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RESEARCH ARTICLE

Boosting hydrogen production from fermentation effluent of biomass wastes in cylindrical single-chamber microbial electrolysis cell

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Abstract

Microbial electrolysis cells (MECs) are considered as green technologies for H_2 production with simultaneously wastewater treatment. Low H_2 recovery and production rate are two key bottlenecks of MECs fed with real H_2 fermentation effluent of biomass wastes. This work established a 1 L cylindrical single chamber MEC and enriched electroactive anodic biofilms from cow dung compost to overcome the bottlenecks. These MEC components (platinum-coated cylindrical titanium mesh cathode and cylindrical graphite felt anode) were arranged in a concentric configuration. A high H_2 production rate of 6.26 ± 0.23 L L^{-1} day^{-1} with H_2 yield of 5.75 ± 0.16 L H_2 L^{-1} fermentation effluent was achieved at 0.8 V. The degradation of acetate and butyrate (main components of H_2 fermentation effluent) could reach to $95.3 \pm 2.1\%$ and $78.4 \pm 3.6\%$, respectively. The microbial community analysis for anode showed the abundance of *Geobacter* (22.6%), *Syntrophomonas* (8.7%), and *Dysgonomonas* (6.3%) which are responsible to complex substrate oxidation, electrical current generation, and H_2 production. These results prove the feasibility of cylindrical single-chamber MEC for high H_2 production rate and high acetate and butyrate removal from H_2 fermentation effluent.

Keywords Reactor configuration · Volatile fatty acid · Bioanode acclimation · Microbial community analysis · Butyrate · Thermally treatment

Introduction

H_2 as one promising green and clean energy carrier has attracted more attention in recent years (Song et al. 2014; Wang and Yin 2019). Meanwhile, the global interests in optimizing sustainable H_2 production are growing. In particular, H_2 production from agricultural wastes (e.g., corn stalk, wheat straw, and grass) by biological method exhibits great advantages due to the agricultural wastes that are abundant and renewable (Li et al. 2020; Tian et al. 2019; Wang and Yin 2019). Among all the biological methods for H_2 generation, the coupling of dark-fermentation process and MEC has been considered as one of highly promising methods, due to its low costs and high H_2 production yield (Desmond-Le Quemener et al. 2019; Gao et al. 2020; Khongkliang et al. 2019; Marone et al. 2017). In dark fermentation process, H_2 production always accompanied with the generation of metabolic end products, such as acetate, butyrate, propionate, and alcohols, which can be used as substrates in MEC for additional H_2 production (Rousseau et al. 2020; Zhang et al. 2019). The coupled process could significantly increase the overall production of H_2 from agricultural wastes (Marone et al. 2017). For example, the overall H_2 production yield of corn stalk in coupled process could be increased up to 4 times when compared to dark fermentation process alone (Li et al. 2014, 2017).

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Numerous previous studies have reported different MEC structures to improve the performance of MEC and reduce the capital cost (Desmond-Le Quemener et al. 2019; Huang et al. 2019; Zhao et al. 2020; Priyadarshini et al. 2021). Normal MEC structures were divided into single-chamber and dual-chamber configurations. The use of double chambers with an ion exchange membrane or proton exchange membrane would increase the capital costs and limit MEC reactor scaling-up and commercialization application (Aiken et al. 2019; Pang et al. 2020). Single-chamber MECs without membrane are more attractive for scale-up due to high H_2 production rate and low capital costs (Huang et al. 2019; Guo et al. 2022). Recently, some studies also demonstrated that the cylindrical configuration up-scaled MECs have more great potential for enhancing H_2 production rate; this type of MEC has some advantages, such as simplified system design, low internal resistance, high current density, low operational costs, and easy maintenance and construction (Guo et al. 2017; Huang et al. 2019). However, high H_2 recovery and production rate in up-scaled MECs were only obtained using acetate as substrate; H_2 production from complex feedstocks (e.g., H_2 fermentation effluent or hydrolysate of biomass wastes) still is challenged. For example, the H_2 production rate could reach to 7.01 $m^3\ m^{-3}\ day^{-1}$ in a 1-L up-scaled cylindrical configuration MECs using sodium acetate as substrate (Guo et al. 2017), while the highest H_2 production rate only was 0.71 $m^3\ m^{-3}\ day^{-1}$ feeding with lignocellulosic hydrolysate in a 10-L up-scaled single chamber MECs (Wang et al. 2021).

Moreover, the performance of MEC also is related to the enrichment strategy of electrogenic bacteria on the anode (Li et al. 2017; Ullery and Logan 2015); most of MEC anodes were enriched with electrogenic bacteria using sodium acetate as sole carbon source in MFC mode (Jatoi et al. 2021); acetate could be degraded completely in MECs. However, most of the butyrate could not be removed and produced H_2 efficiently (Kadier et al. 2014; Yang et al. 2015). For example, the maximum H_2 production rate could reach to 3.43 ± 0.12 $m^3\ m^{-3}\ day^{-1}$ using corn stalk fermentation effluent as substrate; almost of all the acetate can be removed, while the butyrate degradation rate was only 4–6% (Li et al. 2014). When using complex substrate (e.g., fermentation effluent and butyrate) to acclimate the anode biofilm of MECs, the degradation of acetate and butyrate both could be improved efficiently (Yang et al. 2015; Li et al. 2017). For example, Popov et al. (2016) found that the butyrate-acclimated bioanode had obvious advantage for H_2 production when using acetate and butyrate as mixture substrate. It is very important to enhance the conversion of butyrate to H_2 by electrogenic bacteria in MEC, because many complex H_2 fermentation effluents not only contain acetate, but also contain a large number of butyrate (Kumar et al. 2016; Marone et al. 2017).

