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Scaling-up of microbial electrosynthesis with multiple electrodes for in-situ production of hydrogen peroxide

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Summary

Microbial electrosynthesis system (MES) has recently been shown to be a promising alternative way for realizing in-situ and energy-saving synthesis of hydrogen peroxide (H$_2$O$_2$). Though promising, the scaling-up feasibility of such a process is rarely reported. In this study, a 20 L up-scaled two-chamber MES reactor was developed and investigated for in-situ and efficient H$_2$O$_2$ electrosynthesis. The maximum H$_2$O$_2$ production rate of 10.82 mg L$^{-1}$ h$^{-1}$ and cumulative H$_2$O$_2$ concentration of 454.44 mg L$^{-1}$ within 42 h were obtained with an input voltage of 0.6 V, cathodic aeration velocity of 0.045 mL min$^{-1}$ mL$^{-1}$, 50 mM Na$_2$SO$_4$, and initial pH of 3. The electrical energy consumption regarding direct input voltage was only 0.239 kWh kg$^{-1}$ H$_2$O$_2$, which was further much lower compared with lab-scale systems. The obtained results suggested that the future industrialization of MES technology for in-situ synthesis of H$_2$O$_2$ and further application in environmental remediation have broad prospects.

Keywords: Microbial electrosynthesis system; Up-scaled; H$_2$O$_2$ production; In-situ electrosynthesis; Energy consumption.
Introduction

Hydrogen peroxide (H$_2$O$_2$) is a green and environmentally friendly oxidant that has been widely used in industrial and environmental applications (Kelly et al., 2019; Sheng et al., 2020; Zhao et al., 2020). According to statistics, the world's H$_2$O$_2$ production capacity exceeded 5 million tons in 2015 and maintained steady growth, most of which were synthesized based on the anthraquinone method, which has the disadvantage of high energy consumption and high-risk coefficient (Sheng et al., 2020; Zhao et al., 2020). In addition, due to the unstable chemical properties of H$_2$O$_2$, this also provides challenges for transportation and storage and correspondingly increases costs (Chen et al., 2018). For many applications, such as advanced oxidation processes for water treatment, the required concentration of H$_2$O$_2$ was usually below 1000 mg L$^{-1}$. In this context, it is urgent and of importance to develop alternative methods for the in-situ synthesis of H$_2$O$_2$. In the past years, among others, electrochemical methods have received extensive attention due to their relatively high H$_2$O$_2$ synthesis efficiency and easy operation (only need electrical energy as input) (Campos-Martin et al., 2006; Siahrostami et al., 2013). Despite its promise, the electrochemical methods are still suffering from the disadvantage of large energy costs (Perry et al., 2019).

Recently, the microbial electrosynthesis system (MES), in which electrochemically active microorganisms can obtain energy from the oxidation of organics in sewage, have been demonstrated as an efficient and cost-effective means for the production of H$_2$O$_2$ (Hassan et al., 2019; Li et al., 2018; Zhao et al., 2020). Specifically, in a typical two-chamber MES, the anodic electrochemically active microorganisms can oxidize organic matter in wastewater and generate electrons, protons and carbon dioxide via Eq. (1) (Hassan et al., 2019). Among them, the generated electrons and protons are further transferred to the cathode chamber through wires and cation exchange membrane (CEM), respectively, and are further combined with oxygen to generate H$_2$O$_2$ through two-electron reduction via Eq. (2) (Hassan et al., 2019).

$$C_aH_bO_c + (2a-c)H_2O \rightarrow aCO_2 + (b+4a-2c)H^+ + (b+4a-2c)e^- \quad (1)$$
To this end, the microbial fuel cell (MFC) and its derived microbial electrolysis cell (MEC) based MES have been proposed for H\textsubscript{2}O\textsubscript{2} production, and the range of produced H\textsubscript{2}O\textsubscript{2} was 79 to 1300 mg L\textsuperscript{-1} (Li et al., 2017). This production level would be able to satisfy the application in the water treatment process. Nonetheless, it was worth noting that most of the MES studies on the in-situ synthesis of H\textsubscript{2}O\textsubscript{2} including MFC or MEC were conducted with lab-scale reactors ranging from milliliters to hundreds of milliliters. Scaling-up is of importance to prove the feasibility and economic profits of such a process for large scale application. However, a systematic evaluation of the scaling-up of MES for H\textsubscript{2}O\textsubscript{2} synthesis is rarely reported. So far, the only scaling-up study was reported by Sim et al. who developed a 110 L dual-chamber up-scaled MEC reactor (10 L of cathode chamber and 1.6-2.0 V voltage input) to synthesize H\textsubscript{2}O\textsubscript{2}. Nevertheless, the H\textsubscript{2}O\textsubscript{2} titer was less than 100 mg L\textsuperscript{-1} after 20 days of operation, and the corresponding H\textsubscript{2}O\textsubscript{2} conversion efficiency was only about 7.2% in a form of short communication (Sim et al., 2018). Thus, more efforts are required towards the scaling-up of the MES-based H\textsubscript{2}O\textsubscript{2} production. Among others, the electrode material is one of the important factors for a successful scaling-up, which is directly related to the current efficiency (CE), two-electron oxygen reduction reaction (ORR) selectivity (Eq. (2)), and energy consumption during the in-situ electrosynthesis of H\textsubscript{2}O\textsubscript{2} in the cathode. In traditional MFC for bioelectricity production, noble metals (e.g. titanium and platinum) are usually used as electrode materials or catalysts for favoring four-electron oxygen reduction to produce H\textsubscript{2}O via Eq. (3) (Chung et al., 2020; Hassan et al., 2019).

