



# The impact of anode acclimation strategy on microbial electrolysis cell treating hydrogen fermentation effluent



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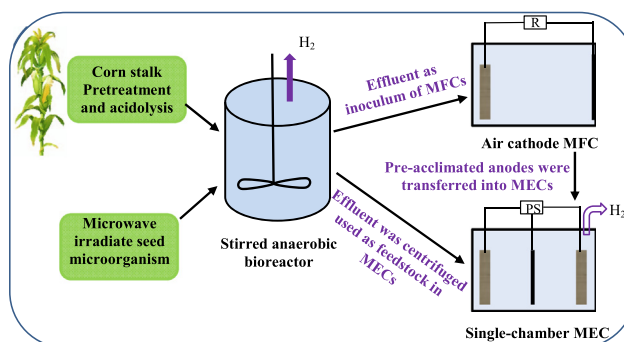
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## HIGHLIGHTS

- The electroactivity of anode microorganisms was affected by acclimation strategy.
- Acclimation strategy affecting the hydrogen fermentation effluent treatment in MEC.
- Butyrate could be efficiently removed in butyrate acclimated MEC.
- The H<sub>2</sub> production rate and yield were enhanced in butyrate acclimated MEC.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 8 February 2017

Received in revised form 23 March 2017

Accepted 28 March 2017

Available online 30 March 2017

### Keywords:

Microbial electrolysis cell (MEC)

Corn stalk fermentation effluent

Pre-acclimation

Hydrogen

Butyrate

Acetate

## ABSTRACT

The impact of different anode acclimation methods for enhancing hydrogen production in microbial electrolysis cell (MEC) was investigated in this study. The anodes were first acclimated in microbial fuel cells using acetate, butyrate and corn stalk fermentation effluent (CSFE) as substrate before moving into MECs, respectively. Subsequently, CSFE was used as feedstock in all the three MECs. The maximum hydrogen yield with the anode pre-acclimated with butyrate ( $5.21 \pm 0.24$  L H<sub>2</sub>/L CSFE) was higher than that pre-acclimated with acetate ( $4.22 \pm 0.19$  L H<sub>2</sub>/L CSFE) and CSFE ( $4.55 \pm 0.14$  L H<sub>2</sub>/L CSFE). The current density ( $480 \pm 11$  A/m<sup>3</sup>) and hydrogen production rate ( $4.52 \pm 0.13$  m<sup>3</sup>/m<sup>3</sup>/d) with the anode pre-acclimated with butyrate were also higher than another two reactors. These results demonstrated that the anode biofilm pre-acclimated with butyrate has significant advantages in CSFE treatment and could improve the performance of hydrogen production in MEC.

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## 1. Introduction

Hydrogen as one promising alternative clean energy source has attracted international attention in recent years (Datar et al., 2007; Guo et al., 2010; Kumar et al., 2016; Li and Fang, 2007). Among all of the available biological routes for H<sub>2</sub> production, biohydrogen

production through dark-fermentation can utilize various crop castoffs as feedstock was considered to be a feasible method due to its low energy consumption and ease of operation (Ghimire et al., 2015). However, during the hydrogen fermentation processes, hydrogen production is accompanied with production of volatile fatty acids (VFAs) and alcohols as by-products in which acetate and butyrate were the main component of fermentation effluent (Pan et al., 2010; Xing et al., 2011). The low conversion efficiency of feedstock and residue organics in fermentation efflu-

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ent are two main bottleneck problems (Marone et al., 2017). Therefore, biohydrogen production is likely to be industrially viable if fermentation processes could be integrated into a combination of processes that are cable of utilizing metabolic end products (Ghimire et al., 2015).

Recently, Microbial electrolysis cell (MEC) as one emerging technology for producing hydrogen from fermentation end products, such as acetate and ethanol, has gained increasing attention (Kadier et al., 2014). Compared with the dark-fermentation the MEC has a higher hydrogen recovery and a wider substrate diversity (Escapa et al., 2016). Most of the MEC studies have relied on the use of pure chemical compounds (primarily acetate) and acidogenic wastewater (fermentation effluent) as the substrate (Kadier et al., 2014). The integration of dark fermentation with MECs has been recognized as a promising method to convert biomass to hydrogen. However, when fermentation effluents were used as substrate, the hydrogen production rate was low and there was substantial methane production. For example, the hydrogen production rate could reach to  $5.56 \text{ m}^3/\text{m}^3/\text{d}$  at applied voltage of 0.8 V in single-chamber MEC using sodium acetate as substrate (Liang et al., 2011), while the highest hydrogen production rate only was  $1.76 \text{ m}^3/\text{m}^3/\text{d}$  feeding with hydrogen fermentation effluent (Liu et al., 2012). It is of great importance to improve the hydrogen production rate from fermentation effluent in MEC.

The performance of MECs is directly related to the substrates. VFAs and alcohols as the main end products in dark fermentation, among which acetate and ethanol were easily degradable while butyrate and propionate could not be oxidized efficiently (Lu et al., 2009; Yang et al., 2015). In a previous study (Li et al., 2014), about  $90 \pm 2\%$  of acetate was removed while the butyrate removal was only  $4 \pm 2\%$  in MEC. It is important to improve the degradation of butyrate by exoelectrogenic bacteria in MECs, as many effluents of hydrogen fermentation not only contain high concentration of acetate but also contain high concentration of butyrate. Recently, Ullery and Logan (Ullery and Logan, 2014) examined the impact of anode acclimation strategy (using different substrate: acetate or domestic wastewater) on the treatment efficiency of cellulose fermentation effluent. It was found that the pre-acclimation strategy of using domestic wastewater or acetate in MECs had no significant difference in COD treatment, current generation, and coulombic efficiency. Popov et al. (Popov et al., 2016) also investigated the influence of pre-acclimation with acetate and butyrate on biofilm structure for enhancing electricity and hydrogen production. It was found that the anode biofilm acclimated to butyrate had a significant advantage in hydrogen production when using butyrate or acetate and butyrate mixture as substrate. However, the effect of pre-acclimation strategy on the MECs treating corn stalk fermentation effluent has never been reported.

