

# Tailoring Microbial Electrochemical Cells for Production of Hydrogen Peroxide at High Concentrations and Efficiencies

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A microbial peroxide producing cell (MPPC) for H<sub>2</sub>O<sub>2</sub> production at the cathode was systematically optimized with minimal energy input. First, the stability of H<sub>2</sub>O<sub>2</sub> was evaluated using different catholytes, membranes, and catalyst materials. On the basis of these results, a flat-plate MPPC fed continuously using 200 mM NaCl catholyte at a 4 h hydraulic retention time was designed and operated, producing H<sub>2</sub>O<sub>2</sub> for 18 days. H<sub>2</sub>O<sub>2</sub> concentration of 3.1 g L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> with 1.1 Wh g<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> power input was achieved in the MPPC. The high H<sub>2</sub>O<sub>2</sub> concentration was

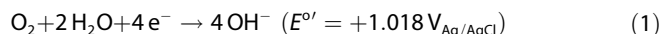
a result of the optimum materials selected. The small energy input was largely the result of the 0.5 cm distance between the anode and cathode, which reduced ionic transport losses. However, > 50% of operational overpotentials were due to the 4.5–5 pH unit difference between the anode and cathode chambers. The results demonstrate that a MPPC can continuously produce H<sub>2</sub>O<sub>2</sub> at high concentration by selecting compatible materials and appropriate operating conditions.

## Introduction

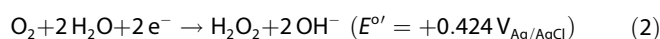
Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is an industrial chemical used in the water and wastewater industries in low concentrations, coupled with the Fenton process or UV treatment for advanced oxidation of complex organic chemicals and emerging contaminants<sup>[1]</sup> and for disinfection.<sup>[2]</sup> For example, Badawy and Ali demonstrated that 550 mg<sub>H<sub>2</sub>O<sub>2</sub></sub> L<sup>-1</sup> was sufficient for 92% COD (chemical oxygen demand) removal and 100% color removal from wastewater diluted to 1600 mg<sub>COD</sub> L<sup>-1</sup>.<sup>[3]</sup> H<sub>2</sub>O<sub>2</sub> is also effective in advanced oxidation processes at removing biological products or contaminants contributing to taste and odor.<sup>[2c,4]</sup> Rajala-Mustonen and Heinonen-Tanski achieved a 3–4 log-units reduction in coliphages when coupling H<sub>2</sub>O<sub>2</sub> with UV radiation for disinfection.<sup>[5]</sup> Yang et al. found that many organic micro-pollutants can be remediated 99+ % by using H<sub>2</sub>O<sub>2</sub> doses in the 50–150 mg L<sup>-1</sup> range and moderate to high UV radiation doses (< 5000 mJ cm<sup>-2</sup>).<sup>[1c]</sup>

Although H<sub>2</sub>O<sub>2</sub> itself is considered environmentally friendly, 95% of the world's H<sub>2</sub>O<sub>2</sub> is produced using the energy-intensive anthraquinone oxidation process, which uses dangerous compounds as catalysts.<sup>[1a]</sup> One potential technology for sus-

tainable H<sub>2</sub>O<sub>2</sub> production is microbial peroxide-producing cells (MPPCs). In MPPCs, anode-respiring bacteria (ARB) consume volatile fatty acids produced during fermentation and respire electrons to an anode, producing electrical current. The electrons pass through an external circuit to a cathode, where they reduce different electron acceptors. In the cathodic oxygen reduction reaction (ORR), O<sub>2</sub> is completely reduced to OH<sup>-</sup> to produce electrical power [Eq. (1)]:



or partially reduced to produce H<sub>2</sub>O<sub>2</sub> [Eq. (2)]:



Above the pK<sub>a</sub> of 11.8, H<sub>2</sub>O<sub>2</sub> is produced as HO<sub>2</sub><sup>-</sup> through [Eq. (3)]:



H<sub>2</sub>O<sub>2</sub> synthesis is advantageous because it requires only a simple carbon catalyst and, depending upon the required rate of H<sub>2</sub>O<sub>2</sub> production, has the potential to be produced with little or no energy input.

Researchers have had limited success producing H<sub>2</sub>O<sub>2</sub> in MPPCs with batch reactors, as summarized in Table 1. Generally, either high concentrations of H<sub>2</sub>O<sub>2</sub> were generated by adding energy to the system or low H<sub>2</sub>O<sub>2</sub> concentrations were produced along with a small output of electrical energy. Rozendal et al. produced 3.9 g<sub>H<sub>2</sub>O<sub>2</sub></sub> L<sup>-1</sup> d<sup>-1</sup> and concentrations as high as 1.3 g L<sup>-1</sup> but required 0.93 Wh per g H<sub>2</sub>O<sub>2</sub>.<sup>[6]</sup> Conversely, Fu et al. produced a low H<sub>2</sub>O<sub>2</sub> concentration of 79 mg L<sup>-1</sup>, but

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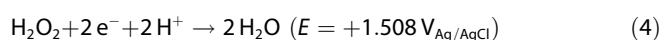
**Table 1.** H<sub>2</sub>O<sub>2</sub> production in microbial electrochemical cells (MECs) as reported in literature. All reactors were dual-chamber systems. Anion exchange membrane = AEM, cation exchange membrane = CEM.