O\textsubscript{2} + 4H\textsuperscript{+} + 4e\textsuperscript{-} → 2H\textsubscript{2}O

(3)

In contrast, carbon-based electrodes without containing noble metals, especially the commonly used graphite-based electrodes such as graphite plate and graphite felt, are often applied in the electrosynthesis of H\textsubscript{2}O\textsubscript{2} given their merits of strong electrical conductivity, high two-electron ORR selectivity, inexpensive, long lifetime, weak H\textsubscript{2}O\textsubscript{2} decomposition effect, and easy to scale up (An et
al., 2019; Chung et al., 2020; Li et al., 2016; Li et al., 2017). In addition, it should be noted that although most of the current lab-scale studies related to bioelectrochemical synthesis of H$_2$O$_2$ used gas diffusion electrodes (GDE) as the cathode to obtain a high H$_2$O$_2$ yield, their mechanical property makes it difficult to be scaled-up (Li et al., 2016). Moreover, the effect of acidic pH (2-3) on the stability of the GDE and the formation of possible refractory by-products are two main challenges for in-situ wastewater treatment (Wang et al., 2020a). In addition, almost all of these lab-scale MES reactors were only equipped with a single electrode including a bioanode and an abiotic cathode in each chamber, which tended to cause system instability.

This study successfully scaled up a MES with multiple graphite electrodes to 20 L (dual-chamber and 10 L of each chamber), and comprehensively investigated the influence of operating parameters including input voltage, cathodic aeration velocity, catholyte pH, and electrolyte nature and concentration on the in-situ synthesis of H$_2$O$_2$. To the best of our knowledge, this is the first scaled-up MES reactor equipped with highly stable graphite plate electrodes for in-situ H$_2$O$_2$ synthesis. The present study not only verified the feasibility of such scaled-up MES for H$_2$O$_2$ synthesis but also accumulated valuable experience for subsequent industrial applications on the treatment of wastewater.

Results and discussion

The performance of 20-L scaled-up MES reactor for H$_2$O$_2$ synthesis

A series of experiments were carried out to demonstrate the feasibility of the 20-L scaled-up MES reactor for H$_2$O$_2$ synthesis. Fig. 1 shows the change of H$_2$O$_2$ concentration with operating time at the cathode in the 20-L scaled-up MES reactor. The concentration of H$_2$O$_2$ increased monotonically with operating time and reached about 454.44 mg L$^{-1}$ at 42 h. Specifically, the synthesis rate of H$_2$O$_2$ in the first 16 h was significantly higher than that in the next 26 hours, especially after 36 hours, the increase of H$_2$O$_2$ concentration was relatively slow. Moreover, the cathodic pH varied during the process (Fig.S1). A rapid rise in pH was observed as the reaction progressed. The
cathodic pH reached 8.58 in the first 16 h, and then gradually increased and finally reached 10.07 at 42 h. The fast increase of pH observed in the first 16 h was due to the fast reduction of two electrons of oxygen to H₂O₂ via Eq. (2). After that, when the cathodic pH turned to alkaline, the main reaction occurring on the cathode surface was the reduction of oxygen to HO₂⁻ via Eq. (4) (Brillas et al., 1997; Moreira et al., 2017).

\[ \text{O}_2 + \text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{HO}_2^- + \text{OH}^- \]  (4)

In addition, the in-situ generated H₂O₂ would be further reduced to H₂O and OH⁻ under acidic or alkaline conditions via Eq. (5) and (6), respectively, and the decomposition of H₂O₂ would also occur simultaneously via Eq. (7) and (8), thus more H₂O₂ would be decomposed in a longer operating time (Nidheesh and Gandhimathi, 2012; Qiang et al., 2002; Xia et al., 2015). In short, the above results also proved the feasibility of the graphite plate as the cathode of this scaled-up reactor for two-electron ORR to generate H₂O₂ via Eq (2).

\[ \text{H}_2\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow 2\text{H}_2\text{O} \]  (5)

\[ \text{HO}_2^- + \text{H}_2\text{O} + 2\text{e}^- \rightarrow 3\text{OH}^- \]  (6)

\[ 2\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}_2\text{O} \]  (7)

\[ \text{H}_2\text{O}_2 + \text{OH}^- \rightarrow \text{HO}_2^- + \text{H}_2\text{O} \]  (8)

Besides, the control experiments including the MES reactor without cathodic aeration (control 1), open circuit (control 1), and without input voltage (control 3) were also conducted and the results were also exhibited in Fig. 1. As expected, no H₂O₂ production was detected when the system was in an open circuit. According to Eq. (2), as no electrons were flowing from the anode to the cathode in the open-circuit state, there were no H₂O₂ produced. Similarly, the reactor without cathodic aeration or input voltage (corresponding to operating at MFC mode) only produced a low concentration of H₂O₂ (below 20 and 70 mg L⁻¹, respectively). The low H₂O₂ production could be attributed to insufficient oxygen or electrons for the synthesis of H₂O₂ based on Eq. (2). Similar results were also found in the previous studies by using a lab-scale MES and electrochemical
system (Li et al., 2017; Yu et al., 2015a). Therefore, the obtained results highlighted that the reactor operating in MEC mode (requiring a small amount of voltage input) was effective and superior in H$_2$O$_2$ productivity compared with operating in MFC mode. However, it is worth emphasizing that around 70 mg L$^{-1}$ of H$_2$O$_2$ was still produced when this scaled-up reactor was operated in the MFC mode, which further proved that the scaled-up reactor can generate enough H$_2$O$_2$ to meet the water and wastewater treatment requirements (e.g. 5-50 mg L$^{-1}$ of H$_2$O$_2$ were needed for water disinfection and micro-pollutant removal) (Chung et al., 2020).

