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The effect of a short term biofilm-aeration treatment on energy recovery in microbial electrolysis cell

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ABSTRACT

In microbial electrolysis cells (MECs), hydrogen production yield is often limited by the occurrence of methanogenesis. For reducing the methane production, air treatment process was applied as a cost-effective approach, however, the reported way using air or oxygen sparging may cause an energy loss because of residual dissolved oxygen in MEC solution. In this study, an air exposure to the biofilm was applied to improve H_2 production in single-chamber MECs. Twelve reactors with 0.8 V applied voltage were operated under four batch conditions (three replicates for each): (a) Biofilm aeration for 10 minutes before medium was refilled (air speed: 0.8-1 $L \cdot min^{-1}$); (b) Biofilm air-exposure for 10 minutes before medium refilled; (c) Fresh medium refilled immediately after reacted medium discharged; (d) Nitrogen gas sparging for 10 minutes after fresh medium refilled (as control treatment). It was found that the H_2 yield increased ~60% after biofilm aeration under condition (a), the hydrogen production rate was up to 1.3 $mL^3 \cdot mL^{-3}$ reactor$\cdot d^{-1}$, while little methane was detected. In contrast, under condition (c) and (d), the maximum production rate of methane was 0.1 mL^3 $CH_4 \cdot mL^{-3}$ reactor$\cdot d^{-1}$, while the production rate of hydrogen decreased to 0.8 $mL^3 \cdot mL^{-3}$ reactor$\cdot d^{-1}$. This work indicated that a short term aeration treatment could substantially affect energy recovery and methanogen communities located in biofilms.

Keywords: Bioconversion, Biogas, Bioreactors, Biofilms, Dissolved Oxygen, Bioelectrochemical

Introduction

Microbial electrolysis technology has been developed to produce bio-hydrogen from wastewater for over a decade[1-4]. In microbial electrolysis cells (MECs), various substrates could be directly degraded by anode respiring bacteria, and bio-electrons are transferred to anode and subsequently transported to cathode, where protons are reduced to H_2 (on the surface of catalyzed cathode) by applying a small external voltage. Several promising advantages have been reported, such as a >90% coulombic efficiency for bioenergy recovery, high conversion efficiency of end-fermentative

products, e.g. acetate, and high purity hydrogen production [2, 4]. However, recent studies have still been carried out to further improve MEC performances by optimizing key factors, such as electrode materials[5], reactor configurations [6, 7] or regulating microbial communities [8-12]. But the occurrence of methanogenesis could not be avoided in single chamber MECs, thus hydrogen yield loss turns out to be a serious issue caused by either substrate competition or hydrogen consumption[13-15].

In bioelectrochemical systems, several pathways of methane generation were reported, including acetoclastic methanogenesis using acetate, hydrogenotrophic methanogens using H_2 and CO_2 [14, 16], synthesis directly using electrons from cathode [17], and direct generation from interspecies electron transfer [18, 19]. Targeting H_2 production enhancement, several approaches have been applied to inhibit methanogenesis in the MECs, such as reducing the pH [20], controlling external voltage [14], low temperature [21], using methanogen inhibitors [22], and using ultraviolet irradiation on cathode [23]. However, the activities of anode respiring bacteria were also inhibited by low pH (or i.e. low temperature). It would not be a feasible practice to use inhibitors or extra energy input on long-term operation. Alternatively, the use of oxygen might be the most simple and cost-effective approach to inhibit methanogenesis. Some studies reported that air diffusion to anode led to inhibit methane generation[4, 24], however, dissolved oxygen in anode solution would cause a possibility of electron consumption and energy loss[25]. Moreover, methane production could be only inhibited for a while because methanogens could survive in the relatively higher oxygen tolerance in anode biofilm[4, 26]. Until now, there are only a few reports on how to exactly manage air exposure to control methanogens with a limited and short impact to energy recovery[27].

