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Improved bio-hydrogen production from glucose by adding a specific methane inhibitor to microbial electrolysis cells with a double anode arrangement

Jingnan Zhang, Yanxia Bai, Yaoting Fan,* and Hongwei Hou

College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450052, PR China

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Improved hydrogen production from glucose was achieved by adding a specific methane inhibitor (such as chloroform) to repress the activity of methanogens in a single-chamber microbial electrolysis cells (MECs) with a double anode arrangement. A maximum hydrogen production of 8.4 ± 0.2 mol H_2/mol-G (G represents glucose), a hydrogen production rate of 2.39 ± 0.3 m^3 $H_2/m_3/d$ and a high energy efficiency (relative to the electrical input) of ηE = 165 ± 5% had been recorded from 1 g/L glucose at a low dosage of chloroform (5‰, v:v) and an applied voltage of 0.8 V. Almost all of the glucose was removed within 4 h, with 66% of the electrons in intermediates (mainly including acetate and ethanol), and methane gas was not detected in the MECs through 11 batch cycles. The experimental results confirmed that chloroform was an effective methane inhibitor that improved hydrogen production from glucose in the MECs. In addition, the cyclic voltammetry tests demonstrated that the electron transfer in the MECs was mainly due to the biofilm-bound redox compounds rather than soluble electron shuttles.

[**Key words:** Microbial electrolysis cells; Biohydrogen; Glucose; Chloroform; Methane inhibitor; Electron transfer mechanism]

As an ideal energy carrier, hydrogen has received significant attention because it has a high specific energy content (122 kJ/g) and zero carbon emissions after combustion. This year, the dark fermentative bio-hydrogen production from different biomasses has attracted considerable attention because it is clean and renewable, but it is also faced a major challenge for the scientific community due to the low conversion efficiency of the substrate and serious environmental issues from the fermentative wastewater, which have not received sufficient attention to date. For instance, hydrogen production is often accompanied by the formation of volatile fatty acids (VFAs) by-products, such as acetate (HAc), butyrate (HBu) and propionate (HPr), during hydrogen fermentation (1,2). Recently, it was shown that hydrogen fermentation can be linked with microbial electrolysis cells (MECs) to produce more hydrogen from the VFAs and improve the conversion efficiency of the substrate, which has been highlighted in the literature. This introduces a new avenue of utilizing different species of substrates to generate hydrogen. For example, several studies have focused on the utilization of different substrates as the primary carbon source, such as acetate, acidogenic wastewater and glucose, to produce bio-hydrogen in the MECs (3–5). Recently, Liang et al. (6) recorded a high hydrogen production rate of 5.56 m^3/m^3/d from 1 g/L acetate at an applied voltage of 0.8 V in the designed single-chamber MECs with a stacking bioanode arrangement. Chookaew et al. (7) reported a hydrogen production of 48.8 ml H_2/g-COD from a glycerol fermentation effluent at an applied voltage of 0.6 V using the two-chamber MECs. Recently, Lu et al. (8) also observed a hydrogen production of approximately 6 mol H_2/mol-glucose and a relatively low cathodic hydrogen recovery (61–68%) from 2 g/L of glucose under 0.6 V applied voltage at 4 °C in the MECs. However, in most cases, the H_2 production from MECs is usually accompanied by the formation of methane gas that significantly represses the activity of the electrogenic flora in the MECs. For instance, Kyazze et al. (9) and Chae et al. (10) observed the emission of methane gas from the acetate-fed MECs during the hydrogen production process.

Different approaches have been attempted to suppress the activity of methanogen in the MECs, such as addition of some antibiotics/specific chemicals, periodic aeration, an increase in the applied voltage, UV irradiation, reduction of the pH of the medium in the MECs (11–15). For example, Chae et al. (16) found that the activity of the methanogens to produce hydrogen from acetate could be nearly completely inhibited following the addition of 572 μM BES (2-bromoethanesulfonate) to the MEC. Catal et al. (11) examined the effect of the CES (2-chloroethanesulfonate) dosage on the growth of methanogens to produce hydrogen from sodium acetate in the MECs, and indicated that the activity of the methanogens could be completely suppressed by the addition of 20 mM CES to the MEC. Recently, the chloroform was also found to be an effective methane inhibitor during the bio-hydrogen production process using dark fermentation (17,18).