In addition to studying the H$_2$O$_2$ synthesis at the cathode, we also monitored the current and cathode potential of the system under different conditions. As depicted in Fig.S1, the stable and relatively high current density output (around 2.86 A m$^{-2}$) together with a stable cathode potential (around -0.6 V) were observed during the entire operation, respectively. It proved that the biological anode of this scaled-up system was stable during the test. Furthermore, since the performance of the in-situ synthesis of H$_2$O$_2$ via MES has been shown to depend on the transfer of effective microbial electrons to the anode (Logan and Regan, 2006), the observed stable current output demonstrated the feasibility of further application of large-scale MES in-situ synthesis of H$_2$O$_2$ in the future. In contrast, the current density outputs obtained in the control conditions (around 1.25, 0, and 0.56 A m$^{-2}$) were significantly lower than the normal operating condition.

Overall, the above results proved that the scaled-up MES reactor with sufficient cathodic aeration and electron flow can provide stable and efficient in situ production of H$_2$O$_2$.

Fig.1 is here

**Effect of operating parameters**

**Input voltage**

The H$_2$O$_2$ synthesis was generally slow in MFC mode due to the low circuit current (Li et al., 2017). As an alternative, H$_2$O$_2$ production can be significantly increased by applying a small amount of applied voltage (normally below 0.8 V) in MEC mode. In general, a higher input voltage would
lead to a higher current, thus the enhanced electron flow would increase the rate of \( \text{H}_2\text{O}_2 \) synthesis. However, it would not be the case, because the higher input could also promote the occurrence of side reactions, resulting in a decrease in current efficiency, and produce similar or even lower \( \text{H}_2\text{O}_2 \) yields compared with lower input voltage (Moreira et al., 2017; Oturan et al., 2018). Therefore, seven groups of different input voltages (0.1, 0.2, 0.4, 0.5, 0.6, 0.7 and 0.8 V) were selected to investigate their influence on \( \text{H}_2\text{O}_2 \) synthesis. As shown in Fig.2a, the cumulative \( \text{H}_2\text{O}_2 \) production in 42 hours were 125.36, 236.36, 314.93, 353.40, 454.44, 408.70, and 385.71 mg L\(^{-1}\), respectively, at the input voltage ranging from 0.1 to 0.8 V. The results indicated that the optimum input voltage was 0.6 V and further increasing the input voltage (0.7 and 0.8 V) led to a decrease in \( \text{H}_2\text{O}_2 \) production. Likewise, the variation trend of current efficiency presented in Fig.2b also in line with the yield of \( \text{H}_2\text{O}_2 \), and the corresponding values were 1.63%, 2.42%, 2.80%, 3.41%, 2.66% and 2.37% at input voltage ranging from 0.1 to 0.8 V, respectively. The system current and cathode potential were shown in Fig.S2, respectively. It was observed that both system current density (from 1.63 to 3.44 A m\(^{-2}\)) and cathode potential (in terms of absolute value, from -0.45 to -0.66 V) increased continuously with the increase of the applied voltage from 0.1 to 0.8 V. Thus, the above results can be explained from two aspects. On the one hand, the increase of current density led to accelerated electron transfer on the surface of the cathode electrode, and thereby promoting oxygen reduced to the \( \text{H}_2\text{O}_2 \) via Eq. (1) (Yu et al., 2015a; Yu et al., 2015b). On the other hand, higher currents may also enhance the occurrence of side reactions, such as accelerating the conversion of the produced \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \) via Eq. (5), (7) and (8), or even reduction of oxygen to \( \text{H}_2\text{O} \) via Eq. (3) (Chen et al., 2015a; Nadais et al., 2018; Wang et al., 2020b), or hydrogen evolution via Eq. (9) (Chen et al., 2015a).

\[
2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2 \quad (9)
\]

Similar results were also observed in the previous studies regarding abiotic electro or MES for \( \text{H}_2\text{O}_2 \) production (Chen et al., 2015a; Li et al., 2016; Yu et al., 2015a). Notably, the highest \( \text{H}_2\text{O}_2 \) yield (at
0.6 V) obtained in this study was around 10.82 mg L$^{-1}$ h$^{-1}$, which was about 3.3 times higher than that observed in the highest value from lab-scale (cathodic working volume of 14 mL) MEC reactor (3.92 mg L$^{-1}$ h$^{-1}$) (Li et al., 2016). Moreover, it was also found that the production rate of H$_2$O$_2$ was slightly higher than that of the lab-scale (cathodic working volume of 100 mL) MES reactor is driven by salinity gradient (10.80 mg L$^{-1}$ h$^{-1}$) (Li et al., 2017). Taking into account the H$_2$O$_2$ production rate, the current efficiency, and the energy consumption, an input voltage of 0.6 V was the optimal value among the investigated voltages.