Ref.	Cathode setup	Membrane type	Cathode HRT [h]	Anolyte/buffer	Catholyte	Maximum H <sub>2</sub> O <sub>2</sub> concentration [g L <sup>-1</sup> ]	H <sub>2</sub> O <sub>2</sub> production rate [g L <sup>-1</sup> d <sup>-1</sup> ]	Maximum current and power/ voltage applied	Power input required [Wh g <sub>H<sub>2</sub>O<sub>2</sub></sub> <sup>-1</sup> ]
[9a]	batch	AEM	4	domestic and hospital wastewaters	50 mM NaCl	0.34	2.0	10 A m <sup>-2</sup> at 0.6 V	2.5
[10b]	batch	3-D CEM	24	domestic wastewater and 12 mM acetate media/50 mM PBS	50 mM NaSO <sub>4</sub>	0.2	0.2	18.4 A m <sup>-2</sup> at 0.04 V produced	0.09 power output
[7]	batch	CEM	3	glucose media/50 mM PBS	50 mM K <sub>3</sub> Fe(CN) <sub>6</sub> +PBS	0.08	0.6	0.2 A m <sup>-2</sup> at 0.4 V produced	0.06 power output
[11]	continuous	CEM	0.023	12 mM acetate media/50 mM PBS	50 mM NaSO <sub>4</sub>	8.5 × 10 <sup>-5</sup>	0.1	6.1 A m <sup>-2</sup> at 0.6 V	56
[10a]	batch	CEM	47	acetate media	50 mM NaCl	5	2.6	2.5 A m <sup>-2</sup> at 3.8 V	2.3
[10a]	batch	CEM	21	domestic wastewater	50 mM NaCl	0.08	0.1	0.4 A m <sup>-2</sup> at 0.9 V	1.8
[8]	batch	CEM	21	6 mM acetate media/10 mM PBS	50 mM NaCl	9.7	11.1	1.7 A m <sup>-2</sup> at 11.8 V	3.0
[6a]	batch	CEM	8	12 mM acetate media/190 mM PBS	50 mM NaCl	1.3	3.9	5.3 A m <sup>-2</sup> at 0.5 V	0.93
[9b]	batch	CEM	2–24	5 mM acetate media/50 mM PBS	deionized water	1.4	7.9 × 10 <sup>-4</sup>	7.7 A m <sup>-2</sup> at 1 V	2.6
[9b]	batch	CEM	2–10	raw domestic wastewater	deionized water	0.15	8.5 × 10 <sup>-5</sup>	0.56 A m <sup>-2</sup> at 6.3 V	28
this paper	continuous	AEM	4	acetate media	200 mM NaCl	3.1	18.6	10.1 A m <sup>-2</sup> at 0.31 V	1.1

generated 0.06 Wh per g H<sub>2</sub>O<sub>2</sub>.<sup>[7]</sup> In an attempt to maximize H<sub>2</sub>O<sub>2</sub> production, Modin and Fukushi produced H<sub>2</sub>O<sub>2</sub> at a concentration of 9 g L<sup>-1</sup> and a rate of 11.1 g L<sup>-1</sup> d<sup>-1</sup> while using 3.0 Wh g<sup>-1</sup> H<sub>2</sub>O<sub>2</sub>, demonstrating that high H<sub>2</sub>O<sub>2</sub> concentrations can be achieved when enough energy is added to the system.<sup>[8]</sup>

Most MPPC research focused more on optimization of one or two variables rather than a systematic investigation of factors affecting H<sub>2</sub>O<sub>2</sub> production, net energy demand, and reactor design. Fu et al.,<sup>[7]</sup> Modin and Fukushi<sup>[8]</sup>, Arends et al.,<sup>[9a]</sup> and Sim et al.<sup>[9b]</sup> focused on maximizing H<sub>2</sub>O<sub>2</sub> production from different wastewater sources at different anode and cathode hydraulic retention times (HRTs). Modin and Fukushi<sup>[10a]</sup> and Chen et al.<sup>[10b]</sup> focused on designing cathode catalysts for H<sub>2</sub>O<sub>2</sub> production. Li et al. used a continuous-flow cathode but focused primarily on optimizing abiotic catalyst performance.<sup>[11]</sup>

Several abiotic studies demonstrated that H<sub>2</sub>O<sub>2</sub> rapidly decomposes to H<sub>2</sub>O when exposed to platinum-carbon catalysts<sup>[12]</sup> through Equation (4):



Although carbon-based electrodes can achieve high H<sub>2</sub>O<sub>2</sub> concentrations and current efficiencies through Equation (2),<sup>[11,12a,13]</sup> carbon electrodes can decompose H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O, particularly when the catalyst layer is thick. Paulus et al. used a rotating-ring-disk electrode (RRDE) to understand catalyst reaction pathways at a variety of operating conditions and catalyst characteristics.<sup>[14]</sup>

pH can be an important source of overpotential in MPPCs. When the pH value in the cathode chamber increases one unit at standard temperature and pressure, the MPPC incurs an approximately 60 mV drop in voltage based on the Nernst equation.<sup>[15]</sup> Because the optimal pH value of ARB media is 7.0,<sup>[16]</sup> a pH 12 cathode incurs approximately 300 mV of concentration overpotential between the cathode and anode. Cathodic pH control is complicated by OH production during H<sub>2</sub>O<sub>2</sub> synthesis through Equations (2) or (3), increasing catholyte pH value and overpotential. Ki et al. tested the effects of pH-reducing buffers at the cathode to reduce pH-related concentration overpotentials and determined that the addition of bicarbonate buffer decreased the catholyte pH value from approximately 12 to 7, reducing the applied voltage by 200 mV.<sup>[17]</sup>

The strong oxidizing power of H<sub>2</sub>O<sub>2</sub> further complicates MPPC design. H<sub>2</sub>O<sub>2</sub> and its ions and radicals present chemical incompatibility problems with the materials typically used in microbial electrochemical cells (MECs), including catalysts, binders, and membranes. Furthermore, the reactions by which H<sub>2</sub>O<sub>2</sub> attacks MPPC materials also lead to H<sub>2</sub>O<sub>2</sub> decomposition. Until now, research has not been performed to determine the compatibility of typical MEC materials with H<sub>2</sub>O<sub>2</sub> at high pH values.