In this study, the anode biofilm was enriched successfully using H_2 fermentation effluent of corn stalk as substrate in MEC mode; the potential of using volatile fatty acids (VFAs) and ethanol mixtures for H_2 production was studied. The key operating parameters of MECs were optimized in batch experiments. The overall COD removal and degradation of different VFAs and ethanol also were studied for further improvement. Moreover, microbial community characteristics of anode biofilm were analyzed. Additional multiple cycles were also tested to investigate the stability of operation and to better understand how operating impacts performance for commercial applications.

Materials and methods

Seed microorganism of MECs

Cow dung compost was used as the inoculum of electrogenic bacteria, which was obtained from the biogas plant (Henan Agricultural University, Henan province, China). Firstly, the cow dung compost was pretreated by thermally treatment (100℃, 10 min) to inhibit the activity of methanogens. Thereafter, the pretreated cow dung compost was pre-incubated with medium in a 1-L batch-stirred anaerobic reactor at 36 ± 1℃ for 12 h, which was used as the inoculum of MEC anode biofilm; the medium contains the following: glucose, 10 g L^{-1}; peptone, 2 g L^{-1}; yeast, 2 g L^{-1}; NH_4HCO_3, 1 g L^{-1}; KH_2PO_4, 0.2 g L^{-1}; and 10 mL mineral salt solution (contained NH_4HCO_3, 2 g L^{-1}; KH_2PO_4, 1 g L^{-1}; $MgSO_4{\cdot}7H_2O$, 0.1 g L^{-1}; NaCl, 0.01 g L^{-1}; $Na_2MoO_4{\cdot}2H_2O$, 0.01 g L^{-1}; $CaCl_2{\cdot}2H_2O$, 0.01 g L^{-1}; $MnSO_4{\cdot}7H_2O$, 0.02 g L^{-1}; $FeCl_2$, 0.03 g L^{-1}).

Characteristics of H_2 fermentation effluent used in MECs

The H_2 fermentation effluent was obtained from one 5-L anaerobic reactor; the reactor was operated as previous approach (Li et al. 2014). In order to remove fermentation residue and microorganisms, the fermentation effluent was pretreated by centrifuging at 13,000 rpm for 10 min before using as feedstock in MEC for H_2 production. The centrifuged fermentation effluent was mainly including acetate 2550 ± 28 mg L^{-1}, butyrate 2330 ± 25 mg L^{-1}, propionate 137 ± 18 mg L^{-1}, and ethanol 210 ± 19 mg L^{-1}, the COD was 8740 ± 50 mg L^{-1}.

MEC reactor construction

The cylindrical single-chamber MECs (10 cm outer diameter, 9.4 cm inner diameter, and 15 cm height) was constructed with working volume of 1 L using acryl glass (see

Fig. 1). A total of 0.5 cm thick of cylindrical graphite felt (8 cm diameter and 10 cm length) was used for anode. The cathode was made of cylindrical Ti mesh (4 cm diameter and 10 cm length) coated with 0.5 mg Pt cm^{-2} (20 wt% Pt/C, JM). Titanium wires were used to connect the anode and cathode. The anode and cathode were connected to battery test system (Neware Battery Testing System TC53, China) that was used as power supply (PS) to supply the applied voltage and record the current of MECs.

MEC start-up and running

The anode was acclimated in MEC mode using the corn stalk fermentation effluent from the anaerobic bioreactor without centrifugation as inoculum; the MEC was inoculated with 1:1 (volume/volume) mixture of fermentation effluent and buffer solution, which contains the following: NH_4Cl, 0.31 g L^{-1}; KCl, 0.13 g L^{-1}; $NaH_2PO_4{\cdot}2H_2O$, 2.27 g L^{-1}; $Na_2HPO_4{\cdot}12H_2O$, 11.54 g L^{-1}; trace mineral, 12.5 mL L^{-1}; vitamin, 12.5 mL L^{-1}. An applied voltage of 0.5 V was supplied to start up the MEC. When the electrogenic bacteria were acclimated on the anode surface, the inoculum was omitted. The MECs was feed with 1 L centrifuged H_2 fermentation effluent, which added with trace mineral 12.5 mL L^{-1}, vitamin 12.5 mL L^{-1}, NH_4Cl 0.62 g L^{-1}, KCl 0.26 g L^{-1}, $NaH_2PO_4{\cdot}2H_2O$ 5.54 g L^{-1}, and $Na_2HPO_4{\cdot}12H_2O$ 23.08 g L^{-1} (equivalent to 100 mM phosphate buffer) to control the pH at 6.8 ± 0.1; the conductivity is 18.32 ms cm^{-1}. The temperature of effluent in MEC was controlled at 36 ± 1℃. Meantime, the MECs was purged with N_2 for 15 min to keep an anaerobic condition.

