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The significance of key operational variables to the enhancement of hydrogen production in a single-chamber microbial electrolysis cell (MEC)

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Abstract

The Microbial electrolysis cell (MEC) is one of the promising and cutting-edge technologies for generating hydrogen from wastewater through biodegradation of organic waste by exoelectrogenic microbes. In MECs the operational parameters, such as applied voltage (E_{ap}), anode surface area, anode-cathode distance, and N_2/CO_2 volume ratio have a significant impact on hydrogen yield and production. In the present study, to enhance current and hydrogen production of MECs, the effects of key operational conditions on the MEC performance were extensively investigated. The optimal operating condition for hydrogen production in MECs was determined as: the optimum applied voltage of 1.1 V, an anode surface area of 94 (cm^2), an anode-cathode distance of 1.5 (cm), and a N_2/CO_2 volume ratio of 4:1. With these optimum conditions, the maximum H_2 volume, current density and hydrogen production rate (HPR) of the MEC reached to 270.09 mL, 314.01 ± 2.81 A/m^3, and 4.25 ± 0.55 m^3 H_2 /m^3 d, respectively. The results obtained in this study imply that a systematic investigation of the key operational variables is an effective strategy to maximize the hydrogen production in single-chamber MECs.

1. Introduction

Fossil fuels including coal, oil, and natural gas are the major energy sources that are being used in the world today [1]. However, the burning of fossil fuels raises serious environmental issues and concerns such as global warming. Furthermore, fossil fuels are finite resources, the cost of which increases sharply as the availability decreases. This has triggered researchers in the energy sector to find carbon-neutral and renewable energy sources to replace fossil fuels. Hydrogen is an ideal and the clean fuel of the future

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which is considered as the most prospective energy carrier, but environmentally benign and commercially affordable production processes are needed. Currently, 96% of commercial H_2 produced comes from fossil fuels via steam reforming, thermo-chemical conversion (pyrolysis) and gasification [2], but results in massive emission of greenhouse gases (GHG) [3]. These processes deplete fossil fuels and consume high energy to produce hydrogen [4], and thus they are not considered a long-term sustainable method of H_2 production. The development of advanced technologies for producing H_2 from biomass and other renewable energy resources which reduce environmental problems is now given high priority. The microbial electrolysis cell (MEC) is an emerging "green" technology which has been recognized for its two prime functions a) biological wastewater treatment and b) value-added products: i.e. H_2, CH_4, ethanol, formic acid, and H_2O_2 [5, 6]. Hydrogen production in MECs represents a novel bio-electrochemical process which has been gaining momentum, the schematic diagram of a typical single-chamber MEC reactor is shown in Fig. 1. In an MEC, electrochemically active microorganisms grow on the anode surface and decompose the organic matter or wastes into carbon dioxide (CO_2), electrons (e^-), and protons (H^+) as a part of its metabolism. The bacteria transfer the electrons to the anode, while the protons are released directly into the MEC solution. An anode reaction is shown below using sodium acetate as an example:

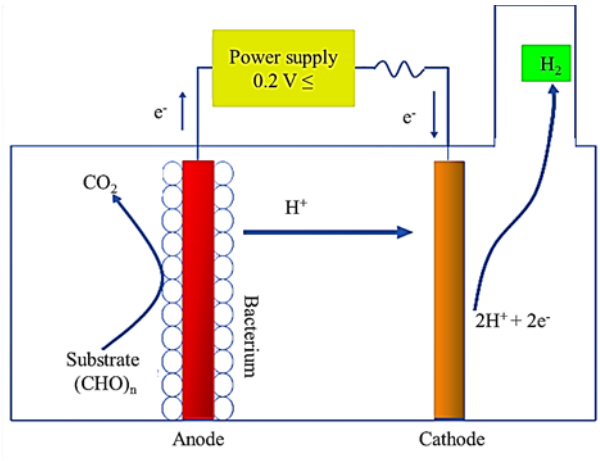

Fig. 1. The schematic diagram of a typical single-chamber MEC reactor.

$$CH_3COO^- + 4H_2O \rightarrow 2HCO^{3-} + 9H^+ + 8e^- \quad (1)$$

The electrons then travel through an electronic wire with the help of a power supply to a cathode and combine with the free H^+ in the solution to generate H_2 (Eq. 2).

$$8H^+ + 8e^- \rightarrow 4H_2 \quad (2)$$

However, this reaction does not occur spontaneously. In order to produce H_2 at the cathode of MEC from the combination of these H^+ and electrons, a cathode potential of at least > -0.414 V vs NHE (normal hydrogen electrode) is needed under standard biological conditions of pH:7, T=25°C, and P_{H2} = 1atm [7, 8]. This is done by the input of externally supplied voltage (≥ 0.2V) via a power supply. However, MECs require relatively low energy input (0.2-0.8 V) compared to typical water electrolysis (1.23-1.8 V).

