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Environmental Technology (ISSN: 0959-3330)

Citation for the published paper:

http://dx.doi.org/10.1080/09593330.2013.788041

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Production of high concentrations of H₂O₂ in a bioelectrochemical reactor fed with real municipal wastewater

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Abstract
Bioelectrochemical systems can be used to energy-efficiently produce hydrogen peroxide (H₂O₂) from wastewater. Organic compounds in the wastewater are oxidized by microorganisms using the anode as electron acceptor. H₂O₂ is produced by reduction of oxygen on the cathode. In this study, we demonstrate for the first time production of high concentrations of H₂O₂ production from real municipal wastewater. A concentration of 2.26 g/L H₂O₂ was produced in 9 hours at 8.3 kWh/kgH₂O₂. This concentration could potentially be useful for membrane cleaning at membrane bioreactor wastewater treatment plants. With an acetate-containing nutrient medium as anode feed, a H₂O₂ concentration of 9.67 g/L was produced in 21 hours at an energy cost of 3.0 kWh/kgH₂O₂. The bioelectrochemical reactor used in this study suffered from a high internal resistance, most likely caused by calcium carbonate deposits on the cathode-facing side of the cation exchange membrane separating the anode- and cathode compartments.

Keywords: Bioelectrochemical systems; Hydrogen peroxide; Membrane bioreactor; Microbial electrolysis cell; Wastewater treatment

1. Introduction
Hydrogen peroxide (H₂O₂) could potentially be used at municipal wastewater treatment plants for disinfection, odor control, and oxidation of recalcitrant pollutants. At membrane bioreactor treatment plants, H₂O₂ would be especially interesting as a cleaning chemical for membranes as it could be an environmentally friendly alternative to chlorine. A previous study has shown that H₂O₂ concentrations of 2-5 g/L would be sufficient for membrane cleaning [1]. To make the membrane cleaning process even more environmentally friendly, H₂O₂ could potentially be generated onsite using a bioelectrochemical reactor in which dissolved organic matter present in the municipal wastewater is used to power the electrochemical production of H₂O₂.

A bioelectrochemical reactor for H₂O₂ production consists of two compartments separated by an ion exchange membrane. A solution containing dissolved organic matter (e.g. wastewater) is fed to the anode compartment where microorganisms oxidize the organics and use the anode as electron acceptor. The electrons flow through an external circuit to the cathode where oxygen is reduced to H₂O₂. The cathode compartment should contain a relatively clean water or salt solution without metals or organics that could lead to decomposition of the produced H₂O₂. The first bioelectrochemical reactor for H₂O₂ production was developed by Rozendal et al. [2]. They fed the anode compartment with an acetate-containing nutrient medium. A H₂O₂ concentration of 1.3 g/L was generated in the cathode compartment at an energy input of 0.93 kWh/kgH₂O₂. Although
bioelectrochemical $\text{H}_2\text{O}_2$ production could take place without an input of electrical energy, a voltage of 0.5 V was applied to increase the reaction rate [2]. We, Modin and Fukushi [3], produced 5 g/L of $\text{H}_2\text{O}_2$ in a reactor that was also fed with an acetate-containing nutrient medium to the anode. When we switched anode feed to real wastewater, a concentration of only 0.08 g/L could be generated because the anode delivered a much lower current. Fu et al. [4] produced 0.079 g/L $\text{H}_2\text{O}_2$ with a glucose-containing medium as anode feed and graphite rod electrodes as cathodes. Their reactor was operated as a microbial fuel cell (MFC), i.e. with simultaneous recovery of $\text{H}_2\text{O}_2$ and electrical energy. Furthermore, MFCs with composite carbon/iron cathodes have been used for generation of Fenton’s reagent [5-7]. Bioelectrochemical reactors have also been investigated for several other applications, for example, electricity generation from wastewater in MFCs [8-10], dye degradation [11], hydrogen and methane production [12, 13], caustic generation [14], and use as biosensors [15, 16].

