Graphical Abstract

1 **Periodic polarity reversal for stabilizing the pH in two-chamber microbial**
2 **electrolysis cells**

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Abstract:

A successful pH control is of critical importance for ensuring efficient H₂ production in two-chamber MECs. In this study, a novel two-chamber MEC is developed with biotic/abiotic hybrid electrodes, which are composed of carbon felt (CF) and stainless steel (SS) mesh. The CF is used for the electrogenic microorganisms attachment catalyzing the oxidation of organic matter (OM); the SS serves as the current collector and the H₂ evolution catalyst. The MEC is operated in the periodic polarity reversal (PPR) mode and two hybrid electrodes work alternately as an anode and a cathode. With the hybrid electrodes and the PPR mode, the pH fluctuation is limited within the range of 6.4–8.6, and the H₂ production is 5.3 times that of control without the PPR mode. The optimal reversal interval is 2 hours for maximal H₂ production. The H₂ production increases with the amplitude of the applied voltage (0.7–1.0 V). The using of hybrid electrodes, combining with the PPR mode, provides a simple but efficient pH control strategy and better H₂ production performance in MECs. The findings of this study are expected to facilitate the design of pilot-scale MECs or similar bio-electrochemical reactors in future research.

Keywords:

Biotic/abiotic hybrid electrodes, polarity reversal, pH control, microbial electrolysis cell

1. Introduction

Microbial electrolysis cell (MEC) is a novel bio-electrochemical device producing hydrogen gas (H_2) from the organic matter (OM) contained in common wastewater. It utilizes electrogenic microorganisms as the anode catalyst for OM oxidizing, which generates carbon dioxide and protons [1, 2]. With the supply of a small voltage (>0.2 V), hydrogen will be produced in the cathode by consuming electrons and protons [3]. Differing in cell configuration, two types of MECs have been most often studied: single-chamber and two-chamber MECs. In a single-chamber MEC, anodic OM oxidation and cathodic H_2 production take place in the same chamber [4]. As a result, the collected H_2 often contains gas impurities such as CO_2 , CH_4 and H_2S . Those gases are released from the activity of anodic biofilms that host diverse microorganisms other than electrogenic bacteria, such as methanogens and sulfate reducing bacteria [5]. Meanwhile, a significant portion of produced H_2 will be lost due to methanogenesis (which converts CO_2 and H_2 to CH_4) [6-9] and the possible re-oxidation of H_2 by electrogenic microorganisms [5, 10-12]. The methanogenesis remains a tremendous challenge facing the long term operation of a single chamber MEC [7]. To prevent such loss and obtain high purity H_2 , the OM oxidation and H_2 production shall ideally be engineered to occur in different (separate) spatial or temporal domains. In a two-chamber MEC, a membrane is used to spatially separate those two processes [5, 13, 14]. However, the transport rate of protons across the membrane is often limited due to the very low concentration of protons as compared to other cations (e.g., Na^+ , K^+ , and NH_4^+), leading to a decrease in pH in the anode chamber while an increase in the cathode chamber [15]. The acidified anolyte then inhibits the activity of electrogenic microorganisms [16], lowering the H_2 production rate and energy efficiency [3]. A successful pH control is therefore of critical

importance for ensuring efficient H₂ production using two-chamber MECs.

Various chemical reagents have been added in the two-chamber MEC to minimize the pH gradient across the membrane. Among them, a phosphate buffer solution of 50–200 mM was often used [17]. However, the pH gradient would build up once the buffer capacity was depleted [18]. Timely adding NaOH in the anode chamber and HCl in the cathode chamber, or even initially alkalizing the well buffered anolyte has been tested, which however would further increase the salinity of the MEC effluent [13, 19, 20]. The other option that has been tested in the laboratory used bicarbonate or ammonia as a carrier of protons [17, 21]. However, the acceleration of methanogenesis [22] and the aim of nitrogen removal could restrict their application. The use of chemical reagents for pH control is therefore neither economical nor environmentally friendly. The pH gradient across the membrane can also be reduced by revising the reactor design of two-chamber MECs. For instance, Sleutels et al. put an additional chamber called “recovery compartment” between the anode and cathode chambers to recover alkalinity, thereby increasing the pH buffer capacity of the wastewater to be treated [23]. However, the problem of pH increase in the cathode chamber remained unsolved; and an increase in the MEC’s internal resistance lowered the overall energy efficiency of the system.