In previous works, Ki et al. designed a flat-plate, two-chamber MEC to maximize current densities and voltage efficiencies by applying a high-surface-area anode with buffered catholytes to reduce the pH value.<sup>[17]</sup> Applied potentials and current densities were greatly affected by two factors: Reduced distance

between the anode and cathode and the use of buffered catholyte to reduce the pH gradient between the anode and cathode chambers. Reduction of the distance between anode and cathode to approximately 0.5 cm minimized Ohmic overpotentials between the electrodes. In addition to these results, Ki et al. found that membrane selection had the smallest effect on system overpotentials.<sup>[17]</sup>

In this work, we outline a methodology for designing MECs for H<sub>2</sub>O<sub>2</sub> production. We modify Ki et al.'s MEC design to construct an MPPC that achieves long-term, continuous H<sub>2</sub>O<sub>2</sub> production.<sup>[17]</sup> We first evaluated various membranes, cathode materials, and catholytes for H<sub>2</sub>O<sub>2</sub> compatibility. We then applied our findings to design and operate a continuous-flow cathode in a flat-plate MPPC to obtain a good balance of H<sub>2</sub>O<sub>2</sub> concentration (> 3.1 g L<sup>-1</sup>) and a low power input (1.1 Wh g<sup>-1</sup>).

## Results and Discussion

### H<sub>2</sub>O<sub>2</sub> stability tests

Figure 1 illustrates that H<sub>2</sub>O<sub>2</sub> was more stable at lower pH values during 120 h stability tests. Phosphate buffers performed well, with pH 4.5 phosphate buffer solution (PBS) resulting in no detectable H<sub>2</sub>O<sub>2</sub> decomposition in 24 h and 7% over 120 h whereas pH 7.5 PBS resulted in 6% reduction in H<sub>2</sub>O<sub>2</sub> concentration over 24 h and 21% reduction over 120 h, which is consistent with the results of Yang et al.<sup>[1c]</sup> A NaCl solution at pH 6.5 also resulted in short-term stability of H<sub>2</sub>O<sub>2</sub> as the H<sub>2</sub>O<sub>2</sub> concentration was reduced by 13% in 24 h and by 62% in 120 h. H<sub>2</sub>O<sub>2</sub> became increasingly unstable as the pH value increased to alkaline conditions in the presence of Na<sub>2</sub>CO<sub>3</sub> and NaCl. H<sub>2</sub>O<sub>2</sub> concentration was reduced in pH 11.5 Na<sub>2</sub>CO<sub>3</sub> by 31% within the first 2 h and by 99% within 24 h. Similarly, 49% of H<sub>2</sub>O<sub>2</sub> degraded in pH 12 NaCl within 24 h. The susceptibility of H<sub>2</sub>O<sub>2</sub> to decomposition under alkaline conditions is consistent with studies by Brown and Abbot<sup>[18a]</sup> and Qiang et al.<sup>[18b]</sup>. The reduced stability in the presence of

CO<sub>3</sub><sup>2-</sup> may be attributed to increased H<sub>2</sub>O<sub>2</sub> decomposition rates caused by the formation of metal–hydrogen–carbonate complexes in alkaline conditions.<sup>[19]</sup>

From a broader perspective, the decreasing stability of H<sub>2</sub>O<sub>2</sub> at higher pH values becomes a processing and storage issue. Our stability tests confirm that alkaline-produced H<sub>2</sub>O<sub>2</sub> cannot be stored long-term without significant decomposition. Thus, maintaining a relatively low or neutral pH value at the cathode is optimal for H<sub>2</sub>O<sub>2</sub> production and stability.

### Membrane material selection

Several factors are considered important in membrane selection. Membrane compatibility with H<sub>2</sub>O<sub>2</sub> is the most important factor because contaminants or functional groups on the membrane may contribute to H<sub>2</sub>O<sub>2</sub> decomposition either catalytically or through a reaction with the membrane itself. H<sub>2</sub>O<sub>2</sub> reacting with the membrane may destabilize the membrane's integrity, leading to membrane failures. Membrane compatibility at different pH values is also important because the OH<sup>-</sup> produced during the ORR [Eq. (2)] has the potential to significantly increase the catholyte pH, and H<sub>2</sub>O<sub>2</sub> deprotonates to the more reactive HO<sub>2</sub><sup>-</sup> at a pK<sub>a</sub> of 11.8. Therefore, we evaluated H<sub>2</sub>O<sub>2</sub> decomposition and membrane weight loss over a 45 day exposure period at pH 7 and 12. We used electrochemical impedance spectroscopy (EIS; detailed in the Supporting Information) to evaluate membrane Ohmic overpotentials. Finally, the choice of using an anion exchange membrane (AEM) or cation exchange membrane (CEM) results in operational trade-offs. HO<sub>2</sub><sup>-</sup> produced at high pH values can potentially diffuse across an AEM. However, a CEM provides preferential diffusion of cations other than H<sup>+</sup> to the cathode to maintain electro-neutrality, potentially lowering the pH value below an acceptable threshold for ARB.

AMI-7001, CMI-700, and FAA membranes were evaluated for stability in a 10 g<sub>H<sub>2</sub>O<sub>2</sub></sub> L<sup>-1</sup> solution at pH 7. After 45 days of exposure, there was negligible H<sub>2</sub>O<sub>2</sub> degradation at pH 7 (Figure S1a in the Supporting Information), and the membranes experienced negligible mass loss (Figure S1b). This stability was likely a result of the suitability of the membranes for use near neutral pH and increased H<sub>2</sub>O<sub>2</sub> stability at pH 7.

As OH<sup>-</sup> production owing to H<sub>2</sub>O<sub>2</sub> formation at the cathode would likely increase catholyte pH during MPPC operations, membranes were evaluated for compatibility and H<sub>2</sub>O<sub>2</sub> degradation at pH 12. Figure S2 illustrates that H<sub>2</sub>O<sub>2</sub> was most stable in the presence of the Nafion membrane: Approximately 11% of the total H<sub>2</sub>O<sub>2</sub> decomposed over 45 days while the electrolyte pH value decreased from 12 at day zero to 2.5 at day 45. This decomposition was significantly lower than the 91% decomposition exhibited during H<sub>2</sub>O<sub>2</sub> stability tests without the membrane (Figure 1). All other membranes exhibited > 85% H<sub>2</sub>O<sub>2</sub> decomposition, values similar to the 91% measured during H<sub>2</sub>O<sub>2</sub>/NaCl stability tests without a membrane, without significant pH change between days 0 and 45. Thus, the lack of degradation with Nafion was likely a result of acidification of the electrolyte.