Although several researchers have investigated bioelectrochemical systems for $\text{H}_2\text{O}_2$ production, no study has so far demonstrated production of high concentrations (>2 g/L) from real municipal wastewater. Thus, the goal of this study was to bioelectrochemically produce practically useful concentrations of $\text{H}_2\text{O}_2$ using real municipal wastewater as feed to the biological anode. We define a practically useful concentration as 2-5 g/L, which potentially could be used for onsite membrane cleaning at membrane bioreactor treatment plants.

2. Experimental

2.1 Bioelectrochemical Reactor

The bioelectrochemical reactor had cylindrical anode and cathode chambers separated by a Nafion 117 cation exchange membrane with a diameter of 2 cm. The anode chamber had a liquid volume of 23 mL, was 9.6 cm long with a diameter of 2 cm, and contained a 0.2 x 3 x 9 cm carbon fiber felt electrode attached to a 9 cm long, 0.615 cm diameter graphite rod. The cathode chamber had a liquid volume of 5 mL, was 1.3 cm long with a diameter of 2 cm. The gas-diffusion cathode was made of carbon fiber paper (Toray TGP-H-060) coated on both sides with a solution of 40% PTFE and graphite powder (200 mesh, Alfa Aesar) to avoid water leakage. The liquid-facing side was coated with carbon nanoparticles and PTFE (30% mass PTFE/mass C).

2.2 Operation

The anode chamber was fed with either a nutrient medium (hereby referred to as synthetic feed) or raw wastewater that had passed the preliminary treatment steps in a wastewater treatment plant in Tokyo, Japan (hereby referred to as real wastewater). The synthetic feed consisted of (mg/L): 500 CH$_3$COONa, 100 NH$_4$Cl, 2925 NaCl, 150 CaCl$_2$*2H$_2$O, 200 MgSO$_4$*7H$_2$O, 461 KH$_2$PO$_4$, 939 Na$_2$HPO$_4$, mixed in tap water. The cathode chamber was fed with 50 mM NaCl irrespective of anode feed.

The reactor was operated for 63 days. During the first 4 days, the reactor was operated as a MFC with a 1000 $\Omega$ resistor connected between anode and cathode. Then, a cell potential of 0.2 V (day 4-10), 0.5 V (day 10-15), and 1 V (day 16-63) was applied to the system and the current was measured across a 10 $\Omega$ resistor. The anode and cathode were fed continuously at flow rates of 280 and 10 mL/d, respectively. The anolyte was also recirculated through the anode chamber at a flow rate of 50 mL/min. $\text{H}_2\text{O}_2$ production was investigated in specific tests with either controlled anode potential or controlled current (Table 1). During a $\text{H}_2\text{O}_2$ test, the cathode was operated as a batch whereas the anode was fed continuously at 90 mL/h. The tests usually lasted for 2, 9, or 21 h and the final $\text{H}_2\text{O}_2$ concentration was measured in the catholyte.
Table 1. H$_2$O$_2$ production tests.

<table>
<thead>
<tr>
<th>Test #</th>
<th>Day</th>
<th>Duration (h)</th>
<th>Control$^a$ ($E_{\text{anode}}$ or I)</th>
<th>Anolyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>2</td>
<td>-0.1 V vs SHE</td>
<td>Synthetic feed</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>2</td>
<td>3 mA</td>
<td>Synthetic feed</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>9</td>
<td>-0.1 V vs SHE</td>
<td>Synthetic feed</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>9</td>
<td>3 mA</td>
<td>Synthetic feed</td>
</tr>
<tr>
<td>5</td>
<td>44-45</td>
<td>21</td>
<td>-0.1 V vs SHE</td>
<td>Synthetic feed</td>
</tr>
<tr>
<td>6</td>
<td>45-46</td>
<td>21</td>
<td>3 mA</td>
<td>Synthetic feed</td>
</tr>
<tr>
<td>7</td>
<td>53</td>
<td>2</td>
<td>-0.1 V vs SHE</td>
<td>Real wastewater</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>2</td>
<td>3 mA</td>
<td>Real wastewater</td>
</tr>
<tr>
<td>9</td>
<td>54</td>
<td>9</td>
<td>-0.1 V vs SHE</td>
<td>Real wastewater</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>9</td>
<td>3 mA</td>
<td>Real wastewater</td>
</tr>
<tr>
<td>11</td>
<td>57-58</td>
<td>21</td>
<td>-0.1 V vs SHE</td>
<td>Real wastewater</td>
</tr>
<tr>
<td>12$^b$</td>
<td>58-59</td>
<td>13.9</td>
<td>~3 mA</td>
<td>Real wastewater</td>
</tr>
</tbody>
</table>