Recently, an operating mode named periodic polarity reversal (PPR) was developed to eliminate the pH gradient across the membrane in two-chamber microbial fuel cells (MFCs) [24, 25], which are structurally similar to two-chamber MECs. The basic idea is that a biotic electrode covered with electrogenic microorganisms, which is regularly used for OM oxidation, can also catalyze the oxygen reduction [26]. Thus, for an MFC with two biotic electrodes, the roles of anode and cathode can be switched periodically – by cutting off the OM supply while

81 introducing O₂, a previous anode can be converted to a cathode, and vice versa. With
82 such switching, the pH changes in the anode and cathode chambers can be controlled
83 within a relatively small range, which helps maintain a “conformable zone” for
84 electrogenic microorganisms. However, there is no perfect switching in reality – OM
85 or O₂ will not be completely gone immediately after switching. A concurrent
86 existence of OM and O₂ in the same chamber during the transition can cause a
87 decrease in the MFC’s electricity generation and energy efficiency. For example, the
88 remaining OM in the cathode chamber can severely lower the dissolved oxygen and
89 impede the oxygen reduction process due to its stimulation to the growth of the
90 heterotrophic bacteria [25]. The different activity of a biotic electrode towards oxygen
91 reduction versus OM oxidation is another issue. With the same biotic electrode, the
92 current density generated from oxygen reduction was considerably smaller than that
93 from OM oxidation [26, 27]. Such unbalanced capacities limit the overall electricity
94 generation of a two-chamber MFC. Moreover, it requires complicated experimental
95 setup and operation to switch the two chambers periodically between anaerobic and
96 aerobic conditions. All those factors hinder the application of PPR to MFCs.

97 Although no previous efforts have been reported, the PPR mode in principle shall
98 also apply to two-chamber MECs. Different from MFCs, in MECs both OM oxidation
99 and H₂ evolution occur under oxygen-free, i.e., anaerobic conditions. A simultaneous
100 existence of OM and protons (which are anode and cathode reactants, respectively) in
101 the same chamber may affect a biotic electrode’s electrochemical activity; but the
102 effect is expected to be minor compared to the case of MFCs. There is no need to
103 switch the chambers periodically between anaerobic and aerobic conditions. The PPR
104 can be achieved by simply changing the direction of the voltage applied on an MEC.

105 In this study, two-chamber MECs were constructed with a special design of biotic/

abiotic hybrid electrodes. Such biotic/abiotic hybrid electrode designs are expected to provide a comparable electrochemical activity for oxygen reduction to that for OM oxidation. The cells were operated in the PPR mode. With that, the accumulated protons in the anode chamber during OM oxidation were *in situ* utilized for cathodic H₂ production after polarity reversal. The pH gradient across the membrane was greatly reduced upon applying the PPR operating mode. The effects of applied voltage and polarity reversal interval on the MEC performance were further investigated. The re-oxidation of produced H₂ by electrogenic microorganisms was also discussed.