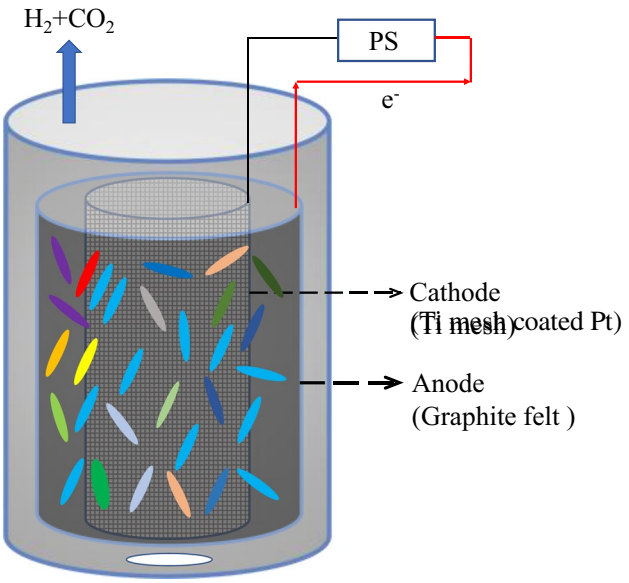

Fig. 1 Schematic of the cylindrical single-chamber microbial electrolysis cell

The MECs was operated at least three times at the same condition, and the values of current density and H_2 production rate in MEC were also consistent over triplicate cycles.

Analytical methods

The concentration of H_2, CH_4, and CO_2 in MEC was detected using a gas chromatograph (GC, Agilent 5890), which was equipped with thermal conductivity detector (TCD) and stainless column packed with Porapak Q (80/100 mesh). The concentration of acetate, butyrate, propionate, and alcohols was detected using GC with a flame ionization detector (FID). The volume of H_2 was calculated by the following Eq. (1):

$$V = V_{i1}x_{i1} + V_{i2}x_{i2} \quad (1)$$

where V is the total H_2 volume at the time (i); V_{i1} is the volume of headspace of the MECs and x_{i1} is the concentration of H_2; V_{i2} is the gas volume discharged from the MECs; and x_{i2} is the concentration of H_2.

The H_2 production rate was calculated by Eq. (2):

$$HPR = V/\left(t \times V_0\right) \quad (2)$$

The t is the time of H_2 production, and V_0 is the working volume of the MECs.

The Coulombic efficiency (C_E) was calculated based on the removal of the VFAs and alcohols; the cathodic H_2 recovery (r_{cat}), overall H_2 recovery (R_{H2}), and the energy efficiency (η_E) were calculated following previous report (Li et al. 2014). The volumetric current density (A m^{-3}) was calculated based on the working volume of MEC; the projected current density (A m^{-2}) was calculated based on surface area of cathode. The COD concentration of fermentation effluent was measured and calculated as the standard methods (American Public Health Association, AWWA 1998).

Anode microbial community analysis

The anode biofilm sample of MECs was collected when the MEC running stability for community characteristic analysis via high-throughput sequencing of 16S ribosomal RNA gene amplicons. The DNA was extracted in triplicate using a Soil DNA Kit (OMEGA, A/S). 16S sRNA gene was amplified with F968-GC (5′-GC-clamp-AACGCGAAGAACCTTAC-3′) and R1401 (5′-GCGTGTGTACAAGACCC-3′) and subjected to sequencing on an Illumina HiSeq2500 sequencer at Magigene Sequencing Lab. Bioanode community analysis was performed as previously described (Zhao et al. 2021).

Results and discussion

Bioanode pre-acclimation in MEC mode with applied voltage

Figure 2 shows the development of current of MEC during anode biofilm acclimating period. During the acclimating period, the anode was firstly pre-acclimated with fermentation effluent as inoculum and supplied 0.5 V applied voltage (cycle 1). The current starts to gradually increase at 18 h after inoculating; this demonstrated that the electrogenic bacteria have been effectively attached to the anode. From cycle 2 to 3, the current would directly increase to stable maximum value (16.3 ± 0.4 mA) after feeding centrifuged fermentation effluent (without lag phase); the corresponding H_2 production rate was 0.73 ± 0.18 L L^{-1} day^{-1}. When the fermentation effluent was switched to butyrate medium at cycle 4, the current firstly decreased to 4.6 ± 0.4 mA, and then increased smoothly peaking (11.1 ± 0.5 mA), thus demonstrating that the anode biofilm was adapting to the butyrate. In the three butyric medium-fed MEC batches (from cycle 4 to 6), the maximum current was kept at 11.1 ± 0.5 mA; the corresponding H_2 production rate only was 0.46 ± 0.09 L L^{-1} day^{-1}. The comparatively slower current to peak in the butyrate medium-fed MEC indicated that the electrogenic bacteria on the anode biofilm cannot degrade butyrate directly. Furthermore, acetate always was detected at the peak current period. These behaviors indicated that the butyrate was likely firstly degraded into acetate by the butyrate degrading acetogenic bacteria in MEC, and then the acetate was oxidized completely to CO_2 and H^+. The phenomenon is similar to previous several studies (Ullery and Logan 2015; Popov et al. 2016; Li et al. 2017). From cycle 7 to 9, the substrate was switched to fermentation effluent again; the current rapidly increased to a maximum of 20.4 ± 0.4 mA, and then stabled at the maximum value, which are higher than that of cycle 2 to cycle 6. Moreover, due to the higher current, the H_2 production rate in cycle 8 and cycle 9 reached maximum of 2.61 ± 0.11 L L^{-1} day^{-1}, which is higher than that of cycle 2 to 6. These results demonstrated that the current can be greatly improved by using butyrate as single substrate with applied voltage of 0.5 V to acclimate anode biofilm in MEC. Moreover, the pre-acclimation method of bioanode in MEC mode also is easy to operate in scaling up MEC reactor.