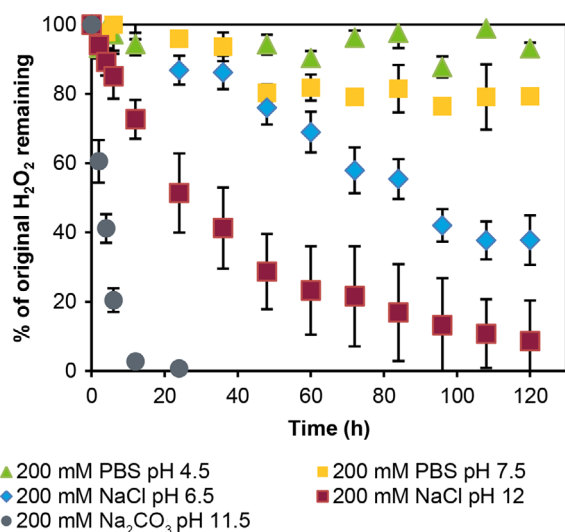
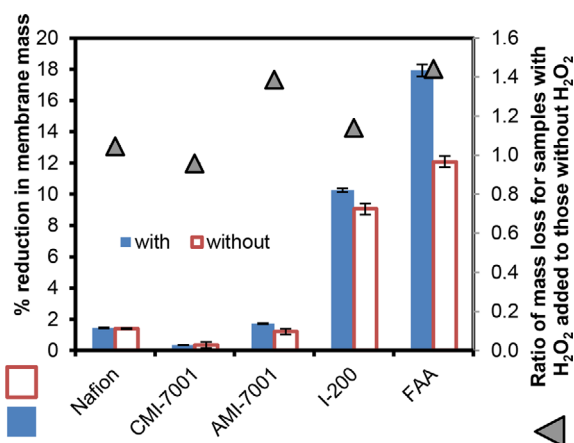


Figure 1. H<sub>2</sub>O<sub>2</sub> stability in various electrolytes at different pHs.



**Figure 2.** (left axis) Percent reduction in membrane mass during batch bottle tests for membrane stabilities with  $\text{H}_2\text{O}_2$  concentration of  $10 \text{ g L}^{-1}$  at pH 12 and (right axis) ratio of mass loss for membranes exposed to  $\text{H}_2\text{O}_2$  versus membranes exposed to pH 12 only. Values  $> 1$  indicate that membranes exposed to  $\text{H}_2\text{O}_2$  lost more weight than membranes exposed to electrolyte only. Values  $< 1$  indicate that membranes exposed to electrolyte only lost more weight than membranes exposed to  $\text{H}_2\text{O}_2$ .

$\text{H}_2\text{O}_2$  degradation did not necessarily correlate with membrane mass loss during the stability tests. Figure 2 illustrates that all membranes lost weight at pH 12, regardless of the presence of  $\text{H}_2\text{O}_2$ . Apart from CMI-7000, all other membranes exposed to  $\text{H}_2\text{O}_2$  experienced higher weight loss than membranes exposed to electrolyte only. The CEM membranes demonstrated lower differences in weights ( $< 5\%$  for both CEMs) than AEMs because the CEMs' negatively charged active sites likely repelled  $\text{HO}_2^-$  ions from the membrane. For AEMs, the I-200 membrane had a 14% difference between the  $\text{H}_2\text{O}_2$ -exposed and electrolyte-only masses, making it the AEM with the smallest change in mass when exposed to  $\text{H}_2\text{O}_2$ . AMI-7001 and FAA lost 39 and 44% more mass, respectively, when exposed to  $\text{H}_2\text{O}_2$  versus samples at high pH only.

Although FAA is rated by the manufacturer as stable at pH 12, FAA's lower thickness ( $130 \mu\text{m}$ ) may have contributed to a lower structural integrity, causing the membrane to disintegrate when exposed to  $\text{H}_2\text{O}_2$  at pH 12 (Figure S3). AMI-7001 experienced the lowest absolute mass loss ( $0.69 \text{ mg cm}^{-2}$  with and  $0.96 \text{ mg cm}^{-2}$  without  $\text{H}_2\text{O}_2$ ), which we speculate is the result of either AMI-7001 being a less reactive material or its greater thickness ( $3.5\times$  thicker than FAA), which hampered  $\text{H}_2\text{O}_2$  permeation into and, therefore, decomposition of the membrane. Total organic carbon (TOC) analyses (detailed in the Supporting Information) revealed that membranes exposed to  $\text{H}_2\text{O}_2$  and/or high pH values consistently produced more TOC over the duration of the experiment, likely caused by the release of complex soluble organics into solution during polymer deterioration (Figure S4).

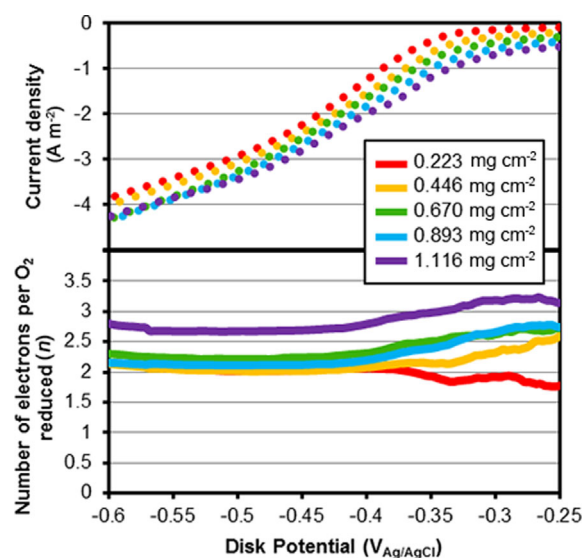
Nafion, AMI-7001, and FAA demonstrated greater  $\text{H}_2\text{O}_2$  stability in the short term, making them the most viable candidates for an MPPC. In addition, all membranes exhibited low Ohmic losses  $< 85 \Omega\text{-cm}^2$  (detailed in the Supporting Information). Based on this evaluation, we opted to use an AEM in the MPPC to provide easy regulation of anode pH value. Based on

its low reactivity with  $\text{H}_2\text{O}_2$ , we chose to utilize AMI-7001 for MPPC experiments. If the pH value increases at the cathode, catholyte choice could help regulate the pH value near neutral to reduce concentration overpotentials.