2. Experimental Material and Method

2.1. Construction of a two-chamber MEC with biotic/abiotic hybrid electrodes

The MEC consisted of an anode and a cathode chamber each with a liquid volume of 21 mL (Fig. 1). The two chambers were separated by a cation exchange membrane (CMI7000, Membranes International Inc., USA). A glass tube (80 mL; flushed with pure nitrogen gas prior to use) was mounted on the top of each chamber for H₂ collection [28]. Carbon felts (CF; 3.0 cm in diameter and 0.5 cm in thickness; Qijie Carbon Co., Ltd., China) were inoculated with effluents from an acetate-fed MFC that had run for >1 year, and were enriched as the anodes in a cube-type MFC [29]. During this enrichment period, the electrogenic microorganisms could gradually attach on the CF anode. The CFs were considered fully acclimated when a stable and reproducible anodic current generated from the oxidation of organic substrate was obtained. A biotic/abiotic hybrid electrode was fabricated by mechanically pressing together a piece of acclimated biotic CF and an abiotic stainless steel (SS; Type 304) mesh (3.0 cm in diameter, 50x50 mesh, and 0.23 mm wire diameter). SS meshes were cleaned with ethanol before use. Two biotic/abiotic hybrid electrode were separately

inserted in the middle of each chamber of an MEC. Also inserted in each chamber was a saturated calomel electrodes (SCE, 242 mV versus a standard hydrogen electrode, SHE; Leici Co., Ltd., Shanghai, China) serving as the reference electrode, and all potentials presented were referenced to the SCE.

Figure 1. Schematic diagram of a microbial electrolysis cell (MEC) with both the anode and cathode being biotic/abiotic hybrid electrodes. The cell was operated in the periodic polarity reversal (PPR) mode. CF: carbon felt; SS: stainless steel mesh.

2.2.MEC startup and operation

The substrate media in the two chambers were the same and were prepared by dissolving 1.032 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.332 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 4.44 g KCl, 1 g sodium acetate, 0.31 g NH_4Cl , 0.02 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.02 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 12.5 mL trace minerals solution (Table S1), and 5 mL vitamins solution (Table S2) [30] in 1 L deionized water. The conductivity of the substrate media was 9.85 ± 0.02 mS/cm. To suppress methane production, 5 mM sodium 2-bromoethanesulfonate was added as the inhibitor. The final phosphate buffered saline (PBS) concentration was as low as 5 mM to prevent abrupt pH variation [25]. A potentiostat (CHI 1030B, Chenhua Instruments Co., Ltd., Shanghai, China) was used as an external power source. The working electrode of the potentiostat was connected to one of the MEC's hybrid

electrodes while both the counter and reference electrodes were connected to the other one. The potentiostat was set up to run in the Multi-Potential Steps mode, with a positive voltage of 0.9 V and a negative voltage of -0.9 V sequentially and periodically applied on the MEC to achieve periodic polarity reversal (PPR). By periodically reversing the polarity of an applied voltage across the MEC, the two hybrid electrodes each worked alternately as anode and cathode. Different PPR intervals from 0.5 to 6 hours were tested; and a separate MEC without PPR was set as the negative control, with a constant voltage of 0.9 V being applied across the cell. The voltage was further changed to 0.7/-0.7 V, 0.8/-0.8 V and 1.0/-1.0 V to evaluate its impact on H₂ production. Each MEC was run as duplicated and all the experiments were performed at a temperature of ~25°C.

2.3. Analysis and calculation

Hydrogen gas was analyzed by gas chromatography with a thermal conductivity detector (GC-TCD) as described in the previous work [28, 31]. The electrolyte pH was monitored using a micro pH electrode (InLab® Micro, Mettler-Toledo, Switzerland). The electrochemical activity of the hybrid electrodes for OM oxidization and H₂ production was investigated by cyclic voltammetry (CV, potential range: 0.2 to -1.1 V vs. SCE, scan rate: 1 mV s⁻¹) using an Autolab electrochemical workstation (PGSTAT 128N, Metrohm Autolab, The Netherlands). The H₂ production rate (Q , m³ m⁻³ d⁻¹), Coulombic efficiency (CE), cathodic hydrogen recovery (R_{cat}), energy efficiency relative to the electrical input (η_E), and the overall energy recovery (η_{E+S}) were calculated to evaluate the MEC performance [3].