As stated above, there is no useful information about the use of chloroform as a specific methane inhibitor to improve hydrogen production from the MECs. Therefore, in this study, glucose was used as a carbon source, and the effects of the chloroform dosage on the activity of methanogens and hydrogen production were specifically investigated in single-chamber MECs with a double anode arrangement. For this purpose, several key parameters that impact

* Corresponding author. Tel./fax: +86 371 67766017.
E-mail addresses: yt.fan@zzu.edu.cn, ytfan1207@163.com (Y. Fan).

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TABLE 1. Half-cell reactions and moles of electrons of per mole substrate.

Compound	Reaction	b
Glucose	$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$	24
Acetate	$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$	8
Formate	$CH_2O_2 \rightarrow CO_2 + 2H^+ + 2e^-$	2
Propionate	$C_3H_6O_2 + 4H_2O \rightarrow 3CO_2 + 14H^+ + 14e^-$	14
Ethanol	$C_2H_6O + 3H_2O \rightarrow 2CO_2 + 12H^+ + 12e^-$	12

b is the moles of electrons of per mole substrate.

hydrogen production in the MECs were carefully optimized in the batch tests. In addition, the possible electron transfer mechanism in the MECs was also briefly analyzed by cyclic voltammetry experiment.

MATERIALS AND METHODS

Construction of the MEC system Single-chamber membrane-less MECs with a stacking bioanode arrangement were constructed as previously described by Liang et al. (6) The cubic chamber constructed of acrylic glass with a total volume of 64 ml and wall thickness of 10 mm was equipped with the bioanodes that were separately placed on both sides of the cathode (Fig. S1). The anode consisted of two pieces of square graphite felts (30×30 mm^2; thickness: 5 mm). The cathode was made from carbon cloth (30×30 mm^2, Hesen, China) coated with 0.5 mg Pt/cm^2 (20 wt% Pt/C, JM) and Nafion (5%, Dupont, USA). The cathode was placed in the middle of the cubic chamber with an average distance of 15 mm between the anodes. Titanium wire was used to connect the electrodes to the circuit. The electrodes were connected to a battery test system. The power supply for MECs was an external battery test system (Neware Battery Testing System TC53, Neware, China), and the generated current from the MECs was recorded at 10-s intervals.

Enrichment and operation The bio-anodes were first enriched in single chamber microbial fuel cells (MFCs) with carbon cloth air cathodes and were initially inoculated with pretreated cow dung. A 50 mM nutrient phosphate buffer solution (NPBS, pH 7.0) (0.31 g/L NH_4Cl, 0.13 g/L KCl, 2.27 g/L $NaH_2PO_4 \cdot 2H_2O$, 11.54 g/L $Na_2HPO_4 \cdot 12H_2O$, 12.5 ml/L trace minerals, and 12.5 ml/L vitamins) containing 1 g/L glucose was used as the electron donor. The tests were conducted in fed-batch mode, and the inoculum was omitted when the output voltage was larger than 0.1 V (over 1 kΩ resistor). A batch cycle was defined as the duration in which the solution was refilled when the voltage was <0.1 V. The graphite felts anodes remained in the MFCs until they exhibited at least three repeatable stable maximum outputs and were then were transferred to the MEC reactors to be used as bio-anodes for H_2 production. The MECs were fed glucose in a 100 mM nutrient phosphate buffer solution (NPBS, pH 7.0) in batch mode. Prior to operation, the solution was sparged with nitrogen gas (99.999%) for 10 min to remove any oxygen, and then fixed voltages from 0.5 V to 0.9 V were applied to the MECs to investigate the hydrogen production from glucose. All of the experiments were performed at 36 ± 1 °C, and each condition was applied in triplicate. The results were reported as the mean values of the triplicate samples.

Analysis and calculations The biogas produced by the MECs was measured using the water replacement method. The gas composition was analyzed using a gas chromatograph (4890D, Agilent, Inc.) equipped with a thermal conductivity detector (TCD) and a 6-foot stainless steel column packed with Porapak Q (80/100 mesh). The liquid samples from the MECs were immediately centrifuged in a high-velocity centrifuge and then analyzed to determine the volatile fatty acid (VFA), ethanol and glucose concentrations. The concentrations of VFAs (including acetate, propionate, butyrate and formate) and ethanol were analyzed using another GC with a flame ionization detector (FID) and an 8-foot stainless steel column packed with 10% PEG-20 M and 2% H_3PO_4 (80/100 mesh). The reducing sugar content was measured using the 3,5-dinitrosalicylic acid (DNS) method (19). The cyclic voltammetry (CV) experiments were conducted with a multichannel potentiostat (CHI630B, Shanghai Chenhua instrument Co., Ltd.) at a scan rate of 1 mV/s and potentials ranging from −0.8 V to 0.2 V using a three-electrode arrangement under anaerobic conditions. Unless stated otherwise, all potentials provided in the cyclic voltammetry (CV) experiments were referenced to a Hg/Hg_2Cl_2 reference electrode (0.2415 V vs. a standard hydrogen electrode, SHE).