$^a$The reactor was operated with either controlled anode potential ($E_{\text{anode}}$) or controlled current (I).
$^b$The anode potential rose rapidly and the potentiostat was not able to provide sufficient voltage to drive a 3 mA current, so the experiment was terminated after 13.9 h.

2.3 Analytical Methods

H$_2$O$_2$ concentrations were measured spectrophotometrically [17]. Concentrations of anions were measured using ion chromatography (Metrohm 761 Compact IC). Concentrations of cations were measured using ICP-AES (Perkin Elmer Optima 3000DV). Dissolved organic carbon (DOC) concentrations were measured using a TOC-V analyzer (Shimadzu). Potentials were recorded using a NI USB-6211 data logger (National Instruments) connected to a PC. Anode potentials are reported against the Standard Hydrogen Electrode (SHE) but were measured against Ag/AgCl reference electrodes (+0.197 V vs SHE, BAS Inc.). Polarization curves were obtained by controlling the current using a potentiostat/galvanostat (KP07, Bank IC) and measuring the resulting potentials. The current was increased from 0 mA in steps of 0.1 mA every six minutes.

3. Results and discussion

3.1 Bioelectrochemical Reactor Performance

The current produced by the reactor during normal operation ($Q_{\text{anode}}$=280 ml/d, $Q_{\text{cathode}}$=10 ml/d) is shown in Figure 1. Bioelectrochemical activity was observed after 4 days when a potential difference developed across the 1000 $\Omega$ resistor connected between the anode and cathode. The current produced in the reactor could subsequently be increased to 3.0-3.5 mA by applying an external potential difference across the cell. When real wastewater was fed to the anode on day 50, the current dropped to about 0.6 mA.
The state of the reactor was investigated using polarization curves (Figure 2). On day 4, the anode potential rose rapidly at a current over 2 mA. On day 25, the ability of the anode had improved as a current of over 5 mA could be delivered at low anode potential. This performance remained stable until day 50. On day 52 with real wastewater as the anolyte, the anode could still deliver currents up to 5 mA, though at a higher potential compared to synthetic feed. On day 60, the performance had deteriorated and the anode potential rose quickly. The internal resistance of the reactor can be estimated from the slopes of the cell potential versus current curves. From day 4 to day 37, the internal resistance increased slowly from 253 to 319 $\Omega$. On day 50, the internal resistance had increased to 536 $\Omega$. Real wastewater feed increased the internal resistance dramatically to 745 $\Omega$ on day 52 and 1153 $\Omega$ on day 60. The increase from 536 $\Omega$ on day 50 to 745 $\Omega$ on day 52 can partly be explained by the lower conductivity of the real wastewater, which was 164 mS/cm whereas in the synthetic feed it was 745 mS/cm. A more rapid rise in anode potential with increasing current also partly explains the higher internal resistance with real wastewater.

Figure 1. Current (thin line) and cell potential (thick line) during the experimental period. Negative cell potential indicate an input using a DC power source.