This study aims to inhibit methane production thus improving hydrogen yield in single chamber MECs. In most reported MEC operations, nitrogen or mixture of carbon dioxide (80%:20%) were primarily used to remove dissolved oxygen[1, 4, 28]. It was considered as a feasible way to protect anodic bacteria and maintain high efficiency on electron recovery while leading to unexpected growth of methanogens[14]. In this study, three additional treatments (at the anode) were performed with different

levels of air exposure. For understanding the effects of air exposure, the yield of hydrogen, methane and carbon dioxide was measured during fed-batch cycles.

Materials and methods

MEC Reactor setup and operation

Single chamber MECs used in this study were consisted by one cylindrical chamber (of polycarbonate).The chamber was with a diameter of 3 cm and 4 cm long, the empty bed volume was 28 mL[29]. The anode was a graphite brush (2.5 cm diameter × 2.5 cm length; 0.22 m^2 surface area) with a specific surface area of 18200 $m^2 \cdot m^{-3}$ and a porosity of 95%, placed in the center of the chamber. The cathode was made of carbon cloth (7 cm^2, YW-50, YiBang; Taiwan) coated with 0.5 $mg \cdot cm^{-2}$ Pt.

Twelve reactors were started up (0.8 V applied voltage) by inoculating effluent of an activated sludge tank from local wastewater treatment plant (Harbin, China). After 48 h inoculation, 1500 mg/L acetate was used as sole carbon in 50 mM phosphate buffer solution (PBS[4], containing NH_4Cl 0.31 g/L, KCl 0.13 mg/L, $NaH_2PO_4 \cdot 2H_2O$ 5.618 g/L, $Na_2HPO_4 \cdot 12H_2O$ 6.155 g/L, pH 7.0) for the whole experiment at room temperature (25 ºC) [30]. Medium solution of 26 mL in MEC chamber was discharged and refilled totally every 24 hours. All reactors were operated at least for 1 month.

Biogas production under four types of medium solution refill

After all reactors operated under stable conditions, they were randomly divided into four groups, with three reactors as biological replicates in each group[30]. Four different approaches were applied to make four levels of air exposure of biofilm at the end of each batch test: (a) Biofilm aeration for 10 minutes before medium refilled, which is that the air gas was blew into one of the sampling inlets at a speed of 0.8-1 $L \cdot min^{-1}$ for 10 minutes. (b) Biofilm air-exposure 10 minutes before medium refilled. This treatment is leaving MEC reactor in air for 10 minutes with all sample inlets open, after emptying solution at the end of each batch operation. (c) Fresh medium refilled immediately after reacted medium discharged. (d) Nitrogen gas sparging for 10 minutes after fresh medium refilled (as control treatment). Fresh solution was sparged for 10 minutes by nitrogen gas (99.9%) to remove dissolved oxygen after

medium refill. The tests were carried out for one month to measure gas production.

Measurement and calculation

The currents and anode potential were automatic monitored by multimeter (Acquisition system; Keithley Instrument model 2700)[29]. The gas was collected by a gas bag (100 mL; Cali5-Bond; Calibrated Instrument Inc). Gas components (H_2, CO_2, and CH_4) were analyzed by a gas chromatography (Fuli, GC9790; Zhengjiang Instrument Inc, China) with a packed column[30] (TDX-01; 2 m length). The volume of gas was measured by a glass syringe. The filtrate was immediately used to analyze VFAs, carbohydrate and protein. The SCOD was conducted in accordance with standard methods. The acetate were analyzed by a gas chromatography (Agilent 4890; J&W Scientific, USA) with a capillary column (19095N-123HP-INNOWAX; 30 × 0.530 mm × 1.00 μm; J&W Scientific, USA)[31]. The coulombic efficiency and hydrogen yield was calculated as previous studies[7, 14], calculated by $CE = Q_c/Q_T \times 100\%$, where Q_c was current coulombs calculated by the integration $Q_c = \int I \cdot dt$ and Q_T was coulombs of consumed acetate. The hydrogen yield was calculated using the equation $Y_{H2} = Q_H/Q_T \times 100\%$. The Q_H presents the electron coulomb used to produce hydrogen according to $Q_H = 2nF$, where F is Faraday's constant (96485 C mol^{-1}) and n is the moles of hydrogen produced, calculated as $n=PV/(RT)$, where P is the atmospheric pressure (101325 Pa), V is the hydrogen volume (m^3), R is the gas constant (8.314 J mol^{-1} K^{-1}), and T is the temperature (K).