### Cathode material characterization

We utilized linear sweep voltammetry in a half cell with  $100 \text{ mM}$  sodium perchlorate to narrow our catalyst and binder choices to Vulcan carbon and Nafion, respectively (method detailed in the Supporting Information). The Vulcan carbon had an approximately  $0.4 \text{ V}$  lower activation potential than graphite carbon for the same binder (Figure S7).

We used RRDE testing to determine the optimal Vulcan carbon loading because it distinguishes between 2-electron and 4-electron ORR. Consistent with Bonakdarpour et al.<sup>[20]</sup> and Paulus et al.,<sup>[14]</sup> lower catalyst loadings resulted in a higher number of electrons being transferred to  $\text{H}_2\text{O}_2$  instead of to  $\text{H}_2\text{O}$ . Between  $-0.37$  and  $-0.57 \text{ V}_{\text{Ag}/\text{AgCl}}$  (Figure 3), loadings of  $0.22$  and  $0.45 \text{ mg cm}^{-2}$  provided the lowest average catalyst loading and yielded an average of  $2.04 \pm 0.03$  electrons per  $\text{O}_2$  molecule being reduced, or almost 100% delivery of electrons to the 2-electron reduction to  $\text{H}_2\text{O}_2$ . The highest catalyst loading ( $1.12 \text{ mg cm}^{-2}$ ) yielded an average of  $2.72 \pm 0.06$  electrons per  $\text{O}_2$  molecule being reduced, meaning that about one-third of the electrons were transferred to  $\text{H}_2\text{O}$ . At higher loadings, the produced  $\text{H}_2\text{O}_2$  must be transported through a thick catalyst layer, providing additional catalyst contact time and increasing the likelihood that  $\text{H}_2\text{O}_2$  is reduced again through Equation (4) to  $\text{H}_2\text{O}$ . Figure 3 also shows the disk potentials because the cell required current input regardless of operating potential to operate. At an  $E_{\text{KA}}$  (the anode potential at one-half of the maximum current density) of  $-0.42 \text{ V}_{\text{Ag}/\text{AgCl}}$  for *Geobacter sulfurreducens*, the MPPC will likely require some small power input to produce  $\text{H}_2\text{O}_2$ .<sup>[21]</sup>



**Figure 3.** Ring current density (dotted lines) and the number of electrons per  $\text{O}_2$  reduced ( $n$ ; solid lines) as a function of disk potential for Vulcan carbon catalyst loadings ranging from  $0.22$  to  $1.12 \text{ mg cm}^{-2}$ .



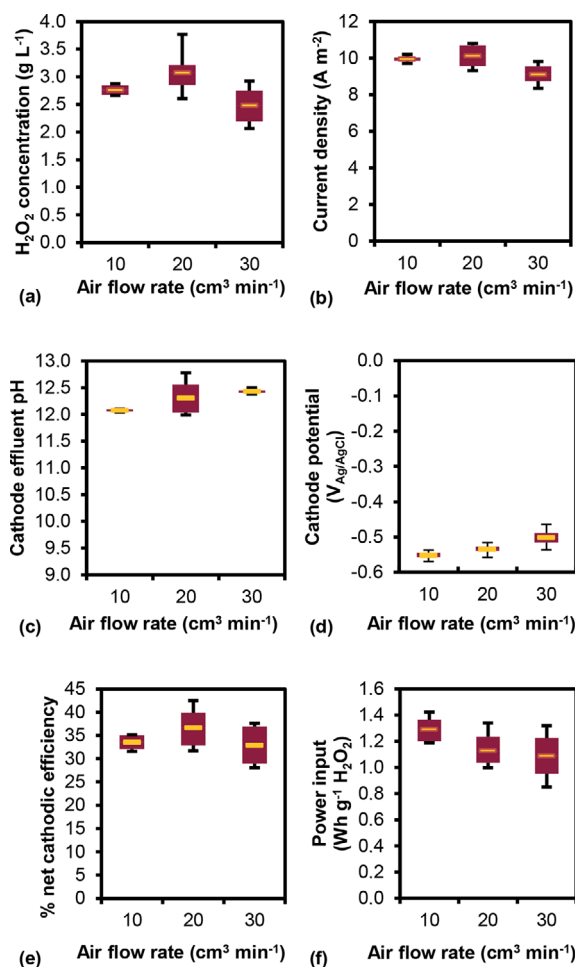
To summarize, the Vulcan carbon/Nafion binder combination produced the lowest cathodic overpotentials with an optimal Vulcan carbon loading of approximately  $0.45 \text{ mg cm}^{-2}$  for the highest  $\text{H}_2\text{O}_2$  production over a wide range of cathode potentials. Based on these results, we applied approximately  $0.5 \text{ mg cm}^{-2}$  of Vulcan carbon to the cathode for MPPC operations.