Fig.2 is here

**Cathodic aeration rate**

As observed in the previous part, the cathode aeration was essential to H$_2$O$_2$ production. However, it was not that the faster the aeration rate, the higher the concentration of H$_2$O$_2$ produced at the cathode. Excess aeration could reduce H$_2$O$_2$ production and also increase energy consumption. Thus, setting an optimal aeration rate could not only maximize the H$_2$O$_2$ production at the cathode but could also save energy consumption. In this part, the aeration rates ranging from 4 to 500 mL min$^{-1}$, corresponding to the cathode aeration velocities of 0.00045 to 0.056 mL min$^{-1}$ mL$^{-1}$, were adopted to study its effect on the H$_2$O$_2$ synthesis at the cathode. As shown in Fig.3, the H$_2$O$_2$ concentration accumulated at the cathode increased monotonically with the increase of the aeration rate in 42 h, which indicated that the selected aeration rate range has no negative effect on the H$_2$O$_2$ production at the cathode. When the aeration velocity was increased from 0.045 to 0.056 mL min$^{-1}$ mL$^{-1}$, the accumulated H$_2$O$_2$ concentration only increased slightly (from 454.44 to 459.30 mg L$^{-1}$).

Additionally, the concentration of cathodic DO also increased with the increase of aeration velocity (Fig. 3). Among them, it can be seen that when the aeration velocity was lower than 0.045 mL min$^{-1}$ mL$^{-1}$, the catholyte was unsaturated, while the catholyte was in a saturated or supersaturated state when the aeration rate reached 0.045 mL min$^{-1}$ mL$^{-1}$ or above. These results were in agreement with previous studies using photocatalytic or photo-assisted electrocatalytic processes for H$_2$O$_2$
The increased aeration rate increased the catholyte DO and promote the mass transfer rate of oxygen in the catholyte, which was beneficial to the production of \( \text{H}_2\text{O}_2 \) (Luo et al., 2015a; Yu et al., 2015a). When the aeration rate was increased from 400 to 500 mL min\(^{-1}\), the insignificant increase in the accumulated \( \text{H}_2\text{O}_2 \) concentration could be due to the following reasons. Firstly, the increase in the number of bubbles generated by aeration would increase the internal resistance of the system (Freakley et al., 2013; Salmerón et al., 2019). Secondly, the further increase in the size of bubbles generated was adverse to their adsorption to the cathode (Nadais et al., 2018). Therefore, it can be inferred that further increasing the aeration rate over 400 mL min\(^{-1}\) may adverse the \( \text{H}_2\text{O}_2 \) production at the cathode. The aeration rate of 400 mL min\(^{-1}\) was selected as the optimized aeration rate for subsequent experiments.

**Fig.3 is here**

**Initial catholyte pH**

Apart from the input voltage and cathodic aeration velocity, initial catholyte pH was another key factor due to the electrosynthesis of \( \text{H}_2\text{O}_2 \) required the participation of protons according to Eq.(2). Accordingly, the impact of initial catholyte pH (3, 5, 7, and 9) on the cathodic electrosynthesis of \( \text{H}_2\text{O}_2 \) was studied. The results showed that the change of initial pH significantly affected the cathodic synthesis of \( \text{H}_2\text{O}_2 \), and cumulative concentration reached approximately 454.44, 412.71, 395.71, and 316.36 mg L\(^{-1}\), respectively after 42 h operation (Fig. 4a). Similarly, the current efficiency and \( \text{H}_2\text{O}_2 \) rates also showed the same variation trend, resulting in the final values were 3.41%, 3.10%, 2.97%, 2.37%, and 10.82, 9.83, 9.42, 7.53 mg L\(^{-1}\) h\(^{-1}\), respectively (Fig. 4b). In addition, the change of catholyte pH value over time was shown in Fig. 4c. The rapid rise of pH indicated that the consumption rate of protons at the cathode for the synthesis of \( \text{H}_2\text{O}_2 \) was faster than the generation rate at the anode (latterly entered the cathode through the CEM). As mentioned above, under alkaline conditions, especially at pH > 9, \( \text{H}_2\text{O}_2 \) mainly existed in the form of \( \text{HO}_2^- \), which could catalyze the decomposition of \( \text{H}_2\text{O}_2 \) via Eq. (10) (Luo et al., 2015b; Sheng et al., 2011).
\[
H_2O_2 + HO_2^- \rightarrow \cdot OH + \cdot O_2^- + H_2O \quad (10)
\]

This also explained why the cumulative $H_2O_2$ concentration was lower with the initial pH of 9 than other pH values. The same experimental results were found in the electrochemical synthesis of $H_2O_2$ using unmodified graphite electrodes (Yu et al., 2015a). Therefore, when the initial pH was set to 3, more $H_2O_2$ can be synthesized at the cathode, and this value conformed to the optimal pH range (2-4) of the traditional electro-Fenton and bio-electro-Fenton processes (Li et al., 2018; Moreira et al., 2017). It should be noted that the cumulative $H_2O_2$ concentration under the neutral and alkaline conditions observed in this study still meet the needs of water treatment, which may broaden the applicability of the scaled-up MES in other fields such as combining other technologies (e.g. UV based advanced oxidation processes) or catalysts for water treatment. In this way, it may also reduce the chemical cost, as there was no need to use acid and alkali to adjust the pH before water treatment.