Results and discussion

Coulombic efficiency and electron transport under different treatments

When repeatable current was performed stably under four types of treatment under a fixed external voltage of 0.8 V, the peak current values presented differently. The highest peak current was achieved ~7.0 mA in the treatment of fresh medium refilled immediately (Fig.1). The next current level presented in the treatment of biofilm air-exposure 10 min after medium discharge. The lowest peak current was ~5.5 mA when biofilm was aerated for 10 min at a blow speed of 0.8-1 L·min^{-1} before medium refill. The change of current showed that aeration to biofilm conducted ~20%

reduction on MEC performances, hinting an inhibition to anode respiring bacteria. But the current was also a decrease even under the condition of N_2 sparging treatment to remove dissolved oxygen in solution. It was indicated that micro oxygen would properly inhibit most of strict anaerobic bacteria which may compete to exoelectrogens on substrate utilization. On one hand, the heterotrophic microbes scavenged the diffused oxygen, thereby reducing the toxic effects to exoelectrogens[26]. On other hand, the oxygen consumption was efficiently conducted by facultative bacteria metabolizing biodegradable substrates in the anodic biofilm[32].

According to little variety on coulombic efficiencies among the four treatments (Fig. 2), averaged coulombic efficiency was around 84.6±0.2% in all reactors. There was not an obvious electron loss detected in the reactors. An average efficiency of 86.8±3.5% was performed with biofilm aeration condition for 10 min, while the lowest efficiency (81.1±3.4%) was observed when biofilm was exposed to air for 10 min. Therefore, coulombic efficiency was principally determined by microbial factors both on exoelectrogenic activities and substrate utilization [7, 9]. The COD removal showed a consequent change according to different conditions. An averaged COD removal ranged from 85±3% to 89±3%, indicating that a short term air-exposure to biofilm did not substantially inhibit activities of anodic communities.

In previous studies, nitrogen or mixed gas of CO_2 and N_2 (20:80) or pure N_2 was used for oxygen removal in MECs [1, 28], as a consideration to prevent coulombic loss from oxygen-caused electron consumption in solution surrounding anodic communities. However, subsequent research indicated that methane production was maintained in continuous batch operation with all solution emptied each time[14], hinting that methanogens were existing on biofilms. Recently relatively study points out that residual O_2 in solution during fed-batch cycle was the key indicator for CH_4 control[24]. Therefore, direct air-exposure to biofilm also inhibited methanogens (and exoelectrogens) while avoiding a long time effects from dissolved oxygen in reaction solution, which can be evaluated by anode potentials.

Anode potential and archaea community structure under different treatments

Although coulombic efficiencies showed no substantial difference among the

four treatments, an increased anode potential was observed as a consequence of dissolved oxygen (Fig. 3). Anode potential (vs. Ag/AgCl) was detected in one 24-h batch operation. Anode potentials were all reduced to < -300 mV (vs. Ag/AgCl) in the first 1 h. The lowest potential obtained was -460 mV (vs. Ag/AgCl), when the refilled solution was sparged by nitrogen gas to removal dissolved oxygen. Although a similar low anode potential was also observed in MECs without nitrogen gas sparging, it took ~6 h to reduce anode potential from initial -400 mV to final -460 mV (vs. Ag/AgCl) (Fig. 3). Clearly, anode potentials were higher (up to -350 mV) after air exposure treatment. The results indicated that anode performance was substantially influenced by initial air treatment, which consequently determined electron transfer ability, which hydrogen production was depended on.