The charge balance was checked to determine the fate of electrons in the MECs. The number of Coulombs (C) can be calculated as:

$$C = nbF \quad (1)$$

where n is the number of moles, b is the moles of electrons per mole of substrate calculated from the half-cell reaction (Table 1), and F is Faraday's constant ($F = 96485$ C/mol).

The total Coulombs (C_T) that can be produced from glucose is calculated as:

$$C_T = C_I + C_S + C_C + C_L \quad (2)$$

where C_I is the Coulombs from the measured by-products (Eq. 1, Table 1), C_S is the Coulombs calculated from the substrate left in the medium, C_C is the Coulombs recovered from the current produced and C_L is the remaining Coulombs lost to non-measured products. C_C can be calculated as:

$$CC = \int_{t=0}^{t} Idt \quad (3)$$

where I is the current in the circuit, and dt is the time interval of current sampling that is recorded by our instrument.

The coulombic efficiency (C_E) (%) based on the total consumption of the substrate, hydrogen production (Y_{H2}) (mol H_2/mol-G, G represents glucose), hydrogen production rate (Q) (m^3 $H_2/m^3/d$), volumetric current density (I_v) (A/m^3), cathodic hydrogen recovery (R_{cat}) (%), overall hydrogen recovery (R_{H2}) (%) and energy efficiency relative to the electrical input (η_E) (%) was calculated as previously described (3,20).

RESULTS AND DISCUSSION

Enrichment of the anodes Approximately six days after start-up (the inoculum was omitted from the third batch), the MFCs were operated at 36 ± 1 °C and reached a maximum voltage, with an average voltage of 547 ± 10 mV, using cow dung as the inoculum. The rule of the output voltage is shown in Fig. S2. This power output was higher than that reported value in the

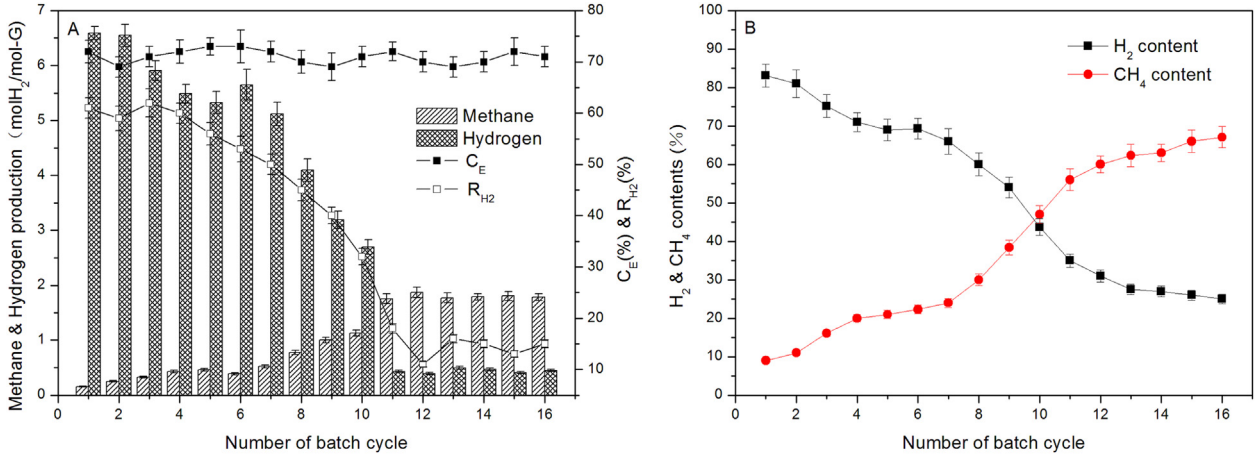

FIG. 1. Changes in methane production, hydrogen production, coulombic efficiency (C_E), overall hydrogen recovery (R_{H2}) (A), hydrogen content and methane content (B) with the number of batch cycle using glucose (1 g/L) as the substrate and an applied voltage of 0.5 V.

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