Effect of applied voltage on the MEC performance

Applied voltage is an important factor of MECs for H_2 production. As shown in Fig. 3, the H_2 production rate almost linearly increased with the increasing of applied voltage from 0.5 to 0.8 V, which increased from 2.61 ± 0.11 to 6.26 ± 0.23 L L^{-1} day^{-1}. Figure 3 depicts the current under different applied voltage; the corresponding stable current density was improved from 20.4 ± 0.4 (1.62 ± 0.03 A m^{-2}) to 36.8 ± 0.4 A m^{-3} (2.93 ± 0.03 A m^{-2}) with the increasing of voltage from 0.5 to 0.8 V. When the applied voltage was further increased to 0.9 V, both H_2 production rate and current density were closed to plateau level (6.36 ± 0.21 L L^{-1} day^{-1} and 37.6 ± 0.4 A m^{-3}). The higher current represents corresponding to the higher H_2 production; this behavior also is similar with mostly previous MEC studies

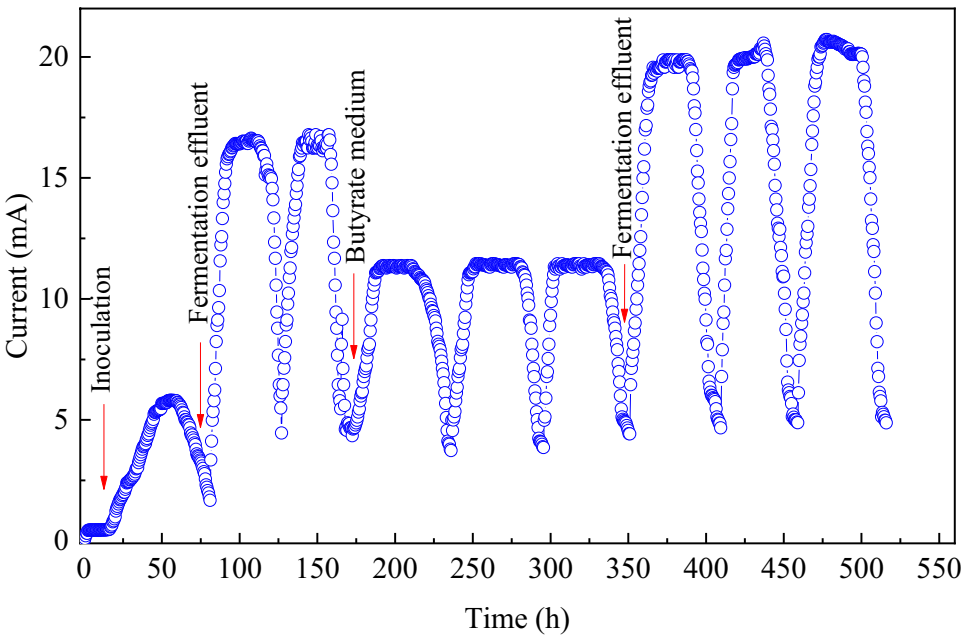

Fig. 2 Current generation of the cylindrical single-chamber MEC during the start-up period. The MECs was started under 0.5 V applied voltage

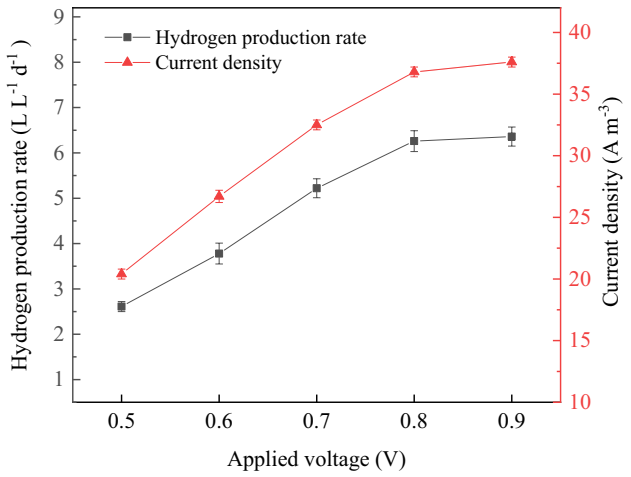

Fig. 3 H_2 production rate and maximum current density of the MEC at different applied voltages

(Liu et al. 2012). However, compared to previous MECs, the higher H_2 production rate was obtained in this MEC at same applied voltage. The maximum cumulative H_2 volume also increased from 4.64 L H_2 L^{-1} fermentation effluent to 5.75 ± 0.16 L H_2 L^{-1} fermentation effluent with the applied voltage increasing from 0.5 to 0.8 V. When further increase the applied voltage from 0.8 to 0.9 V, the H_2 volume was steady around 5.75 ± 0.16 L H_2 L^{-1} fermentation effluent. The initial and final pH of fermentation effluent was kept at 6.8 ± 0.1; the lack of pH change due to the high buffering capacity of the phosphate buffer used for the fermentation effluent. The initial conductivity of fermentation effluent was 18.32 ms cm^{-1}; the final conductivity under different applied voltage are shown in table S1. It has been reported that the H_2 production is mainly by the oxidative degradation of substrate by the bioanode (Li et al. 2017; Zhang and Li 2020). The increasing of H_2 production rate and cumulative H_2 volume with the increasing of applied voltage, indicating the bioanode oxidating ability, was enhanced in the range of 0.5–0.8 V. However, the oxidative ability was not improved accordingly when the applied voltage increased to 0.9 V. Moreover, there only were H_2 (91%) and CO_2 (9%) were detected in gas phase; no CH_4 was observed in the MECs. These results indicated that the MECs can produce addition H_2 with high production rate from the H_2 fermentation effluent. Cyclic voltammeter (CV) test was conducted to analyze the microbial activity of bioanode in the MECs (Fig. S1). Voltammograms showed that oxidation peaks were observed from − 200 to − 300 mV. Electrochemical impedance spectroscopy (EIS) spectrum was fitted to equivalent circuits (Fig. S2) to identify the inner resistances of the MECs; the calculated polarization resistance (R_{pol}) for anode is 84.94 Ω, the biofilm resistance (R_{film}) is 1.92 Ω, and the inner resistances of MECs contain solution (R_{sol}) is 3.02 Ω.