Figure 2. Polarization curves showing cell potential (left) and anode potential (right) as functions of current for different days during the experimental run.
As we increased the potential input to the system in the beginning of the experiment, the magnitude of the current flowing in the cell also increased. However, when the input potential was kept constant at 1 V, the current decreased from about 3.5 mA on day 16 to 1.5 mA on day 50. The decrease in current can be explained by an increased internal resistance caused by white deposits building up on the cathode-side of the cation exchange membrane. To get a qualitative indication of the composition of the deposits, the membrane was submerged in 50 mL 1% HNO₃ for 1 day. The ionic composition of the HNO₃ solution was then analyzed. Ca²⁺ was the most significant cation with a concentration of 21.4 mM. Mg²⁺ was present at 3.6 mM, Na⁺ at 3.8 mM, and K⁺ at 0.5 mM. For anions sulfate was present at 1.0 mM, chloride at 0.7 mM and phosphate 0.02 mM. These results suggest that calcium carbonate was the most important membrane deposit. Since calcium ions were not present in the catholyte originally, they must have migrated from the anode compartment through the cation exchange membrane. Migration of ions between anode- and cathode compartments occurs in electrochemical systems to maintain charge balance. Since the oxidation of organics at the anode liberates protons and the reduction of oxygen to hydrogen peroxide at the cathode consumes protons, migration of other ions than protons or hydroxide ions will lead to pH shifts [see e.g. 18, 19]. A high pH in the catholyte leads to a shift in the carbonate system equilibrium towards CO₃²⁻ which together with calcium ions forms calcium carbonate precipitates. In future work on bioelectrochemical H₂O₂ production, we should try to minimize calcium carbonate deposits on the membrane. One option may be to use an anion exchange membrane as separator to prevent calcium ions from migrating from the anode chamber to the cathode chamber. Another option could be to use an acidic catholyte to prevent carbonate from dissolving into the liquid.

3.2 H₂O₂ Production

Production of hydrogen peroxide was investigated in specific tests (Table 1 and Table 2). The highest concentration of 9.67 g/L H₂O₂ was achieved after a 21-h test with controlled anode potential and synthetic feed to the anode. With real wastewater, the highest concentration was 2.3 g/L, which was achieved in 9 hours with a controlled current of 3 mA. This is significantly higher than the 0.08 g/L in 21 hrs, which we obtained with real wastewater in a previous study [3]. The reason is the larger anode surface area (58.8 cm² vs 31 cm²) and higher current (3 mA vs 0.65 mA) that could be generated by the anode in this study compared to the previous study. This shows the importance of correctly dimensioning the anode surface area in relation to the cathode compartment volume.

Despite the high internal resistance of the bioelectrochemical reactor used in this study, H₂O₂ could be produced at a rather low energy cost. The electrical energy input was 1.8 to 3.0 kWh/kgH₂O₂ with synthetic feed and 2.2 to 8.3 kWh/kgH₂O₂ with real wastewater. Comparing the cathodic coulombic efficiency (i.e. the efficiency with which electric current is converted to H₂O₂) in the tests operated with constant current shows a slight decrease with increasing test duration. Longer test duration leads to higher H₂O₂ concentration. A higher H₂O₂ concentration would make H₂O₂ more likely to self-decompose or be reduced to water on the cathode surface, which would lower the coulombic efficiency.
Table 2. H₂O₂ production tests. The table shows average current, final H₂O₂ concentration and pH in the catholyte, cathodic and anodic coulombic efficiency (CE), acetate consumption in anolyte, percentage of acetate used to reduce acetate, and energy input.