**Fig.4 is here**

**Electrolyte nature and concentration**

Generally, research processes related to electrochemical systems required high conductivity in the electrolyte to enhance the flow of electrons. In this study, we have selected three widely used electrolytes including $Na_2SO_4$, $NaCl$, and $Na_2CO_3$ with an initial concentration of 50 mM to explore their effects on the synthesis of $H_2O_2$ at the cathode. As presented in Fig.5a, when $Na_2SO_4$ was used as the electrolyte, the accumulated $H_2O_2$ concentration in 42 h was 454.44 mg L$^{-1}$, followed by $Na_2CO_3$ and $NaCl$ (358.50 and 262.17 mg L$^{-1}$, respectively). The reasons why $Na_2SO_4$ as an electrolyte was superior to $Na_2CO_3$ and $NaCl$ can be explained as follows: 1) chloride ion in NaCl could react with $H_2O_2$ to generate higher reactive HClO and/or chlorine derivatives via Eq.(11)-(18) (De Laat et al., 2004; Lai et al., 2020; Moreira et al., 2017); 2) $CO_3^{2-}$ itself and its hydrolysis product $HCO_3^-$ (main role) could cause the decomposition of $H_2O_2$ via Eq.(19)-(22) (Attiogbe and Francis, 2011; Fabian, 1995; Xia et al., 2020).
\[ \text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{ClO}^- + \text{H}_2\text{O} \quad (11) \]
\[ \text{H}_2\text{O}_2 + \text{ClO}^- \rightarrow \text{Cl}^- + \text{O}_2 + \text{H}_2\text{O} \quad (12) \]
\[ \text{H}_2\text{O}_2 + \text{e}^- \rightarrow \cdot \text{OH} + \text{OH}^- \quad (13) \]
\[ \cdot \text{OH} + \text{Cl}^- \rightarrow \cdot \text{ClOH}^- \quad (14) \]
\[ \cdot \text{ClOH}^- + \text{Cl}^- \rightarrow \cdot \text{Cl}_2^- + \text{OH}^- \quad (15) \]
\[ \cdot \text{Cl}_2^- \rightarrow \cdot \text{Cl} + \text{Cl}^- \quad (16) \]
\[ \cdot \text{Cl}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot \text{HO}_2^- + \text{H}^+ + 2\text{Cl}^- \quad (17) \]
\[ \cdot \text{Cl} + \text{H}_2\text{O}_2 \rightarrow \cdot \text{HO}_2^- + \text{H}^+ + \text{Cl}^- \quad (18) \]
\[ \text{CO}_3^{2-} + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{OH}^- \quad (19) \]
\[ \text{CO}_3^{2-} + \cdot \text{OH} \rightarrow \cdot \text{CO}_3^- + \text{OH}^- \quad (20) \]
\[ \text{HCO}_3^- + \text{H}_2\text{O}_2 \rightarrow \text{HCO}_4^- + \text{H}_2\text{O} \quad (21) \]
\[ \cdot \text{CO}_3^- + \text{H}_2\text{O}_2 \rightarrow \text{HCO}_3^- + \cdot \text{HO}_2 \quad (22) \]