### MPPC operation

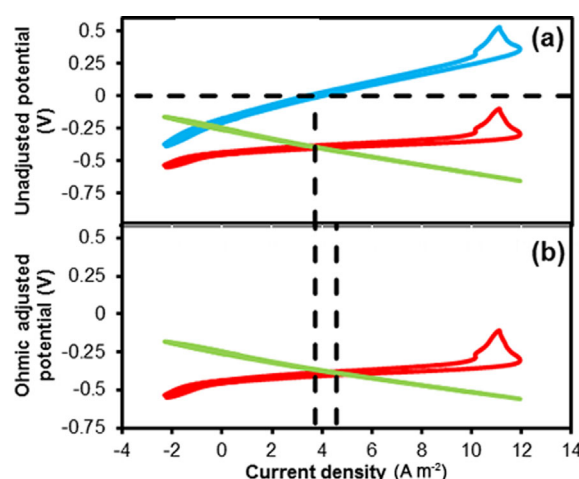
We assembled a MPPC using the optimal conditions from our previous testing:  $200 \text{ mM NaCl}$  at  $\text{pH } 7$ , which demonstrated good stability with  $\text{H}_2\text{O}_2$  in the short term; the AMI-7001 AEM, which showed low mass loss; and  $0.5 \text{ mg cm}^{-2}$  of Vulcan carbon catalyst with Nafion binder, which maintained the lowest cathodic overpotentials over the largest range while transferring the highest number of electrons to  $\text{H}_2\text{O}_2$ . We operated the MPPC continuously for 18 days with a 4 h HRT in the liquid cathode chamber and air-flow rates of 10, 20, and  $30 \text{ cm}^3 \text{ min}^{-1}$  through the air cathode chamber. Prior to anode inoculation, we quantified the total cell abiotic Ohmic overpotential as  $75.0 \Omega \text{ cm}^{-2}$  using EIS.

As demonstrated in Figure 4, the MPPC showed good performance with the optimized design variables. Variation of the air supply rate had minimal effect on  $\text{H}_2\text{O}_2$  concentration: The average effluent  $\text{H}_2\text{O}_2$  concentration ranged from  $2.5 \pm 0.4$  to  $3.1 \pm 0.4 \text{ g L}^{-1}$ , with the highest  $\text{H}_2\text{O}_2$  concentration ( $3.8 \text{ g L}^{-1}$ ) achieved at  $20 \text{ cm}^3 \text{ min}^{-1}$ . These high concentrations were achieved even though the effluent catholyte pH ranged from 12.1 to 12.4. Although the cathode potential decreased from  $-0.54$  to  $-0.50 \text{ V}_{\text{Ag}/\text{AgCl}}$ , the  $\text{H}_2\text{O}_2$  concentration decrease from 20 to  $30 \text{ cm}^3 \text{ min}^{-1}$  was likely a result of the  $1 \text{ A m}^{-2}$  decrease in current density. For comparison, our cell used 18% more energy to produce three times the amount of  $\text{H}_2\text{O}_2$  compared to Rozendal et al.<sup>[9]</sup>

We used cyclic voltammetry (CV) to understand our MPPC's performance. The CVs illustrated that the MPPC achieved higher energy-neutral current densities (i.e., the current at which the reactor experiences  $0 \text{ V}$  cell potential) than other systems because of reduced overpotentials, and cathodic overpotentials are still the limiting factor in MPPC performance. Figure 5 shows a CV of our MPPC at a  $1 \text{ mV s}^{-1}$  scan rate. Correcting for Ohmic losses in the system, the open-circuit potential was  $0.20 \text{ V}$  for all scans, which is lower than the theoretical potential of  $0.56 \text{ V}$ . The approximately  $0.3 \text{ V}$  difference is associated with the equilibrium pH difference between the anode and cathode because there was an approximately 5 pH unit difference between the anode and cathode. Energy-neutral operations occurred at  $3.72 \pm 0.29 \text{ A m}^{-2}$ , which is significantly larger than that achieved by Rozendal et al.<sup>[6]</sup> at  $1.6 \text{ A m}^{-2}$  and Modin and Fukushi<sup>[10a]</sup> at  $0.54 \text{ A m}^{-2}$ . The superior energy-neutral performance indicates that  $\text{H}_2\text{O}_2$  could be produced at up to  $3.72 \text{ A m}^{-2}$  without requiring energy input and the cell had significantly lower overpotentials than in previous studies. Based on the theoretical potentials at the anode and cathode and not corrected for Ohmic losses, Figure 5a demonstrates that, at energy-neutral conditions, the anode overpotential was  $0.149 \text{ V}$  and cathode overpotential was  $0.379 \text{ V}$ . Concentra-



**Figure 4.** Performance during continuous operations with varying air-flow rate: (a)  $\text{H}_2\text{O}_2$  concentration, (b) current density, (c) pH, (d) cathode potential, (e) percent net cathodic efficiency (as  $\text{H}_2\text{O}_2$ ), and (f) power input required to produce  $1 \text{ g}$  of  $\text{H}_2\text{O}_2$ .



**Figure 5.** Cyclic voltammetry of the MPPC for (a) unadjusted cell and (b) Ohmic corrected potentials, with cell (blue), anode (red), and cathode (green) potentials. For the cell potentials, positive voltages represent power consumption and negative voltages represent power production.

tion overpotential due to pH differences between the anode and cathode chambers accounts for 0.27 V or 51 % of cathode overpotentials. Therefore, 0.029 V Ohmic overpotential exists at energy-neutral conditions, reiterating that our cell design significantly decreases Ohmic losses within the MPPC. However small, these Ohmic losses significantly affected cell performance: Adjusted for Ohmic losses, Figure 5b shows that the anode and cathode overpotentials increased to 0.157 and 0.403 V, respectively, and that the cell could operate at  $4.58 \text{ A m}^{-2}$  before drawing additional energy to produce  $\text{H}_2\text{O}_2$ . At our operating current density of approximately  $10 \text{ A m}^{-2}$  and pH 12.08–12.43, the cathode overpotential increased to 0.524 V, 58 % of which was due to pH differences between the anode and cathode chambers. As discussed by Popat and Torres, the production of  $\text{OH}^-$  ions during the oxygen reduction reaction increases pH at the cathode, making it more difficult to reduce the operating pH value of the cathode and, consequently, concentration overpotential.<sup>[22]</sup>

The superior performance of the MPPC we report here likely was the result of a combination of several factors. The improvement in energy-neutral operations was largely driven by the decreased distance between anode and cathode: When the distance was decreased from 1 to 0.5 cm in the MPPC, the energy-neutral current density increased from  $1.63 \pm 0.03$  to  $3.72 \pm 0.29 \text{ A m}^{-2}$  (Figure S5). Catalyst selection tailored to  $\text{H}_2\text{O}_2$  production resulted in low cathode potentials and good net cathodic efficiencies with little power input. The use of AMI-7001 membrane provided long-term, stable performance while having minimal effect on  $\text{H}_2\text{O}_2$  production compared to other membranes, as shown in Figure S5. Nevertheless, performance could be further improved by reducing the pH gradient between the anode and cathode chambers, consequently reducing the MPPC's concentration overpotential.