<table>
<thead>
<tr>
<th>Test #</th>
<th>Avg. I (^a) (mA)</th>
<th>H₂O₂ conc. (^b) (g/L)</th>
<th>Final cat. pH</th>
<th>Cat. CE (^c) (%)</th>
<th>ΔAc (^d) (mM)</th>
<th>An. CE (^c) (%)</th>
<th>SO₄(^{2-}) red. (^e) (%)</th>
<th>Energy input (^f) (kWh/kgH₂O₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.5±0.1</td>
<td>0.70</td>
<td>11.7</td>
<td>80</td>
<td>1.2±0.2</td>
<td>15.3±0.2</td>
<td>32.7±2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>3.0</td>
<td>0.60</td>
<td>11.7</td>
<td>78</td>
<td>0.8±0.2</td>
<td>19.9±4.4</td>
<td>45.6±8.7</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>4.0±0.1</td>
<td>3.50</td>
<td>12.3</td>
<td>76</td>
<td>1.1±0.2</td>
<td>19.5±3.5</td>
<td>36.4±10.2</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>3.0</td>
<td>2.51</td>
<td>12.2</td>
<td>73</td>
<td>0.9±0.3</td>
<td>18.0±1.8</td>
<td>48.4±2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>4.7±0.1</td>
<td>9.67</td>
<td>12.9</td>
<td>78</td>
<td>1.2±0.2</td>
<td>21.1±1.3</td>
<td>41.5±9.1</td>
<td>3.0</td>
</tr>
<tr>
<td>6</td>
<td>3.0</td>
<td>5.18</td>
<td>12.3</td>
<td>64</td>
<td>1.1±0.1</td>
<td>14.3±1.2</td>
<td>53.6±2.5</td>
<td>2.6</td>
</tr>
<tr>
<td>7</td>
<td>1.0±0.2</td>
<td>0.23</td>
<td>11.0</td>
<td>86</td>
<td>0.5</td>
<td>9.5±1.4</td>
<td>78.9</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>3.0</td>
<td>0.62</td>
<td>11.8</td>
<td>81</td>
<td>0.7</td>
<td>21.7</td>
<td>58.4</td>
<td>5.5</td>
</tr>
<tr>
<td>9</td>
<td>1.1±0.0</td>
<td>0.90</td>
<td>10.9</td>
<td>73</td>
<td>0.7±0.1</td>
<td>7.4±0.6</td>
<td>63.6±6.2</td>
<td>2.9</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
<td>2.26</td>
<td>11.9</td>
<td>66</td>
<td>0.7±0.0</td>
<td>21.4±1.0</td>
<td>61.7±1.3</td>
<td>8.3</td>
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<tr>
<td>11</td>
<td>0.9±0.2</td>
<td>1.51</td>
<td>10.7</td>
<td>64</td>
<td>0.7±0.0</td>
<td>5.8</td>
<td>83.2</td>
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<tr>
<td>12</td>
<td>3.0</td>
<td>1.73</td>
<td>11.8</td>
<td>37</td>
<td>X(^i)</td>
<td>X(^i)</td>
<td>X(^i)</td>
<td>18.7</td>
</tr>
</tbody>
</table>

\(^a\)Values with error margins (±) were from experiments with controlled anode potentials, others were run with controlled current of 3.0 mA.

\(^b\)Cathodic coulombic efficiency (percentage of current charge equivalents used for H₂O₂ production).

\(^c\)Anodic coulombic efficiency (percentage of the charge equivalent from the consumed acetate used to produce current).

\(^d\)Percentage of charge equivalents from the consumed acetate used to reduce sulfate.

\(^e\)On the first sampling occasion the influent acetate was 0.7 mM, the subsequent three sampling occasions had influent acetate concentration of 0.0 mM.

\(^f\)Test #12 was terminated early and measurements on effluent concentrations were not carried out.

Tests were carried out with either controlled anode potential or controlled current operation. Controlled current operation was expected to give a predictable concentration of H₂O₂ in the catholyte irrespective of anode feed whereas controlled anode potential was expected to maximize current generation (and thereby the H₂O₂ production rate) without exceeding the capacity of the electrochemically active microorganisms in the anode chamber. The controlled current operation did indeed give a predictable H₂O₂ concentration in the catholyte. The concentrations produced with synthetic feed and real wastewater were similar when constant current operation was employed (Figure 3). Operation with constant anode potential, however, led to varying H₂O₂ concentrations depending on the current the anode could deliver. With synthetic feed, the anode delivered an average current of 4.7 mA in test #5, which led to a H₂O₂ concentration of 9.67 g/L after 21 h. When the anode was fed with real wastewater, the same test duration only led to a H₂O₂ concentration of 1.51 g/L since the delivered current was much smaller. Thus, for reliable H₂O₂ production, constant current operation would be the best choice. However, constant current operation can lead to problems in bioelectrochemical systems. If the biological anode cannot deliver the drawn current through oxidation of organics, other abiotic reaction will occur, which will lead to a dramatically increased anode potential. This occurred in test #12, which had to be interrupted after 13.9 h since the potentiostat could not provide a large enough potential (>5 V) to support a 3 mA current. Moreover, abiotic oxidation reactions at the anode may lead to the formation of oxygen or other oxidants, which may permanently damage the biological activity at the anode. This seems to have occurred in test #12 since the polarization curve done on day 60 (the day after test #12) showed a dramatically deteriorated anode performance (Figure 2).
The type of wastewater fed to the anode determined the current generation at controlled anode potential. With synthetic feed, the average current ranged from 3.5 to 4.7 mA when the anode was controlled at -0.1 V vs SHE. The synthetic feed had an average influent acetate concentration of 5.5±0.5 mM acetate. The anodic coulombic efficiency (CE) (i.e. the fraction of the consumed acetate utilized for current production) ranged from 14.3 to 21.1%. The fraction of acetate used to reduce sulfate ranged from 32.7% in test #1 to 53.6% in test #6. Other processes such as aerobic oxidation in the effluent collection vessel and methanogenesis that may have been responsible for the remaining portion acetate loss were not quantified.