After new medium solution was refilled without air treatment, the anode potential decreased to -400 mV (vs. Ag/AgCl) which reflected the processes of electron transfer by anode respiring bacteria[7]. The oxygen led to an increase of anode potential because the electron acceptor was partly changed from anode (-520 mV vs. Ag/AgCl) to oxygen (+840 mV)[33]. As a result, different anode potentials had an determined influence on the selection of microbial communities in the MECs [34]. The different initial anode potential, which is determined by air treatment of the biofilm, might determine a selection towards facultative aerobes and anaerobes [33]. The higher anode potential will favour lower redox facultative anaerobes. However, strict anaerobes were possibly enriched at low anode potential. Otherwise, facultative anaerobes (*Shewanella*) and strict anaerobes (*Geobacter*, *Clostridium,* methanogens) will be changed in community structure under different treatment with air exposure. Biofilm aeration treatment was a feasible way to inhibit hydrogen consumption under the 24-h batch operation.

The archaea community composition analysis was based on the OTU numbers detected from anode biofilm (Table 1). There were 80 detected for N_2 sparging treatment, 30 for solution refill, 6 for air exposure, and 9 for biofilm aeration. The most detected methanogens were using acetate as electron donors, including *Methanosaeta*, *Methanoregula* and *Thermogymnomonas*. Totally acetate consumption

methanogens accounted for 66% for N_2 sparging treatment, 77% for solution refill, 67% for air exposure, and 44% for biofilm aeration. A small part of methanogens was identified using hydrogen and carbon dioxide as substrate, including four species *Methanospirillum*, *Methanobrevibacter*, *Methanobacterium*, and *Methanomicrobia*. In previous study, hydrogen consumption methanogens were considered as the main reason for hydrogen lose in single chamber MECs[14]. However, it was much reasonable that hydrogen consumption methanogens were dominant on cathode surface or suspended solution rather than anode biofilm. It was interesting that no hydrogen consumption methanogens were detected in the biofilm sample with air exposure. The results showed similar inhibition effect on hydrogen consumption methanogens as anaerobes when air treatment was exposed to biofilm.

The methanogens were the most effectively inhibited in 24 h under biofilm aeration treatment. But there is a relative high existing of methanogens in biofilm, surviving 7.5% of acetoclastic methanogens, 15.4% of hydrogenotrophic methanogens and 21.4% of unclassified archaea (Table 1). Actually, some researchers have pointed out that O_2 stress strongly inhibited CH_4 production[35] but many methanogens can survive exposure to air several hours or longer without losing viability[36]. It has been revealed that anodic biofilm was functioned by fermentative bacteria and exoelectrogens, which are able to build complex networks on more tolerant to environmental fluctuations[26], and more accommodating to a variety of substrates[29]. Methanogens are quite sensitive by exposure to air, but the most important factor contributing to the tolerance of acetoclastic methanogens was the oxygen consumption by facultative bacteria metabolizing biodegradable substrates[32]. Therefore, methanogens in anodic biofilm have some tolerance to oxygen and simply exposing the anode to air does not cause efficient oxygen diffusion into the liquid surrounding the methanogens because of the low solubility of oxygen[26].

Biogas production detected in MECs

After 1 month operation, hydrogen yield attained 0.80±0.07 $mL^3 \cdot mL^{-3}$ reactor$\cdot d^{-1}$ and methane was up to 0.09 $mL^3 \cdot mL^{-3}$ reactor$\cdot d^{-1}$ in the MECs with nitrogen sparging to remove dissolved oxygen in replaced medium (Fig. 4). It was not substantially