3. Results and Discussion

3.1. Enhanced H₂ production by PPR

The total volume of produced H_2 with the applying of the PPR operating mode (7.4 ± 0.5 mL) was 5.3 times that without PPR (1.4 ± 0.2 mL) after 40 hours (Fig. 2A). The H_2 production rate with PPR (0.1 ± 0.02 m³ m⁻³ d⁻¹) remained relatively high during the test (Fig. S1). A periodic change in the electrolyte pH was noted. But with PPR, the fluctuation was controlled within the range of ~6.4–8.6 (Fig. 2B). In contrast, the H_2 production rate without PPR dropped to $0.01 (\pm 0.002)$ m³ m⁻³ d⁻¹ after 40 hours (Fig. S1), and the electrolyte pH decreased to 5.4 in the anode chamber while increased to 9.3 in the cathode chamber after only 8 hours (Fig. 2B). For the MEC operated in the PPR mode, the potential of the two hybrid electrodes and the direction of the current both exhibited strong periodicity. As the MEC ran at a constant voltage, the potential of the two hybrid electrodes remained stable while a sharp decline in the current was observed in the first 8 hours of operation (Fig. S2 and Fig. S3). CV analysis of the two hybrid electrodes revealed that, with PPR applied, there was no significant change in the electric current for OM oxidation (from -0.5 to +0.2 V) or the electric current for H_2 production (from -0.8 to -1.1 V), suggesting that PPR can help preserve the hybrid electrodes' bioelectrochemical activity. Comparatively, without PPR, while the current for H_2 production showed no noticeable difference, the electrochemical activity for OM oxidation (as characterized by the current at -0.3 V) declined greatly after 40 hours (Fig. S4). The CV analysis results, in combination with pH fluctuation data, affirmed that the PPR mode provided a simple but efficient pH control strategy and maintained the electrochemical activity of the hybrid electrodes [16, 32], thereby enhanced the H_2 production of MECs.

Figure 2. (A) Accumulative H₂ production and (B) changes in the electrolyte pH in microbial electrolysis cells with (PR) and without (NPR) periodic polarity reversal. The applied voltage was 0.9/-0.9 V. C1 and C2 represents the two chambers, respectively.

3.2. Effect of PPR intervals

Of all the intervals (0.5, 1, 2, 4 and 6 hours) tested, the optimal PPR interval for maximal H₂ production was 2 hours (Fig. 3A). The amplitude of pH changes increased with the PPR interval (Fig. 3B). A neutral pH is essential for maintaining the activity of electrogenic microorganisms [15, 32]. By this regard, a shorter PPR interval would be better. In contrast, H₂ production peaks at lower pH [13], suggesting that the protons accumulated during anodic OM oxidation can promote the subsequent cathodic H₂ production after polarity reversal. This may also explain the enhanced H₂ production rates by PPR. However, the cathodic hydrogen recovery (R_{cat}) decreased from 41±6% (without PPR) to only 5±1% (with PPR and an interval of 0.5 h). A possible reason is that the produced H₂ was not released in a timely manner. Part of

the H₂ gas stayed on the electrode surface and/or in the electrolyte, and this part of H₂ could possibly be re-oxidized by electrogenic microorganisms after polarity reversal (Fig.3C). The occurrence of H₂ re-oxidation is proved by a *CE* higher than 100% (Table 1), because only the substrate was considered for *CE* calculation. Such H₂ recycling can lead to a decrease in *R_{cat}* and H₂ production rate. A similar finding was reported by previous studies on single-chamber MECs [10, 12] where OM oxidation and H₂ production occurred simultaneously in the same chamber. Ruiz et al. recently reported a H₂ production rate as low as 0.006 m³ m⁻³ d⁻¹ and the *R_{cat}* of only 4% with a single-chamber MEC [33]. As the ratio of the remaining (not released) H₂ versus the total produced H₂ decreases with the PPR interval, the loss of H₂ due to the recycling is expected to be lower at larger intervals.