With real wastewater, the current was 0.9-1.1 mA when the anode was controlled at -0.1 V vs SHE. The real wastewater had an average influent DOC concentration of 3.2±0.2 mM. About half of the DOC in the real wastewater was present as acetate, which had an average concentration of 0.9±0.1 mM. The high fraction acetate in the wastewater DOC could have been caused by fermentation taking place after collecting it at the wastewater treatment plant and storing it in the laboratory. When the real wastewater was fed to the anode, the reduction in DOC concentration between the influent and effluent was similar to the reduction in acetate concentration. This means that even with real wastewater as anode feed, acetate was the main source of carbon and energy for the electrochemically active microorganisms. The anodic coulombic efficiency was 5.8-9.5% in the tests with controlled anode potential. With the current controlled at 3.0 mA, it was over 21%. Compared to synthetic feed, a larger fraction (58.4-83.2%) of the removed acetate was used for sulfate reduction in the real wastewater.

The pH in the catholyte increased during the H$_2$O$_2$ production tests (Table 2). Test #5, which had the highest current, also had the highest final catholyte pH of 12.9. Test #11, which had the lowest current, had the lowest pH of 10.7. Since Nafton 117 is a cation exchange membrane, cations migrated from the anode compartment to the cathode compartment when current was flowing in the system. The concentrations of Na$^+$, Ca$^{2+}$, Mg$^{2+}$, and K$^+$ were analyzed in the anolyte and in the catholyte. The distribution of these four ions in the anolyte and their increase in the catholyte are shown in Figure 4. In the synthetic feed, Na$^+$ made up approximately 90% of the four cations, and also made up about 90% of the ionic charge transferred to the catholyte. In the real wastewater, Mg$^{2+}$ and Ca$^{2+}$ made up a larger fraction of the total charge, around 10% each. However, in the catholyte, the Ca$^{2+}$ fraction was very small. This is because the calcium ions that migrated to the
cathode compartment were to a large extent deposited as calcium carbonate on the membrane. The percentage distribution of cations in the membrane deposits are shown in Figure 4.

![Figure 4. Percentage distribution of charge equivalents of Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), and K\(^+\) in the anolyte, catholyte, and membrane deposits. The catholyte distribution refers to ions transferred from the anolyte.](image)

4. Conclusions

This is the first study to show that a H\(_2\)O\(_2\) concentration which could be practically useful for membrane cleaning in membrane bioreactor treatment plants can be generated in a bioelectrochemical reactor with real municipal wastewater as anode feed. A concentration of 2.26 g/L was produced in 9 hours at an energy input of 8.3 kWh/kgH\(_2\)O\(_2\). With an acetate-containing nutrient medium as anode feed, a concentration of 9.67 g/L could be generated in 21 hours with an energy input of 3.0 kWh/kgH\(_2\)O\(_2\).

To reduce the energy requirements for H\(_2\)O\(_2\) production, the internal resistance of the reactor must be lowered. In this study, a high internal resistance was partly caused by calcium carbonate deposits on the cathode-facing side of the cation exchange membrane separating the anode- and cathode compartments.

Acknowledgements

The project was supported by Japan Society for Promotion of Science (JSPS) KAKENHI (21360250). The first author was supported by a post-doctoral fellowship from JSPS.

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