different in the MECs with medium replacement only, in which the hydrogen production was 0.83±0.08 $mL^3.mL^{-3}reactor \cdot d^{-1}$, with methane production of 0.06 $mL^3.mL^{-3}$ $reactor \cdot d^{-1}$. However, biogas production noticeably changed when anode biofilm was exposed to air for a short period before medium solution was refilled. Hydrogen production increased to 1.01±0.12 $mL^3.mL^{-3}$ $reactor \cdot d^{-1}$ and methane was reduced to 0.05±0.01 $mL^3.mL^{-3}$ $reactor \cdot d^{-1}$ when emptying MEC chamber in air for 10 minutes, before medium solution was refilled. Hydrogen production was further increased to 1.30±0.11 $mL^3.mL^{-3}$ $reactor \cdot d^{-1}$ and methane was reduced to as little as 0.01 $mL^3.mL^{-3}$ $reactor \cdot d^{-1}$, by aerating MEC biofilm with air for 10 minutes (0.8-1.0 $L \cdot min^{-1}$) before medium solution refilled. Thus, air exposure of the biofilm at each beginning of a 24-h batch operation effectively inhibited methane production, while strict anaerobic condition will commonly favour methanogenesis in single chamber MEC.

Under the condition of biofilm aeration, H_2 yield increase ~60% compared to nitrogen sparging, with little methane production. The methane concentration was less than 1% in final biogas production, which was much effective than the method by increasing external voltage [14]. The highest hydrogen yield was increased to 3.4±0.3 mol H_2/mol acetate (Fig. 4). In this study, biofilm aeration after medium discharge showed greater effect on saving treatment time and inhibiting methane production compared to dissolved oxygen inhibition from medium solution. Chae et al found that a simple exposure of the anode to air for 10-30 min had no significant effect on the methanogenic activity[26]. But direct aeration of the anode medium for 3 min or immersion of the anode into an oxygen saturated medium for 30 min presented a significant suppression of methane production from 46–50% to 3%. As air exposure in anode medium effectively suppressed hydrogenotrophic methanogens, which led to a methane inhibition in single chamber reactors[27].

Conclusion

In this study, it was demonstrated that a short-term biofilm aeration could enhance H_2 production in single chamber MECs. Although air exposure increased initial anode potential to -350 mV (vs. Ag/AgCl), leading to the lowest coulombic

efficiency, the hydrogen production rate was up to 1.30±0.11 $mL^{3}{\cdot}mL^{-3}$ reactor$\cdot d^{-1}$, while little methane production was detected. The highest hydrogen yield was increased to 3.4±0.3 mol H_2/mol acetate by aerating bioanode to air compared to 2.1 mol H_2/mol acetate (with 0.24 mol CH_4/mol acetate) using nitrogen to removal dissolved oxygen of refilled solution. A short-term biofilm aeration treatment was a feasible way to reduce methanogenesis but less impact to energy recovery with little residual dissolved oxygen in MECs.

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331 Table 1 The OTU count of archaea community detected from anode biofilm

Archaea	OTU	Taxonomy	N_2 sparging	Solution refill	Air exposure	Biofilm aeration
Acetate utilization	Otu18758	*Methanoregula*	1	0	0	0
	Otu18845	*Methanoregula*	1	0	0	0
	Otu18710	*Methanoregula*	2	0	0	0
	Otu18563	*Methanosaeta*	0	1	1	0
	Otu18570	*Methanosaeta*	1	1	0	1
	Otu18562	*Methanosaeta*	11	4	0	2
	Otu18524	*Methanosaeta*	34	16	0	0
	Otu18646	*Thermogymnomonas*	3	1	3	1
H_2 utilization	Otu18712	*Methanospirillum*	4	3	0	0
	Otu18727	*Methanospirillum*	1	0	0	0
	Otu18748	*Methanospirillum*	1	0	1	0
	Otu18750	*Methanospirillum*	1	0	1	1
	Otu18518	*Methanobrevibacter*	1	0	0	0
	Otu18605	*Methanobacterium*	1	0	0	0
	Otu18617	*Methanomicrobia*	4	2	0	1
unclassified	Otu18626	Archaea	1	0	0	1
	Otu18651	Archaea	1	0	0	0
	Otu18668	Archaea;"Euryarchaeota"	0	1	0	1
	Otu18529	Archaea;"Euryarchaeota"	1	0	0	0
	Otu18591	Archaea;"Euryarchaeota"	1	0	0	0
	Otu18597	Archaea;"Euryarchaeota"	1	0	0	0
	Otu18614	Archaea;"Euryarchaeota"	1	0	0	0
	Otu18735	Archaea;"Euryarchaeota"	1	0	0	0
	Otu18631	Archaea;"Euryarchaeota"	3	1	0	0
	Otu18615	Archaea;"Euryarchaeota"	4	0	0	1

