It was noticeable that the microbiological results revealed higher standard deviations than we usually achieve in experiments with conventional water disinfection methods like ozone, chlorine or UV irradiation. For example, according to the National Standard ÖNORM M 5873 on UV disinfection, a standard deviation of not more than log 0.2 is required when measuring the log reductions in performance testing (Austrian Standards Institute 2001). As can be seen in Table 1, this prerequisite would not be fulfilled in the experiments described.

**Effect of the EAOP on selected chemical parameters**

The chloride concentration decreased on average 67 ± 12\% at the higher flow rate (2.18 ± 0.14 L h\textsuperscript{-1}) and 79 ± 9\% at the lower rate (1.38 ± 0.12 L h\textsuperscript{-1}). The other ion concentrations tested did not show significant changes.

Since no specific methods for individual electrochemically formed oxidants exist, measurements were made using the DPD methods (Table 2). In addition to detection of active and total chlorine, chlorine dioxide and ozone, these methods also detect other types of disinfection agent, such as hydrogen peroxide and peroxodisulfates (Rychen \textit{et al.} 2003; Jeong \textit{et al.} 2009). The DPD 1 test is the standard method for measuring active chlorine and chlorine dioxide; the DPD1 + DPD3 (= DPD4) test is used to measure total chlorine and ozone, and glycine is added to destroy ozone in the presence of chlorine. In the latter case a difference
Moreover, the results of experiments on inactivation of the test bacterium, *P. aeruginosa*, revealed standard deviations that were higher than expected, based on our experience with standard methods of water disinfection. Thus, in order to use such systems safely, operating parameters need to be defined and accurately controlled.

The EAOP is sometimes referred to as chemical-free water treatment since no chemicals are added. However, the principle of this technology is based on chemical reactions such as formation of hydrogen peroxide, ozone or chlorine. No specific tests for monitoring the concentrations of EAOP oxidants are available so far. For a safe application of the process these have to be developed. To ensure that harmful disinfection by-products are not formed in critical concentrations, toxicological investigations are necessary.

### CONCLUSIONS

The experiments were designed to test a prototype of an EAOP flow-through cell under practical conditions. Accordingly, we used water rather than a buffer solution for suspending the test bacterium. The prototype was developed especially for treatment of water with low ion concentration, in particular low amounts of chloride as used in water distribution systems in industry and laboratories, for example.

The inactivation performance was strongly dependent on the operation conditions (flow rate, type of current, contact time of the oxidants and type of quenching reagents). Moreover, the results of experiments on inactivation of the values obtained by DPD 4 and DPD4 + glycine tests gives the concentration of ozone.

Measurements using DPD 1 showed that neither active chlorine nor chlorine dioxide was present at a detectable concentration (limit of determination 0.1 mg L$^{-1}$). This could have several explanations. Bergmann & Koparal (2005) stated that losses of chlorine dioxide in the percentage range may occur in less than 1 min, and in the study by Jeong et al. (2009) BDD electrodes produced very low amounts of active chlorine, ranking fourth out of five tested electrode materials. The results of the tests with DPD 1 combined with DPD 3 equal those with DPD 4 within the measuring uncertainty. The addition of glycine had no influence on the measured values, suggesting that no ozone was generated. This finding is in agreement with Michaud et al. (2005), who stated that because BDD electrodes have an inert surface only negligible amounts of ozone are formed. Moreover, our test prototype exhibited only low voltage (2.5–4 V). Hydrogen peroxide occurred in concentrations of 50 mg L$^{-1}$ with DC and 25 mg L$^{-1}$ with AC.

### REFERENCES


Matsunaga, T., Nakasono, S., Takamuku, T., Burgess, J. G., Nakamura, N. & Sode, K. 1992 Disinfection of drinking water by using a novel electrochemical reactor employing carbon-

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Measurements of oxidants using DPD methods (mg L$^{-1}$, mean ± standard deviation) in experiments with differing flow rates (test water: ultrapure water; average current density: 210 mA cm$^{-2}$) and differing types of current (test water: mixture of tap water and ultrapure water (chloride &lt; 8 mg L$^{-1}$); average current density: 140 mA cm$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rates (mean of four test series)</td>
<td></td>
</tr>
<tr>
<td>2.18 ± 0.135 L h$^{-1}$</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>1.38 ± 0.12 L h$^{-1}$</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Types of current (mean of three test series)</td>
<td></td>
</tr>
<tr>
<td>DC</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>AC</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

### CONCLUSIONS

The experiments were designed to test a prototype of an EAOP flow-through cell under practical conditions. Accordingly, we used water rather than a buffer solution for suspending the test bacterium. The prototype was developed especially for treatment of water with low ion concentration, in particular low amounts of chloride as used in water distribution systems in industry and laboratories, for example.

The inactivation performance was strongly dependent on the operation conditions (flow rate, type of current, contact time of the oxidants and type of quenching reagents). Moreover, the results of experiments on inactivation of the value obtained by DPD 4 and DPD 4 + glycine tests gives the concentration of ozone.

Measurements using DPD 1 showed that neither active chlorine nor chlorine dioxide was present at a detectable concentration (limit of determination 0.1 mg L$^{-1}$). This could have several explanations. Bergmann & Koparal (2005) stated that losses of chlorine dioxide in the percentage range may occur in less than 1 min, and in the study by Jeong et al. (2009) BDD electrodes produced very low amounts of active chlorine, ranking fourth out of five tested electrode materials. The results of the tests with DPD 1 combined with DPD 3 equal those with DPD 4 within the measuring uncertainty. The addition of glycine had no influence on the measured values, suggesting that no ozone was generated. This finding is in agreement with Michaud et al. (2005), who stated that because BDD electrodes have an inert surface only negligible amounts of ozone are formed. Moreover, our test prototype exhibited only low voltage (2.5–4 V). Hydrogen peroxide occurred in concentrations of 50 mg L$^{-1}$ with DC and 25 mg L$^{-1}$ with AC.

### REFERENCES


Matsunaga, T., Nakasono, S., Takamuku, T., Burgess, J. G., Nakamura, N. & Sode, K. 1992 Disinfection of drinking water by using a novel electrochemical reactor employing carbon-