3) compared with NaCl and Na\textsubscript{2}CO\textsubscript{3}, Na\textsubscript{2}SO\textsubscript{4} has higher conductivity (9.02 ms cm\textsuperscript{-1} vs. 8.02 ms cm\textsuperscript{-1} of Na\textsubscript{2}CO\textsubscript{3} and 5.66 ms cm\textsuperscript{-1} of NaCl) and correspondingly higher current (see Fig. S5) when the initial concentration was 50 mM, and thus, it has the highest production of H\textsubscript{2}O\textsubscript{2} (Zhou et al., 2007). Although the side reactions listed above that can affect the synthesis of H\textsubscript{2}O\textsubscript{2} will also produce some free radicals with the strong oxidizing ability (e.g. \cdot Cl and \cdot OH), the amount those strong oxidizing agents produced is very small. Notably, the successful application of bioelectrochemical synthesis of H\textsubscript{2}O\textsubscript{2} for the disinfection of greywater and wetland effluent has been recently reported (Arends et al., 2014; Murawski, 2018). However, the dose of H\textsubscript{2}O\textsubscript{2} needs to reach the target fecal coliform level in a real municipal effluent from the wastewater treatment plant ranged from 106 to 285 mg L\textsuperscript{-1}, which made the direct application of H\textsubscript{2}O\textsubscript{2} as a disinfectant very inappropriate (Wagner et al., 2002). Furthermore, our previous study has also shown that the bioelectrochemical synthesis of H\textsubscript{2}O\textsubscript{2} has a poor killing effect on E. coli with an initial concentration of 10\textsuperscript{7} CFU mL\textsuperscript{-1}, while the combination of H\textsubscript{2}O\textsubscript{2}-producing MEC with Fenton process, named bio-electro-Fenton process,
exhibited a significant improvement on the E. coli inactivation (Zhou et al., 2018). Besides, a recent study exhibited that the strong alkaline catholyte (pH > 13) produced by the MFC-based electroosmosis process can effectively pathogens inactivation, but considering its pH also limits its subsequent application to the disinfection of real wastewater (Gajda et al., 2020). In general, although beneficial attempts have been made to directly apply bioelectrochemical systems for water disinfection, considering the treatment efficiency, integration of bioelectrochemical synthesis of H$_2$O$_2$ with other advanced oxidation processes (e.g. Fenton process) may be a more promising way for simultaneous water disinfection and micro-pollutants removal. It was also worth noting that although the application of Na$_2$CO$_3$ and NaCl also yielded higher H$_2$O$_2$ production, the presence of chloride ions (only when solution pH below 7.2) and carbonates may affect the subsequent application of the synthesized H$_2$O$_2$ for wastewater treatment (e.g., they have a scavenging effect on the free radicals (e.g., *OH) in Fenton reaction (Chen et al., 2019; Kläning and Wolff, 1985; Zhang et al., 2018)). Based on the above, Na$_2$SO$_4$ was used as an electrolyte in the subsequent experiments. Besides the electrolyte nature, the electrolyte concentration which was related to the conductivity was also crucial for the electrosynthesis of H$_2$O$_2$. Thus, different concentrations of Na$_2$SO$_4$ (10, 25, 50, and 100 mM) corresponding to the conductivities of 2.44, 4.95, 9.02 and 16.36 ms cm$^{-1}$, respectively, were tested. As shown in Fig.5c, the increase of Na$_2$SO$_4$ from 10 to 100 mM increased the continuous improvement of cathodic H$_2$O$_2$ accumulation. When the Na$_2$SO$_4$ concentration was further increased from 50 to 100 mM, the increase in the H$_2$O$_2$ synthesis was getting slower. The correlation between electrolyte concentration and H$_2$O$_2$ synthesis can be explained as follows. Firstly, the conductivity was higher with higher electrolyte concentration applied, which further increased the current (see Fig. S6) and thereby promoting the production of H$_2$O$_2$ (Zhou et al., 2007). Secondly, to a certain extent (e.g. below 200 mM), increasing the Na$_2$SO$_4$ concentration could increase the mass transfer, and thereby increasing the H$_2$O$_2$ synthesis (Jin et al., 2014). Thirdly, excessive Na$_2$SO$_4$ may be adsorbed on the surface of the electrode, resulting in a decrease
in the active site that can bind to oxygen molecules on the electrode, which in turn reduces to produce H₂O₂ (Chen et al., 2015b). Lastly, excessive Na₂SO₄ could also consume the generated H₂O₂ via Eq.(23)-(24) (Jin et al., 2014).

\[ \text{SO}_4^{2-} + \cdot \text{OH} \rightarrow \cdot \text{SO}_4^- + \text{OH}^- \] (23)

\[ \cdot \text{SO}_4^- + \text{H}_2\text{O}_2 \rightarrow \text{SO}_4^{2-} + \text{H}^+ + \cdot \text{HO}_2 \] (24)

Although the addition of 100 mM Na₂SO₄ resulted in the highest concentration of H₂O₂, it was considered that there was only a small increase compared to that at 50 mM. In addition, considering the cost of chemicals and the subsequent cost of further desalination, 50 mM Na₂SO₄ was selected for the subsequent test.

**Energy consumption and performance comparison**

To comprehensively understand the feasibility of the scaled-up MES for the in-situ electrosynthesis of H₂O₂, the energy consumption required for the entire process under the selected optimal conditions was further calculated. The energy consumption calculation can be divided into two aspects, one was from the input voltage required by the reactor itself and another was from cathodic aeration using a pump. For the first aspect, the calculated energy consumption was 0.298 kWh kg⁻¹ H₂O₂ (Supplemental Information). Moreover, for the second aspect, the energy consumption by the pump within 42 h was recorded as 0.42 kWh, equal to 99.291 kWh kg⁻¹ H₂O₂. Therefore, the total energy consumption was 99.587 kWh kg⁻¹ H₂O₂, of which approximately 99.70% of energy consumption was consumed by the pump for the cathodic aeration. It was mainly due to extremely low oxygen utilization efficiency (calculated value was 1.43% based on the formula reported in the previous literature) (Yu et al., 2015b). However, low oxygen utilization efficiency was a common phenomenon in which graphite plate was used as electrodes (Yu et al., 2015b). Recently, Zhang et al. reported a superhydrophobic natural air diffusion electrode, which had an oxygen utilization efficiency of up to 65% when used in the synthesis of H₂O₂ (Zhang et al., 2020). This provided a solution for the subsequent application of the scale-up MES system to efficiently synthesize H₂O₂.
and further reduce the energy consumption caused by aeration. In addition, it should be pointed out that the energy consumption for H$_2$O$_2$ synthesis based on electrochemical systems or bioelectrochemical systems reported in the literature almost did not take into account the energy consumption including pumping for aeration, and most of the aeration rates adopted in the literature were similar to or slightly higher than that adopted in this study.