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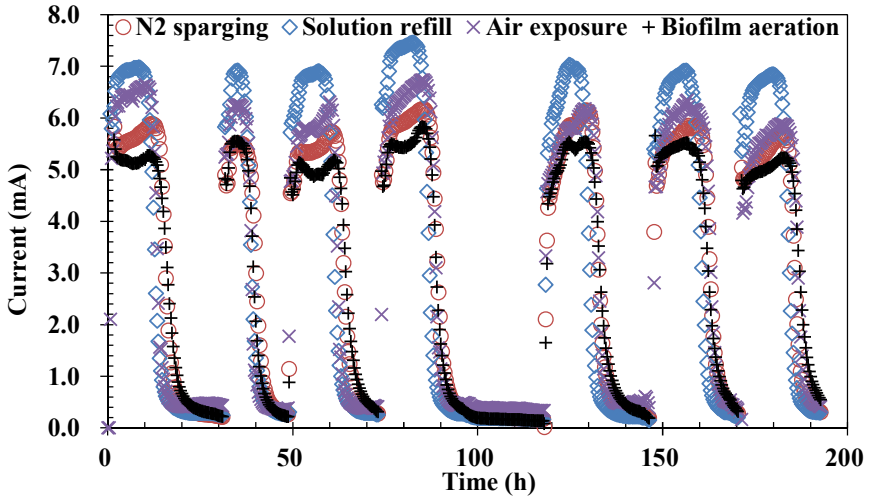

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335 **Figure 1** Current change in MECs of four typical treatments (0.8 V, 10 ohm)

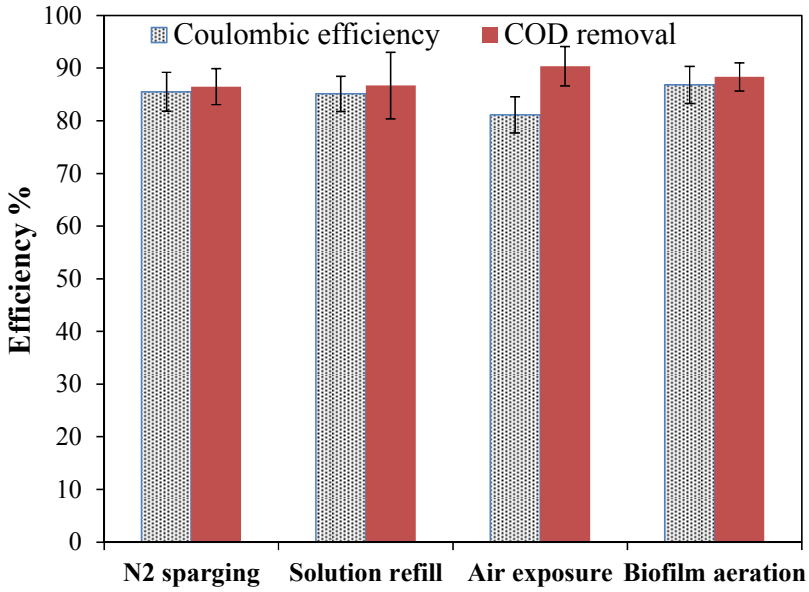

Figure 2 Coulombic efficiency for electrons recovered as current from COD removal efficiency under different treatments.
Error bar was calculated among the data of 3 batches from 3 replicate reactors for each condition.