Figure 4 shows the variation of energy efficiency (n_E), Coulombic efficiency (C_E), cathodic recovery (R_{cat}), and overall H_2 recovery (R_{H2}) of this MEC under different applied voltage. The C_E ranged from 76 ± 3 (0.5 V) to $79 \pm 2\%$ (0.9 V), which were not significantly different. The η_E gradually decreased along with the increase of applied voltages, which were decreased from 285 ± 13 at 0.5 V to $163 \pm 8\%$ at 0.9 V. The η_E was higher than 100%, which demonstrated that the energy contained in the H_2 produced by the MEC was higher than the electrical energy input. This trend of η_E was in agreement with most previous MEC studies (Wu et al. 2013; Guo et al. 2017; Li et al. 2017; Zhang et al. 2019). The R_{cat} showed a favorable increase with the increase in applied voltage, which increased from $73 \pm 3\%$ at 0.5 V to $81 \pm 2\%$ at 0.8 V. The R_{H2} increased from 61 ± 2 to $71 \pm 3\%$ with the increasing of applied voltage from 0.5 to 0.8 V, and then slightly increased to $72 \pm 3\%$ at 0.9 V. These results demonstrate that this single chamber MEC allows high H_2 recovery with high H_2 production rate at 0.8 V. Thus, considering to obtain high H_2 production rate and H_2 yield, 0.8 V was considered as the most promising applied voltage for this MEC. Furthermore, in order to better understand how operating impacts performance for commercial applications, more than 30 cycles were tested to investigate the stability of operation; the performance of MEC is very stable.

VFAs and COD removal of fermentation effluent in MECs

Figure 5a illustrates the removal of VFAs (e.g., acetate, butyrate, propionate), COD, and ethanol with different applied voltage. Seen from Fig. 5a, among all the applied voltages, the COD removal efficiency reached $69 \pm 2\%$. The

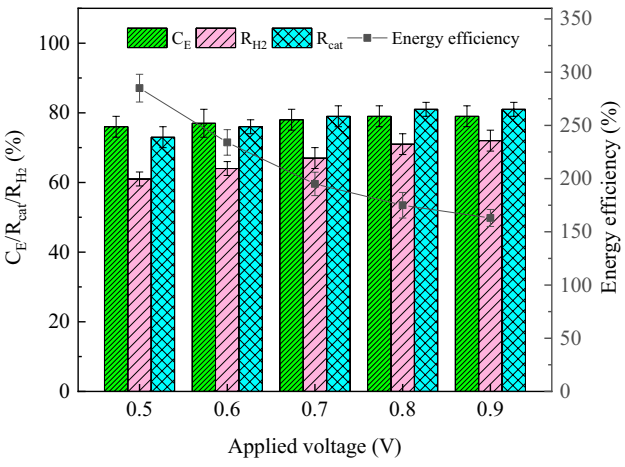

Fig. 4 Energy efficiency (n_E), Coulombic efficiency (C_E), cathodic recovery (R_{cat}), and overall H_2 recovery (R_{H2}) in the MEC under different applied voltages

removal of acetate, propionate, butyrate, and ethanol could reach 95.3 ± 2.1%, 98.2 ± 1.3%, 78.4 ± 3.6%, and 100%, respectively. Even though the butyrate could not be removed completely, the butyrate removal has a great improvement, which is about 18.7–20 times higher than 4 ± 2% in previous study (Li et al. 2014), in which the MEC bioanodes were enriched using sodium acetate as sole carbon source in MFC. The butyrate removal also is higher than previous study using single-chamber MECs with square electrodes treating H_2 fermentation effluent (mainly contains acetate and butyrate), generally ranging from 14 to 32% (Khongkliang et al. 2019). These results also show that acclimating MECs to H_2 fermentation effluent provided a new strategy for H_2 production simultaneously with treatment of fermentation effluent. In addition, the low COD removal is due to the fermentation effluent of corn stalk that contains some complex components, e.g., lignin cleavage fragment, pigment, and furfural generated due to the hydrolysis of corn stalk; they could not be efficiently degraded by the electrogenic bacteria.

It has been clearly studied that the current density and H_2 production rate in MEC are proportional to the substrate utilization rate (Karthikeyan et al. 2017); the butyrate has been well known to difficulty utilized in MECs compared to acetate (Yang et al. 2015; Ullery and Logan 2015; Popov et al. 2016; Li et al. 2017). In order to provide greater insight on the relation of H_2 production rate, VFA degradation, and current. One example of one cycle of H_2 production rate, VFA degradation, and current of the MEC with 0.8 V applied voltage is shown in Fig. 5b. The current and H_2 production rate decreased from maximum to 7.4 ± 0.5 mA and 0.76 ± 0.13 L L^{-1} day^{-1} after 24 h; the acetate concentration was stable at 123 ± 26 mg L^{-1}. Moreover, when the current reached maximum, the acetate remove rate was 113 ± 12 mg L^{-1} h^{-1}; the butyrate remove rate reached 88 ± 9 mg L^{-1} h^{-1}. After 20 h, the current starts to decrease due to the acetate decreasing to 153 ± 26 mg L^{-1}; the butyrate removal rate also decreased to 43 ± 7 mg L^{-1} h^{-1}. When the H_2 production stopped, the acetate has been degraded almost completely. The removing trend of acetate and butyrate in this MEC is similar with previous studies (Li et al. 2014, 2017); the acetate was firstly degraded quickly. The butyrate needed to be degraded firstly to acetate, H^+, and CO_2 by the electrogenic bacteria, and then the acetate was further degraded to H^+ and CO_2. The degradation process of butyrate to acetate is the rate-limiting step (Li et al. 2017), which resulting in butyrate could not be degraded completely when the H_2 production stopped.