## Conclusions

In this work, we outlined a methodology for designing microbial electrochemical cells (MECs) for  $\text{H}_2\text{O}_2$  production. We described the material characterization required to design MECs to continuously produce  $\text{H}_2\text{O}_2$ . The Vulcan carbon/Nafion binder combination provided chemical stability with  $\text{H}_2\text{O}_2$  while producing minimal activation overpotentials compared to graphite catalyst. Using a rotating-ring-disk electrode (RRDE), we determined that the optimal catalyst loading to achieve the 2-electron oxygen reduction reaction (ORR) was approximately  $0.5 \text{ mg cm}^{-2}$ . Anion-exchange-membrane (AEM) stability tests established AMI-7001 as the optimal membrane to resist  $\text{H}_2\text{O}_2$  degradation and promote long-term MPPC performance due to its high structural integrity.

We combined these findings into a continuous-flow flat-plate microbial peroxide-producing cell (MPPC), which we operated using 200 mM NaCl catholyte at different air-flow rates to optimize  $\text{H}_2\text{O}_2$  concentration. The air-flow rate did not drastically change MPPC performance: The MEC produced  $\text{H}_2\text{O}_2$  at a concentration as high as  $3.8 \text{ g L}^{-1}$  and an average of  $2.5 \pm 0.4$  to  $3.1 \text{ g L}^{-1}$  at different air-flow rates. The MPPC's Ohmic overpotentials were small at  $69.1 \Omega \text{ cm}^2$ . During operation, anodic

overpotentials were approximately 42 % lower than cathodic overpotentials, and  $>58\%$  of cathodic overpotential was caused by the pH gradient between the anode and cathode chambers. Thus, we assert that continuous  $\text{H}_2\text{O}_2$  production in MECs is achievable if materials are optimized for compatibility with and production of  $\text{H}_2\text{O}_2$ .

For perspective, our MPPC is capable of producing  $\text{H}_2\text{O}_2$  at concentrations more than adequate for water and wastewater treatment.  $\text{H}_2\text{O}_2$  concentrations of  $2.5\text{--}3.0 \text{ g L}^{-1}$  are five- to ten-fold higher than the doses required to remove 90 + % chemical oxygen demand (COD) from wastewater streams (Badawy and Ali<sup>[3]</sup>, Ksibi<sup>[2c]</sup>), 20-fold greater than required for 99 + % removal of micropollutants and 10–100-fold higher than required for UV disinfection.<sup>[1c, 2e, 5]</sup>

## Experimental Section

### $\text{H}_2\text{O}_2$ measurements and stability tests

For membrane stability experiments, we measured  $\text{H}_2\text{O}_2$  concentration by using the National Diagnostics Hydrogen Peroxide Assay Kit. For all other experiments, we analyzed  $\text{H}_2\text{O}_2$  according to the method of Graf and Penniston.<sup>[23]</sup> Colorimetric measurements were performed using a Cary 50-Bio UV/Vis spectrophotometer (Varian, Palo Alto, CA). When compared, these two methods demonstrated equivalent results (not presented here).

We evaluated  $\text{H}_2\text{O}_2$  stability using different electrolytes. We prepared 200 mL solutions of  $\text{H}_2\text{O}_2$  ( $10 \text{ g L}^{-1}$ ) in five different electrolytes: pH 4.5 PBS (200 mM), pH 7.5 PBS (200 mM), NaCl at pH 6.5 (200 mM), NaCl at pH 12 (200 mM), and  $\text{Na}_2\text{CO}_3$  at pH 11.5 (200 mM). Each electrolyte combination (65 mL) was placed in three 100 mL glass-serum bottles and corked with butyl-rubber stoppers (Bellco Glass, NJ) and aluminum caps. We removed 2 mL samples using a needle and syringe from each bottle after 2, 4, 6, and 12 h and every 12 h subsequently for 120 h. Samples were analyzed for pH value and  $\text{H}_2\text{O}_2$  concentration.

### Membrane stability tests

We characterized five ion exchange membranes to determine their compatibility with  $\text{H}_2\text{O}_2$ : three AEMs including AMI-7001 (Membranes International, Inc.), Excellion I-200 (SnowPure LLC), and fumasep<sup>®</sup> FAA (fumatech GmbH); and two CEMs, that is, CMI-7000 (Membranes International, Inc.) and Nafion-117 (Dupont<sup>™</sup>). We tested the stability of unconditioned membrane in solutions with  $\text{H}_2\text{O}_2$  and pH 7 and 12. Membranes with surface areas of  $9 \text{ cm}^2$  were cut, left at ambient lab conditions for 48 h, and weighed. The membranes were placed in 25 mL serum bottles filled with 20 mL of 100 mM NaCl at pH 12, 100 mM NaCl with  $10 \text{ g L}^{-1}$   $\text{H}_2\text{O}_2$  at pH 12, or 100 mM NaCl together with a  $\text{H}_2\text{O}_2$  solution at  $10 \text{ g L}^{-1}$  and at pH 7. We adjusted the electrolyte pH value to 12 as it is the highest pH value anticipated during MPPC operations. Bottles were prepared in triplicate, capped with butyl rubber stoppers and aluminum crimps, and degassed regularly. After 45 days, we emptied the bottles' contents and analyzed the solutions for  $\text{H}_2\text{O}_2$  concentrations and pH values. Membranes were washed with deionized water and dried under ambient laboratory conditions prior to measuring the final mass.