Figure 3. (A) Hydrogen production, (B) pH fluctuation, and (C) cathodic hydrogen recovery (*R_{cat}*) at different reversal intervals. The applied voltage was 0.9/-0.9 V.

3.3. Effect of the applied voltage

The optimal PPR interval of 2 hours was employed in this investigation. The volume of produced H₂ increased from 2.8±0.3 mL to 11.5±0.9 mL when the applied

voltage increased from 0.7 to 1.0 V (Fig. 4A), which is consistent with previous MEC studies [4, 12, 31]. However, the pH fluctuation became more significant (Fig. 4B). The R_{cat} increased from $17\pm3\%$ at 0.7 V to $25\pm3\%$ at 1.0 V (Fig.4C), indicating a larger voltage was beneficial for H₂ recovery.

Figure 4. (A) Hydrogen production, (B) pH fluctuation, and (C) cathodic hydrogen recovery (R_{cat}) at different applied voltages. A polarity reversal interval of 2 hours was used in all the tests.

The results in this study were compared with those of previous studies on two-chamber MECs, as summarized in Table 1. The performance of two-chamber MECs strongly depends on the pH of both the anolyte and catholyte, which can be reflected as the PBS concentrations in the anode and cathode chamber. For example, the H₂ production rate decreased substantially from 0.118 to 0.024 m³ m⁻³ d⁻¹ when lowering the PBS concentration in the cathode chamber from 100 to 10 mM while it remained constant at 100 mM in the anode chamber [34]. The H₂ production rate dropped to 0.015 m³ m⁻³ d⁻¹ when the PBS concentration was decreased to 10 mM in the anode chamber even increased to 1000 mM in the cathode chamber [35]. The

MEC performance also depends on cathode catalyst. Low-cost stainless steel (SS), especially type 304, has rarely been tested as the H₂ evolution catalyst in previous two-chamber MEC research [36]. With the novel hybrid electrodes (CF+SS304) implemented, the performance of our constructed MECs was comparable to or even better than that of regular MECs with platinum (Pt) as the H₂ evolution catalyst (with lower activation overpotentials than SS), buffered by higher concentrations of PBS, and running at larger electrolysis voltages [13, 34, 37].

The PPR mode provides a practical way to control the electrolyte pH in two-chamber MECs as it exempts the use of high-concentration buffer solution. It should be noted, however, that the applying of the PPR mode may result in decreased R_{cat} . Part of the H₂ gas failing to diffuse into the collection glass tube could be re-oxidized by electrogenic microorganisms, and this H₂-recycling would generate an additional electric current, causing a surge in *CE* (Note: Only the substrate, e.g., acetate in this study, was considered for *CE* calculation with Equation (1)).

$$CE = \frac{8 \int_0^t Idt}{Fv\Delta COD} \quad (1)$$

where I is the current, F is the Faraday's constant, and v is the volume of liquid in the anode of MEC. The *CE* was calculated to be 129–151% for the two-chamber MECs in this study, which is lower than those of single-chamber MECs (191–463%) [10, 11, 33]. The calculated percent indicates relatively low but still significant H₂-recycling that took place on the hybrid electrodes during PPR operation.

The MECs constructed in this study provide three major advantages. First, the protons produced during the OM oxidation process (by electrogenic microorganisms attached on the CF surface) can be *in situ* utilized for H₂ production on the SS mesh after polarity reversal. Secondly, the SS serves as not only the cathode catalyst for H₂ production but also the current collector. As SS has better electrical conductivity than