Comparison to other high-performance MECs

Several previous studies of MECs fed with various wastewater for H_2 production were compared with this MEC, and the comparisons are summarized in the Table 1. As shown in Table 1, the H_2 production rate and butyrate removal were much higher than that of other literatures (Liu et al. 2012; Li et al. 2014; Khongkliang et al. 2019). Especially, when comparing with previous MECs fed with fermentation effluent of biomass waste, this cylindrical single-chamber MEC has a higher H_2 production rate. For example, Wu et al. reported one single-chamber MECs fed with fermentation effluent, which maximum H_2 production rate was only 1.31 ± 0.04 L L^{-1} day^{-1} at 0.6 V applied voltage (Wu et al. 2013). Moreover, Nam et al. also reported H_2 production from cellulose fermentation wastewater in one double-chamber MECs; the maximum H_2 production rate was only 0.49 ± 0.05 L

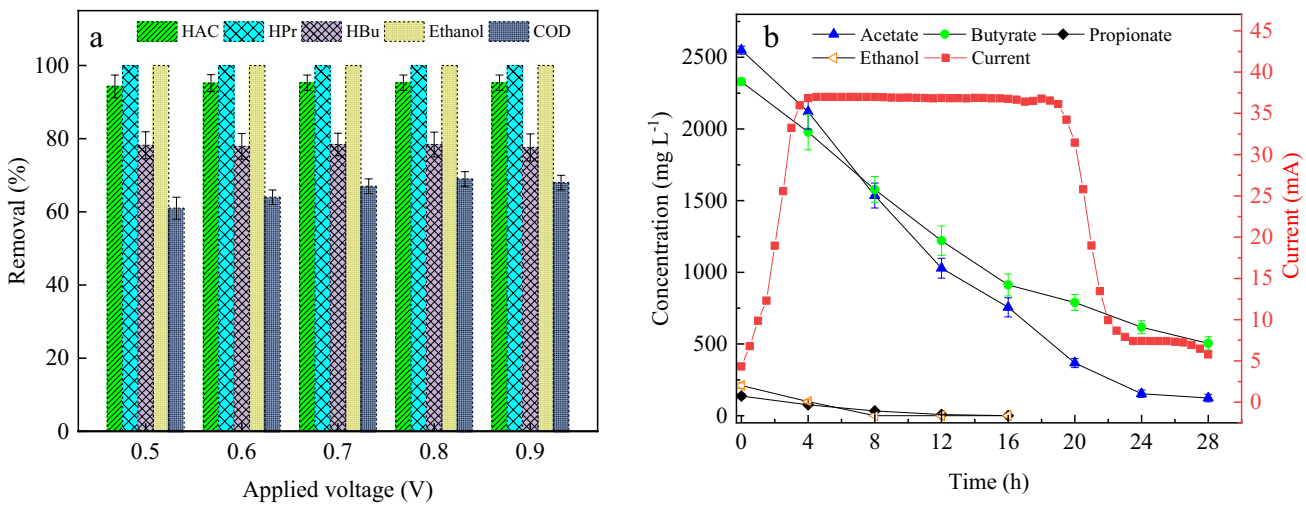

Fig. 5 Remove rate of VFAs (HAC, acetate; HPr, propionate; HBu, butyrate), ethanol, and COD with different applied voltages (**a**) and the current, removal trend of VFAs, and ethanol under 0.8 V (**b**)

Table 1 An summarize of performance of different MEC structures fed with different wastewater

MEC reactor	Substrate	Applied voltage (V)	Current ($A\ m^{-3}$)	Butyrate removal	Energy efficiency (%)	H_2 production rate ($L\ L^{-1}\ d^{-1}$)	References
Single chamber MEC (volume: 44 mL)	Sludge fermentation liquid	0.8	-	54%	155 ± 5	1.76 ± 0.03	(Liu et al. 2012)
Single chamber MEC (volume: 26 mL)	Molasses fermentation liquid	0.8	158	-	170	1.52	(Lu et al. 2009)
Single chamber MEC (volume: 60 mL)	Corn stalk fermentation effluent	0.8	340 ± 4	4%	166 ± 10	3.43 ± 0.12	(Li et al. 2014)
Two chamber MEC (volume: 137 mL)	Cellulose fermentation effluent	0.9	99.3 ± 2.9	-	-	0.49 ± 0.05	(Nam et al. 2014)
Cylindrical two chamber MEC (volume: 4 L)	Saline effluent	-	199.1 ± 4.0	-	-	0.9	(Carmona-Martinez et al. 2015)
Single chamber MEC (volume: 60 mL)	Corn stalk fermentation effluent	0.8	480 ± 11	62 ± 3%	155 ± 10	4.52 ± 0.13	(Li et al. 2017)
Double-chamber tubular MEC (volume: 1 L)	Acetate wastewater	0.8	-	-	-	3.09	(Guo et al. 2017)
Single chamber MEC (volume: 70 mL)	Fermentation effluent	0.7	-	30%	471	7.81	(Khongkliang et al. 2019)
Cylindrical two chamber MEC (volume: 98 mL)	Lignocellulosic fermentation effluent	0.8	-	-	215.33	0.055	(Zhang et al. 2019)
Cylindrical single chamber MEC (volume:1 L)	Corn stalk fermentation effluent	0.8	36.8 ± 0.4	78.4 ± 3.6%	163 ± 8%	6.26 ± 0.23	This study