This study aims to investigate the impact of pre-acclimation of anode biofilm using different substrates in MFC mode on hydrogen production in MEC with corn stalk fermentation effluent (CSFE) as feedstock. The anode biofilm was first enriched using acetate, butyrate and CSFE as substrate, respectively. Subsequently, the potential of using such biofilm for hydrogen production from CSFE in double anodes MEC was investigated. The corresponding operational parameters were optimized in batch tests. In addition, the VFAs and ethanol removal in CSFE along with the current and coulombic efficiencies were evaluated.

## 2. Materials and methods

### 2.1. Seed microorganism

Cow dung compost as the seed of hydrogen-producing microflora and exoelectrogens was obtained from the dairy in biogas

plant. Prior to use, the mixture of water and cow dung compost with liquid/solid ratio of 4:1 (w/w) was sealed in serum bottle, and then treated using microwave irradiated for 1.5 min to suppress the activity of hydrogen-consuming bacteria and methanogens (Song et al., 2012). Thereafter, pre-incubated with basal medium in an anaerobic reactor at  $36^\circ\text{C}$  for about 9 h as the inoculum of corn stalk fermentation. The basal medium contains: sucrose, 10 g/L;  $\text{NH}_4\text{HCO}_3$ , 1 g/L;  $\text{KH}_2\text{PO}_4$ , 0.2 g/L and 10 mL mineral salt solution (comprising 2 g/L  $\text{NH}_4\text{HCO}_3$ , 1 g/L  $\text{KH}_2\text{PO}_4$ , 0.1 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g/L NaCl, 0.01 g/L  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , 0.01 g/L  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.015 g/L  $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$  and 0.0278 g/L  $\text{FeCl}_2$ ).

### 2.2. Characteristics of effluent samples as feedstock of MECs

The effluent was taken from a 5 L batch stirred anaerobic bioreactor, where the batch dark fermentation experiment was performed according to the method previously described (Li et al., 2014; Wang et al., 2012). The flowchart of the integrated hydrogen production process was shown in Fig. S1 (Supplementary Data). The corn stalk as the substrate of fermentation was smashed by a vegetation disintegrator with 40-mesh screen before using. The milled corn stalk and  $\text{H}_2\text{SO}_4$  solution (0.5%) with solid to liquid ratio of 1:10 (w/v) was autoclaved 60 min at  $121^\circ\text{C}$ . Thereafter, the pH was adjusted to 7 with 1 M  $\text{Ca}(\text{OH})_2$  solution for the dark fermentation. The stirred anaerobic bioreactor was filled with 3 L mixture containing the pre-incubated inoculum and pretreated corn stalk of 20 g/L. The bioreactor was flushed with nitrogen gas for 15 min and then was operated at  $37^\circ\text{C}$  with 120 rpm stirring speed. At the end of fermentation, the pH of fermentation effluent was adjusted to 7.0 using NaOH. The effluent was then collected by centrifugal separation to remove the fermentation residue and was further used as feedstock in MECs for  $\text{H}_2$  production. The effluent had a COD of  $8842 \pm 48 \text{ mg/L}$ , with the following constituents identified: acetate,  $3101 \pm 21 \text{ mg/L}$ ; butyrate,  $2602 \pm 24 \text{ mg/L}$ ; propionate,  $88 \pm 12 \text{ mg/L}$ ; ethanol,  $452 \pm 22 \text{ mg/L}$ .

### 2.3. MECs construction

The cubic single-chamber membrane-less MECs were constructed with a total volume of 64 mL as shown in Fig. 1. The MECs were equipped with the bioanode separately placed on both sides of cathode. The anode consisted of two pieces of square graphite felts. The cathode was made of a square carbon cloth coated with 0.5 mg Pt/cm<sup>2</sup> (20 wt% Pt/C, JM) and a Nafion (5%, Dupont). The cathode was placed in the middle of the cubic chamber with 15 mm average spacing to the anodes. Titanium wire was used to connect the electrodes to the circuit. The electrodes were connected to a battery test system (Neware Battery Testing System TC53, Shenzhen, China), which was used as a power supply (PS) to control the applied voltage and record the current generated from MECs.

### 2.4. MEC start-up and operations

All anodes were enriched in MFCs inoculated with a 1:1(v/v) mixture of hydrogen fermentation effluent from the 5 L batch stirred anaerobic bioreactor and nutrient buffer solution (NBS). The NBS contained:  $\text{NH}_4\text{Cl}$  0.31 g/L, KCl 0.13 g/L,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  2.27 g/L,  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  11.54 g/L, trace mineral 12.5 mL/L and vitamin 12.5 mL/L. When the exoelectrogens colonized on the anode surface indicated by a reproducible maximum voltage, the inoculum was omitted. The MFCs was then fed with 1000 mg/L acetate (HAc-MFC), 1000 mg/L butyrate (HBU-MFC) and CSFE (CSFE-MFC) as substrate, separately. A resistor of 1000  $\Omega$  was used as external load during MFCs operation. After one month, the anodes were transferred into MECs. Then an applied voltage rang-

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