carbon felt and thereby a more uniformly-distributed anode potential, this hybrid electrode design promotes the OM oxidation by electrogenic microorganisms on the CF surface, especially for scaled-up MEC reactors [40]. Thirdly, a combination of the two-chamber design and the PPR operating mode enables OM oxidation and H₂ production to always occur in different temporal or spatial domains – in the same chamber, the two processes take place sequentially and periodically; while, at the same moment, the two processes take place in different chambers. The novel biotic/abiotic hybrid electrodes fabricated in this study using CF for electrogenic microorganisms attachment and the SS for H₂ evolution. So the biofilm attachment and H₂ gas emission actually occurred on different part of the hybrid electrode. Compared with previous studies in MEC using biocathode for hydrogen production, the biofilm/bacteria separation from electrode by force of H₂ gas emission will no longer be a threaten to the MEC operation. The application of the low cost material, SS, in bioelectrochemical system could be partly restricted by the corrosion when working at improper electrode potential, which is notable when served as the anode. However, there was no corrosion visible on the SS after use in this study. The potential of stainless steel anode was measured (Fig. S2), and it was generally in the range of -0.1 to -0.3 V vs. SCE. A recent study by Ketep [41] reported that the corrosion of SS based anode can be avoided as the potential remained below 0 V vs. SCE. It was believed that the corrosion of SS was avoided in our MEC operated in the PPR mode. Future research is suggested to develop an automated collection system to periodically collect the produced H₂ gas from two chambers based on the PPR time sequence.

294 **Table 1.** Performance of two-chamber microbial electrolysis cells (MECs) reported in the literature.

PBS ^a (mM/mM)	Applied voltage (V)	Cathode Materials (Switching intervals, h)	<i>CE</i> (%)	<i>R_{cat}</i> (%)	η_E (%)	η_{E+S} (%)	Q^b (m ³ m ⁻³ d ⁻¹)	Ref.
100/100	1.0	Pt	93	41	52	32	0.250	[13]
50/50	0.8	Pt	45.2	80.0	-	-	0.052	[38]
100/100	0.8	Pt	34.6	89.2	-	-	0.118	[34]
100/10	0.8	Pt	8.0	69.9	-	-	0.024	[34]
10/1000	0.8	Pt	30.6	48.0	-	-	0.015	[35]
10/1000	0.8	MoS ₂	21.6	46.2	-	-	0.012	[35]
50/50	0.6	Pd	56.0	46.4	-	-	0.003	[39]
100/100	0.8	N-Fe/Fe ₃ C@C	43.6	79.8	-	-	0.09	[37]
5/5	0.8	SS 304 (2)	129	20.6	37.8	18.6	0.09	This study
5/5	0.9	SS 304 (2)	134	21.8	38.5	18.5	0.11	
5/5	1.0	SS 304 (2)	151	25.1	49.5	22.4	0.17	
5/5	0.9	SS 304 (None ^c)	87	40.7	67.1	27.6	0.02	

295 ^a PBS (phosphate buffered saline) concentrations in the anode and cathode chambers, respectively; “/” were used to separate the concentration
296 values in the two chambers.

297 ^b Hydrogen production rate was normalized by the total liquid volume in the two chambers combined.

298 ^c No polarity reversal. The batch test lasted for 40 hours.

4. Conclusions

A two-chamber MEC was constructed with biotic/abiotic hybrid electrodes made of carbon felt and stainless steel meshes, and it was run in the periodic polarity reversal (PPR) mode. The accumulative H_2 production with PPR was ~5.3 times higher than that without PPR after 40 hours of operation. Such enhanced H_2 production was attributed to: (1) a better control of the electrolyte pH in the two chambers with PPR, and (2) the protons produced during anodic OM oxidation being *in situ* utilized for subsequent H_2 production after polarity reversal. The optimal PPR interval for maximal H_2 production was 2 hour. A shorter PPR interval offered a better pH control but led to a decreased R_{cat} due to H_2 -recycling. The H_2 production rate increased when raising the applied voltage across the MEC from 0.7 to 1.0 V. In summary, the performance of two-chamber MECs can be improved by a combination use of the hybrid electrodes and the PPR operating mode. Future research will focus on optimizing the design of biotic/abiotic hybrid electrodes and developing an automated H_2 collection system particularly for PPR operating mode.

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