The current density ($A\ m^{-3}$) calculated based on working volume of MEC; the H_2 production rate ($L\ L^{-1}\ day^{-1}$) calculated based on the MEC working volume

L^{-1} day^{-1} at 0.9 V (Nam et al. 2014). These results indicated that the cylindrical single-chamber MEC system has a higher H_2 production efficiency and faster oxidation of the substrate.

The existence of methanogenic bacteria has been confirmed that is one of the limiting factors for MECs producing H_2 (Kadier et al. 2018; Karthikeyan et al. 2017). Numerous researchers have studied single-chamber MECs for H_2 production using fermentation effluent; the methanogenic activity would disturb the H_2 production significantly by the methanogenic bacteria on the bioanode (Karthikeyan et al. 2017). Especially with the long-term operation of MECs, the competition would be dominant by the methanogenic bacteria over the electrogenic bacteria. Different methods of methanogenic bacteria inhibition have been studied in single MECs, e.g., 2-bromoethanesulfonate, chloroform, acetylene, UV radiation, and low temperature (Karthikeyan et al. 2017; Wang et al. 2019). Even though thermal treatment is known not be able to completely suppress methanogens, especially for long-term operation MEC (Hu et al. 2008), thermally treating (100 ℃) the mixed microorganism has been reported to inhibit the methanogenic bacteria during dark fermentation process for H_2 production (Fu et al. 2020; Liao et al. 2021). No CH_4 was observed in this MEC, indicating that the thermally treatment also might be an efficient method to inhibit the methanogenic bacteria when using the cow dung compost as inoculum. Furthermore, compared with several previous studies (see Table 1), the H_2 production rate were improved in this study; it demonstrated that thermal treatment (100 ℃) could not inhibit the electrochemical performance of electrogenic bacteria when using cow dung compost as the seed of electrogenic bacteria.

The by-products of fermentation process mainly focus on the acetate, ethanol, and butyrate. The amounts and relative abundances of these by-products varied depending on the types of fermentation, substrates, and bacteria. Acetate and ethanol have been widely studied as promising substrates for H_2 production in MEC; how to enhance the degradation of butyrate is one of the main challenges for H_2 production using fermentation effluent. In this study, the butyrate removal could reach 78.4 ± 3.6%, which is higher than that of previous studies (details see Table 1). Moreover, compared to the previous MECs, the current density based on liquid volume was significantly lower in this study, possibly due to the use of H_2 fermentation effluent as substrate in the MECs.

Numerous studies also have reported tubular and cylindrical MECs for boosting H_2 production (Carmona-Martinez et al. 2015; Guo et al. 2017; Nguyen et al. 2020). For example, Guo et al. (2017) reported one tubular two-chamber MECs with anion exchange membrane, in which the maximum H_2 production rate could reach 7.1 L L^{-1} day^{-1} using acetate as substrate. Although the maximum H_2 production rate in here was lower than it, the cylindrical single-chamber MECs without membrane could reduce the capital cost of MEC and improve the MEC operating economics.

Analysis of microbial communities on bioanode

Microbial community information is another important information to explain the result. Currently, majority of the similar investigations would include data from high-throughput sequence or next-generation sequencing based on genomic DNA of samples. In this study, RNA-based sequencing was used to detect the metabolically active population (Fig. 6). The microbial samples were taken from bioanode at the end of the H_2 production. A total of 15 phyla were detected, and 7 phyla are shown in Fig. 6a. The most abundant phylum was *Proteobacteria* (52.3%), followed by *Bacteroidetes* (18.2%), *Firmicutes* (14.3%), *Actinobacteria* (3.1%), and *Synergistetes* (2.7%) (Fig. 6a). *Proteobacteria* are well known playing important roles in extracellular electron transfer (Fykse et al. 2016). *Bacteroidetes* were related to methanogenesis (Cheng et al. 2021). The dominant genera in anodic communities were characterized as *Geobacter* (22.6%), *Syntrophomonas* (8.7%), *Dysgonomonas* (6.3%), *Comamonas* (4.3%), *Pseudomonas* (4.2%), and *Clostridium* (3.5%) (Fig. 6b). *Geobacter* as well-known electroactive bacteria using acetate as primary substrate has been researched widely (Zhu et al. 2020). *Syntrophomonas* as syntrophic bacteria has not been studied in previous MECs, which was able to oxidize acetate, butyrate, and more complex substrates (Guzman et al. 2019). Popov et al. (2016) also demonstrated that *Syntrophomonas* number increasing would enhance the H_2 production performance and feedstock removal rate by using butyrate to acclimate anode. *Dysgonomonas* also has high ability to degrade different kinds of complex chemicals. *Pseudomonas* had critical functionalities in electrogenic biofilm by promoting acclimation biofilm and enhancing MEC performance for H_2 production (Guzman et al. 2019; Zhao et al. 2021). *Comamonas* and *Clostridium* are classified as fermentative bacteria, and can participate in the degradation of complicated organics to degrade to more biodegradable substrates that can be metabolized by *Geobactor* for electricity generation and H_2 production (Wang et al. 2021). Moreover, *Comamonas* also is a well-known exoelectrogens and has strong ability to degrade organics and generate electricity in MFC (Gao et al. 2020). Besides the high butyrate and acetate removal performance, such high current densities and H_2 production rate for this MEC could also be attributed to the dominance of *Geobacter* and these co-cultures. Moreover, even though previous study has indicated that the methanogens could not be completely inhibited by heat treatment (Hu et al. 2008), no CH_4 was detected in this study. It is possibly a result of inhibiting methanogens in the anode microbial community