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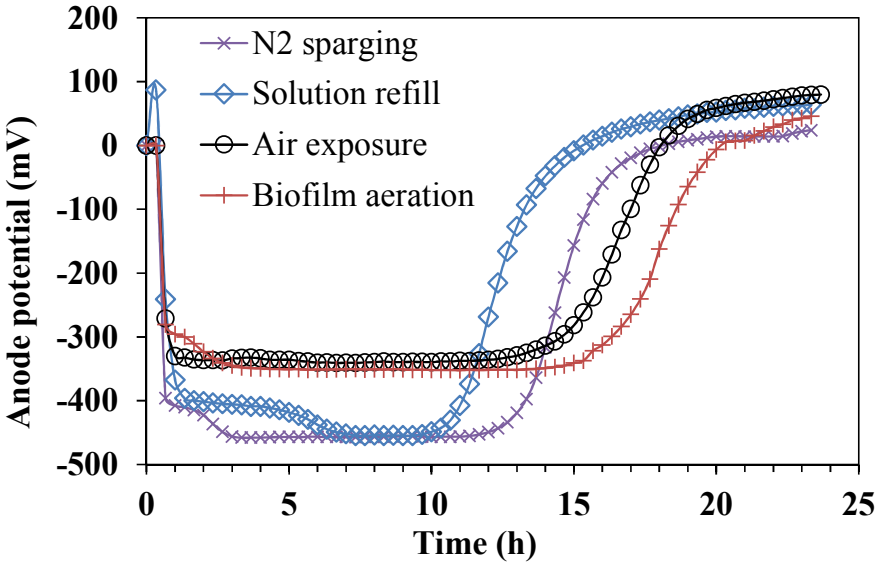

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345 **Figure 3** Anode potential (vs. Ag/AgCl) as an indicator of anodic biofilm
346 performance under four typical treatments

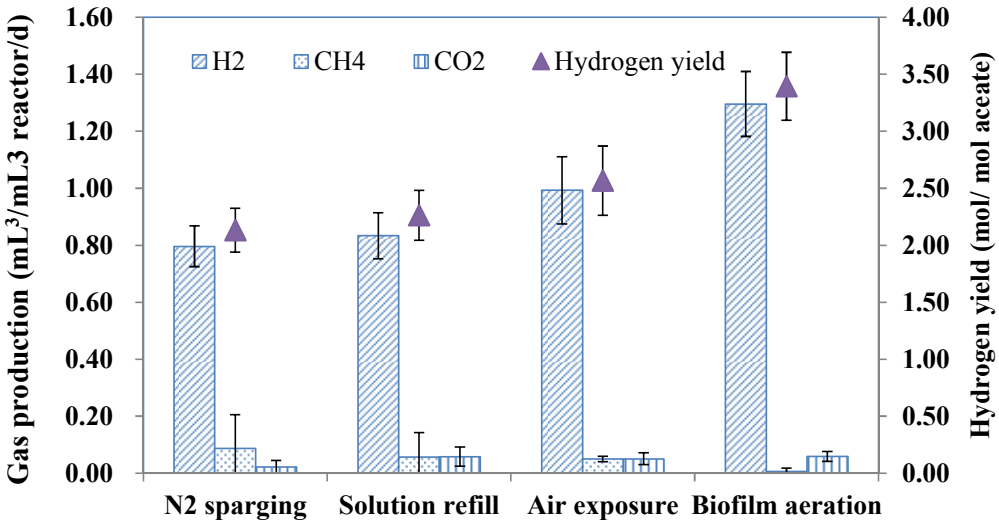

Figure 4 Effect of biofilm treatments on biogas production rates (H_2, CH_4, CO_2) and hydrogen production rates at the end of batch cycles in MECs

Error bar was calculated among the data of 3 batches from 3 replicate reactors for each condition.

Highlights

>H_2 production was improved by air treatment to anode biofilm in MECs.

> H_2 yield increased under biofilm aeration condition than N_2 sparging treatment.

> Biofilm aeration affected initial anode potential but not coulombic efficiency.

> A short-term air exposure impacted methanogen communities located in biofilms.