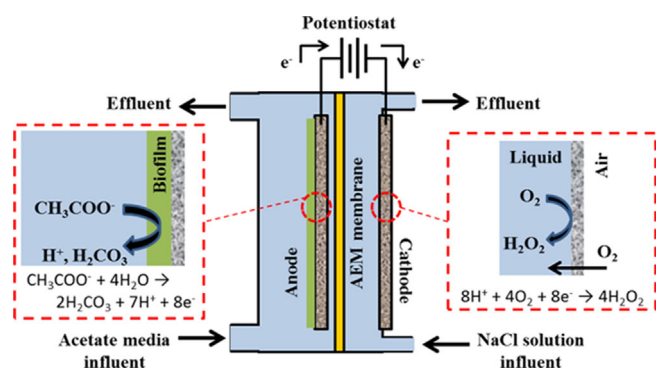
### Catalyst/binder characterization

We performed RRDE tests using a RRDE-3 A rotating ring disk electrode apparatus (ALS-Japan) to determine potential  $\text{H}_2\text{O}_2$  production efficiency for different catalyst/binder combinations. We used a Vulcan carbon catalyst/Nafion binder mixture to coat the catalyst layer on the disk electrode at catalyst loadings of 0.22, 0.44, 0.67, 0.89, and  $1.12 \text{ mg cm}^{-2}$ . The ring-disk electrode was rotated at 1200 rpm for approximately 30 min until the ink dried. Once dried, the ring was submerged in NaCl (100 mM) sparged with  $\text{O}_2$  for >30 min to saturate the solution. We measured the ring and disk current densities at a disk speed of 1600 rpm and ring potential of  $0.08 \text{ V}_{\text{Ag/AgCl}}$  and the disk potential was varied from  $-0.60$  to  $0 \text{ V}_{\text{Ag/AgCl}}$  at a scan rate of  $10 \text{ mV s}^{-1}$  to determine the number of electrons transferred to  $\text{O}_2$ .

### MPPC setup and operation

Scheme 1 illustrates our two-chamber, flat-plate MPPC operated at  $30^\circ\text{C}$ . The anode, cathode, and membrane had geometrical areas of  $49 \text{ cm}^2$ . The anode chamber volume was 200 mL. The anode was composed of carbon fiber (24 K Carbon Tow, Fibre Glast, OH, USA) woven through a titanium frame, as detailed by Ki et al.<sup>[17]</sup> An AMI-7001 membrane was preconditioned in NaCl (2 M) for 1 day prior to use. The cathode chamber consisted of a 18 mL liquid serpentine flow cell and a serpentine air chamber supplied with air from a EcoPlus® Eco Air 3 commercial pump. The two cathode chambers were separated by the carbon cloth cathode (FuelCellStore GDL-CT). The liquid-exposed side of the cathode was coated with a slurry of Vulcan carbon powder ( $0.5 \text{ mg cm}^{-2}$ ) using a paint brush. The catalyst slurry consisted of Vulcan carbon powder (0.5 g), 5% Nafion dispersion in alcohol (3.5 mL,  $0.83 \text{ mL cm}^{-2}$ , D52, FuelCellStore). The air-exposed MPL side was coated with two layers of  $16 \text{ mg cm}^{-2}$  Teflon PTFE DISP 30 cured 15 min at  $200^\circ\text{C}$  and 1 h at  $280^\circ\text{C}$  to improve cathode hydrophobicity. The distance separating the anode and cathode was approximately 0.5 cm. We used a Bioanalytical Systems, Inc. RE-5B Ag/AgCl reference electrode ( $+0.27 \text{ V}$  versus standard hydrogen electrode in acetate media at  $30^\circ\text{C}$ ) and a Bio-Logic VMP3 potentiostat to control anode potential at  $-0.30 \text{ V}_{\text{Ag/AgCl}}$  and monitored current production. We measured the pH value using Thermo Scientific, Inc.'s Orion 2 Star pH meter.

We inoculated the anode chamber with effluent from operating MECs in our laboratory to develop current-producing biofilms. The MPPC's anode was poised at  $-0.3 \text{ V}_{\text{Ag/AgCl}}$  and operated in batch mode until biofilms were formed and then placed into continuous-flow mode and fed with acetate medium (100 mM, approximately



**Scheme 1.** A schematic of the microbial peroxide producing cell (MPPC) configuration used to produce  $\text{H}_2\text{O}_2$  in the liquid cathode chamber.

pH 7; detailed in Parameswaran et al.<sup>[24]</sup>) at 0.5 h HRT. The cathode was operated in continuous-flow mode and fed NaCl catholyte (200 mM) with 4 h HRT to the liquid chamber and air ( $20 \text{ cm}^3 \text{ min}^{-1}$ ) to the air chamber. The MPPC operated in the peroxide-producing mode for more than three weeks.

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**Keywords:** anode-respiring bacteria · cathodes · electrochemistry · hydrogen peroxide · microbial electrochemical cells

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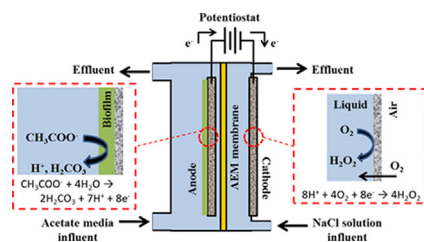
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## FULL PAPERS

**Never-ending H<sub>2</sub>O<sub>2</sub> production:** A methodology for designing microbial electrochemical cells (MECs) for H<sub>2</sub>O<sub>2</sub> production is outlined and the materials characterization is described. Continuous H<sub>2</sub>O<sub>2</sub> production at high concentrations more than adequate for water and wastewater treatment is achieved using a microbial peroxide-producing cell (MPPC) by selecting compatible materials and appropriate operating conditions. H<sub>2</sub>O<sub>2</sub> can be produced at high concentrations at the highest rates thus far achieved in MPPCs.



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B. E. Rittmann, C. I. Torres\*



**Tailoring Microbial Electrochemical  
Cells for Production of Hydrogen  
Peroxide at High Concentrations and  
Efficiencies**