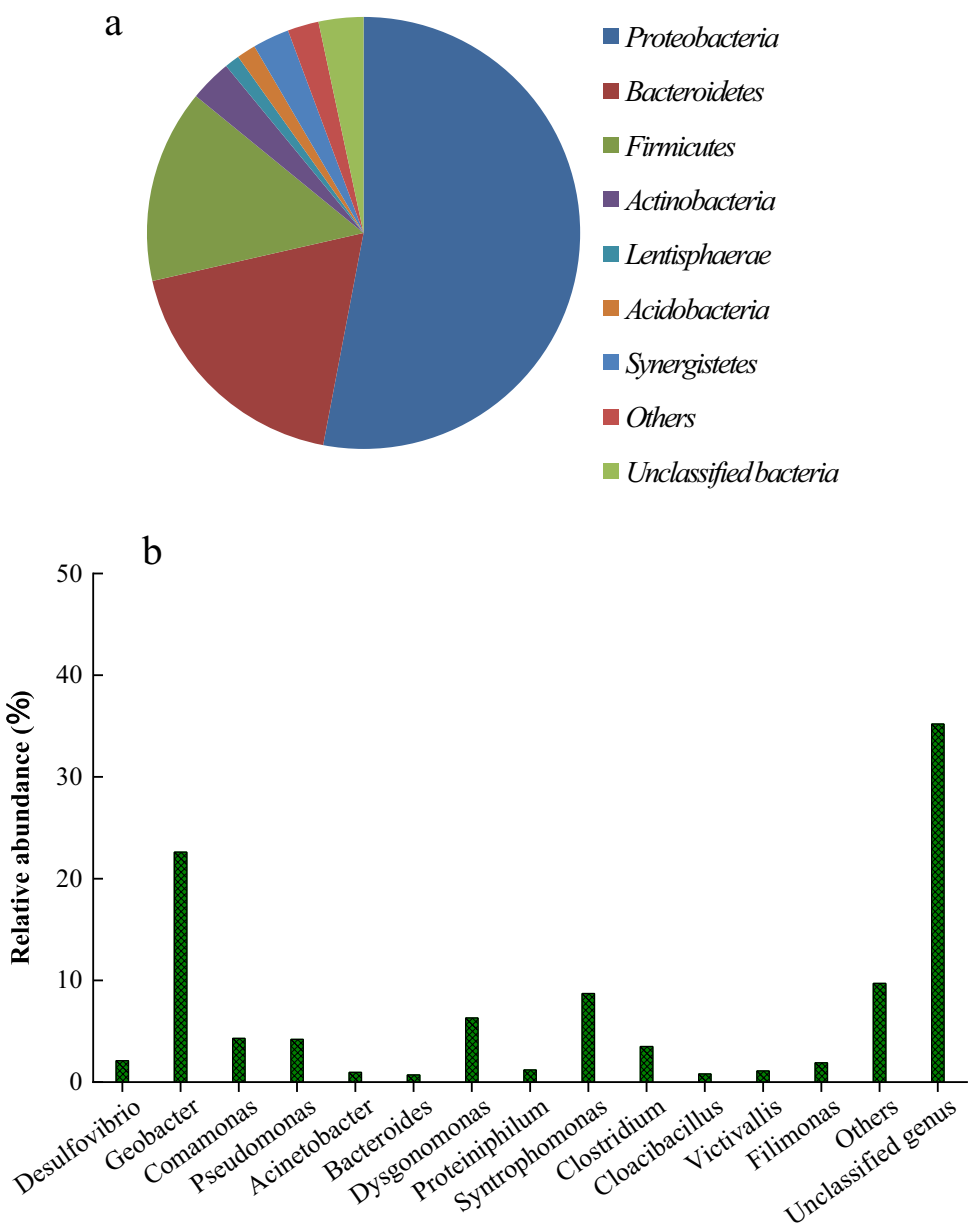

Fig. 6 The relative abundance distribution of microbial community on MEC bioanode at the phylum (**a**) and genus (**b**) levels

that compete with the exoelectrogens for feedstock and space (Wang et al. 2019; Zhao et al. 2021).

Conclusion

The feasibility of using fermentation effluent of corn stalk for H_2 production in a 1-L cylindrical single-chamber MEC was investigated in this work. The H_2 production rate could reach to 6.26 ± 0.23 L L^{-1} day^{-1} and total H_2 yield reach to 5.75 ± 0.16 L H_2 L^{-1} fermentation effluent under applied voltage of 0.8 V. The corresponding COD and butyrate removal in MECs reached 69 ± 2% and 78.4 ± 3.6% separately. Analysis results of anodic microbial communities showed that the microbial culture acclimated to butyrate offered a significant advantage in H_2 production and a role in the prevention of methanogenesis. Compared with other MECs, this study may provide an efficient MEC reactor and operating strategy for H_2 production and simultaneous with H_2 fermentation effluent treatment.

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authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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