Additionally, Table 1 compared the energy consumption of this scaled-up MES with previously published studies. First of all, although the traditional electrochemical systems (e.g. electro-Fenton) in the lab-scale using graphite as the cathode have slightly higher H$_2$O$_2$ production rate compared to the scaled-up MES in this study, the energy consumption was much higher in the traditional electrochemical systems. Secondly, compared with lab-scale MFC systems that did not require additional voltage input, the scaled-up MES exhibited a faster H$_2$O$_2$ production rate with little energy consumption (0.298 kWh kg$^{-1}$ H$_2$O$_2$). Lastly, compared with the lab-scale MEC systems, the scaled-up MES exhibited not only lower energy consumption but also faster H$_2$O$_2$ production rate. Besides, Table 1 also showed that the H$_2$O$_2$ production rate (88.20 vs 10.82 mg L$^{-1}$) of some MEC systems was higher than that of this study because modified GDE was used in these studies. The reason can be attributed to the fact that the pumped oxygen can be absorbed by the porous structure of the GDE instead of being dissolved in the catholyte, which overcomes the limitation of low H$_2$O$_2$ production usually caused by low oxygen solubility (Yu et al., 2015b). However, using GDE may increase the capital cost in the practical application and may cause water leakage when applied to the scaled-up systems (Wang et al., 2020a). There was only one literature in the form of short communication that reported a MEC system with equivalent cathodic volume as in this study, but the reported H$_2$O$_2$ production rate was much lower and the energy consumption was expected to be higher because of the higher aeration rate and input voltage adopted. Finally, it should be pointed out that the cathode potential measured during the whole experiment is higher than -0.86V, which
indicated that the corrosion of the graphite electrode can be effectively avoided in this scaled-up system, thereby achieving a long lifespan (Qiao et al., 2018).

**Limitation of the study**

Though promising, there are still several bottlenecks that need to be further addressed. First, in this study, the electrolyte (e.g. 50 mM Na$_2$SO$_4$) was added to increase the system current and therefore the production of H$_2$O$_2$, which would lead to an increase in operating cost. The future large-scale applications should be concentrated on high-conductivity wastewater (e.g. textile wastewater) or combined with a membrane technology to treat concentrates (e.g. reverse osmosis). Moreover, the cathodic oxygen utilization rate was low, which was manifested as the cathodic aeration provided by the pump occupied around 99.70% of the total energy consumption. This challenge could be solved in the future through optimization of the reactor configuration and the development of new electrode materials with higher oxygen mass transfer efficiency.

**Resource Availability**

**Lead contact**

Further information and requests for resources and materials should be directed to and will be fulfilled by the Lead Contact, Yifeng Zhang (yifz@env.dtu.dk, yifzmfc@gmail.com).

**Materials Availability**

This study did not yield new unique reagents.

**Data and Code Availability**

There is no dataset or code associated with this work.

**Methods**

All methods can be found in the accompanying Transparent Methods supplemental file.

**Supplemental Information**

Supplemental Information can be found online.

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Author Contributions


Declaration of Interests

The authors declare no competing interests.

References


Figure and Table legends

Figure 1. Feasibility verification of this 20 L scaled-up MES reactor regarding H$_2$O$_2$ production. Operating conditions: input voltage of 0.6 V, cathode aeration velocity of 0.045 mL min$^{-1}$ mL$^{-1}$, initial catholyte pH of 3 and electrolyte nature and concentration of 50 mM Na$_2$SO$_4$, respectively. Control 1: without cathodic aeration. Control 2: open circuit. Control 3: without input voltage.

Figure 2. Effect of input voltage on (a) H$_2$O$_2$ production and (b) current efficiency in the scaled-up reactor. Operating conditions: cathode aeration velocity of 0.045 mL min$^{-1}$ mL$^{-1}$, initial catholyte pH of 3 and electrolyte nature and concentration of 50 mM Na$_2$SO$_4$, respectively.

Figure 3. Effect of cathodic aeration velocity on (a) H$_2$O$_2$ production and (b) current efficiency in the scaled-up MES reactor. Operating conditions: input voltage of 0.6 V, initial catholyte pH of 3 and electrolyte nature and concentration of 50 mM Na$_2$SO$_4$, respectively.

Figure 4. Effect of initial catholyte pH on (a) H$_2$O$_2$ production, (b) current efficiency in the scaled-up MES reactor and (c) pH variation during the process. Operating conditions: input voltage of 0.6 V, cathodic aeration velocity of 0.045 mL min$^{-1}$ mL$^{-1}$, and electrolyte nature and concentration of 50 mM Na$_2$SO$_4$, respectively.

Figure 5. Effect of electrolyte nature and concentration on (a), (c) H$_2$O$_2$ production and (b), (d) current efficiency in the scaled-up MES reactor. Operating conditions: input voltage of 0.6 V, cathodic aeration velocity of 0.045 mL min$^{-1}$ mL$^{-1}$, and electrolyte nature (NaCl, Na$_2$SO$_4$ and Na$_2$CO$_3$) and initial Na$_2$SO$_4$ concentration of 10, 25, 50 and 100 mM, respectively.
Table 1. Previous studies on H$_2$O$_2$ production through (bio)electrochemical systems.
Table 1. Previous studies on H$_2$O$_2$ production through (bio)electrochemical systems.

<table>
<thead>
<tr>
<th>System types</th>
<th>Cathode materials</th>
<th>Operating conditions</th>
<th>Reactor volume</th>
<th>H$_2$O$_2$ production rate</th>
<th>Energy consumption</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electro-Fenton</td>
<td>Graphite</td>
<td>pH of 2, 50 mM NaClO$_4$, cathodic aeration rate of 8747 mL min$^{-1}$, and applied cathode potential of -0.5 vs SCE</td>
<td>7.65 L (3.15 L of cathode)</td>
<td>40.6 mg L$^{-1}$ h$^{-1}$</td>
<td>7.8 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>(Qiang et al., 2002)</td>
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<tr>
<td>MEC</td>
<td>Vulcan carbon coated GDE</td>
<td>pH of 7, 200 mM NaCl, cathodic aeration rate of 20 mL min$^{-1}$, and applied voltage of 0.31 V</td>
<td>218 mL (18 mL of cathode)</td>
<td>8.8 mg L$^{-1}$ h$^{-1}$</td>
<td>1.1 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>(Young et al., 2016)</td>
</tr>
<tr>
<td>MEC</td>
<td>GDE</td>
<td>pH of 7, 50 mM NaCl, and applied voltage of 0.9 V</td>
<td>18.8 mL (9.4 mL of cathode)</td>
<td>4.2 mg L$^{-1}$ h$^{-1}$</td>
<td>1.8 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>(Modin &amp; Fukushi, 2012)</td>
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<tr>
<td>MEC</td>
<td>Electrochemically modified graphite particle</td>
<td>pH of 7, 50 mM Na$_2$SO$_4$, and applied voltage of 0.4 V</td>
<td>96 mL (64 mL of cathode)</td>
<td>88.2 mg L$^{-1}$ h$^{-1}$</td>
<td>0.66 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>(Chen et al., 2015)</td>
</tr>
<tr>
<td>MEC</td>
<td>Carbon black and graphite hybrid air cathode</td>
<td>pH of 7, 50 mM NaCl, cathodic aeration rate of 1500 mL min$^{-1}$, and applied voltage of 0.6 V</td>
<td>42 mL (14 mL of cathode)</td>
<td>3.3 mg L$^{-1}$ h$^{-1}$</td>
<td>56 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>(Li et al., 2016)</td>
</tr>
<tr>
<td>MEC</td>
<td>Carbon GDE</td>
<td>Tap water, cathodic aeration rate of 2000 mL min$^{-1}$, and applied voltage of 1.0-1.6 V (CEM) or 1.6-2.0 V(AEM)</td>
<td>110 L (10 L of cathode)</td>
<td>0.019 mg L$^{-1}$ h$^{-1}$ (AEM) and 0.274 mg L$^{-1}$ h$^{-1}$ (CEM)</td>
<td>-</td>
<td>(Sim et al., 2018)</td>
</tr>
<tr>
<td>System types</td>
<td>Cathode materials</td>
<td>Operating conditions</td>
<td>Reactor volume</td>
<td>$\text{H}_2\text{O}_2$ production rate</td>
<td>Energy consumption</td>
<td>References</td>
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<tr>
<td>MFC</td>
<td>3D graphite cathode</td>
<td>pH of 7 and 50 mM Na$_2$SO$_4$</td>
<td>82 mL (50 mL of cathode)</td>
<td>1.5 mg L$^{-1}$ h$^{-1}$</td>
<td>-</td>
<td>(Li et al., 2018)</td>
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<td>MFC</td>
<td>Graphite</td>
<td>pH of 7 and 100 mM Na$_2$SO$_4$ and cathodic aeration rate of 191 mL min$^{-1}$</td>
<td>160 mL (80 mL of cathode)</td>
<td>6.6 mg L$^{-1}$ h$^{-1}$</td>
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<td>(Fu et al., 2010)</td>
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<tr>
<td>MFC</td>
<td>Electrochemically modified graphite/carbon black/active carbon particle</td>
<td>pH of 7 and 50 mM Na$_2$SO$_4$</td>
<td>204.5 mL (120 mL of cathode)</td>
<td>6.8/7.1/8.1 mg L$^{-1}$ h$^{-1}$</td>
<td>-</td>
<td>(Chen et al., 2014)</td>
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<td>MREC</td>
<td>Graphite</td>
<td>pH of 7, 35 g L$^{-1}$ NaCl, cathodic aeration rate of 12 mL min$^{-1}$, and salt and fresh water flow rate of 0.5 mL min$^{-1}$</td>
<td>140 mL (40 mL of cathode)</td>
<td>10.80 mg L$^{-1}$ h$^{-1}$</td>
<td>0.45 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>(Li et al., 2019)</td>
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<td>20 L scaled-up MES reactor</td>
<td>Graphite</td>
<td>pH of 3, 50 mM Na$_2$SO$_4$, cathodic aeration rate of 400 mL min$^{-1}$, and applied voltage of 0.6 V</td>
<td>20 L (10 L of cathode)</td>
<td>10.82 mg L$^{-1}$ h$^{-1}$</td>
<td>0.298 kWh kg$^{-1}$ H$_2$O$_2$</td>
<td>In this study</td>
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</table>
Highlights

- Up-scaled microbial electrosynthesis with multiple electrodes to synthesize H$_2$O$_2$.
- The H$_2$O$_2$ yield was higher than that of lab-scale systems using graphite cathode.
- Energy consumption was lower than that of lab-scale (bio)electrochemical systems.
- Systematic evaluation of the influence of operating parameters on H$_2$O$_2$ production.