The performance of MECs, such as hydrogen production rate (HPR) and current generation, have improved considerably in only a few years since their discovery, but the lower HPR is the main bottlenecks for its practical application. In order to improve performance of the MECs it is critical to elucidate the limiting factor for hydrogen production of MEC. One efficient approach to understand the limiting factor is to study the influence of the key operational parameters on the MEC performance. It is noted from the literature that most of the MECs were operated at applied voltages (E_{ap}) of 0.3-1.0 V [9-11]. Applied voltages >1.2V are not recommended because the electrical energy input is so large that the MEC becomes closer to a water electrolysis process. Additionally, it might inhibit the growth of micro-organisms. Although, hydrogen production was detected at E_{ap} = 0.2 V [9], applied voltages lower than 0.3 V may result in low HPR and erratic system performance [8, 12]. The applied voltage of ≥ 0.7 V was chosen because this range of applied voltage allowed for relatively fast cycle times compared to those obtained with lower applied voltages [13]. Besides the applied voltages,

there are a number of factors that might affect the performance of MEC including initial pH, temperature [14, 15], catholyte concentration [16], substrates and electrode surface area [8], microbial anode potential (MAP) [17], electrode spacing [13], electrolyte [16, 18, 19], and activated sludge concentration [20]. To achieve a high volumetric HPR the present study aimed to shed light on the effect of applied voltage, anode surface area, anode-cathode distance, and the volume ratio of N_2 and CO_2 on hydrogen production and current generation of pure culture MECs.

2. Materials and Methods

2.1. MEC reactor configuration

A single-chamber bottle type MEC with a high surface area of anode and shorten electrode spacing was designed and fabricated to investigate the effect of the key operational conditions on the hydrogen and current production of the MEC system. The MEC was fabricated with protect wide mouth graduated laboratory bottles (total volume, 750 mL) with a diameter of 10.1 (cm) and height of 15.2 (cm). The anodes of MEC were isomolded graphite plates with a thickness of 0.64 (cm) Grade GM-10 (GraphiteStore.com Inc., Illinois, USA). In order to remove the impurities on the anode surface all the anodes were polished using sandpaper (grit type 400), soaked in 1N HCl and NaOH solution for 2 hours, and stored in MilliQ water before use. The cathodes were type B carbon cloth containing 0.5 mg/cm^2 Pt catalyst (www.fuelcell.com, USA). The surface area of the cathodes was 78 cm^2 (6.5 cm long and 6 cm wide). The anode and cathode were held together with plastic screws spaced 15 mm apart. Titanium wire of 0.5 mm, (0.02 in) diameter, and 99.98% Metal basis (Alfa Aesar, USA) was used to connect the electrodes with the electrical circuit. An Ag/AgCl reference electrode (RE-5B; BASi) was placed in the reactor, and the anode potential was recorded using a multimeter.

2.2. Bacterial cultures and growth medium

Geobacter sulfurreducens PCA strain (ATCC 51573) used in this study was purchased from the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, USA. The stock culture was stored at -80ºC until ready to use. According to enclosed instructions from ATCC, the macronutrients solution contained the following (per litre): CH_3COONa (electron donor, 1.64 g), NH_4Cl (1.5 g), NaH_2PO_4 (0.6 g), KCl (0.1 g), $NaHCO_3$ (2.5 g), $Na_2C_4H_2O_4$ (electron acceptor, 8 g), and 10 mL solutions of vitamin and trace mineral each. The medium components were procured from Sigma-Aldrich, Malaysia. All the chemicals used in this study were of analytical grade and weighed by a Swiss made (XB 220A) analytical balance.

All the growth medium ingredients (except the vitamin and trace mineral solution or heat labile substrates such as $NaHCO_3$, sodium fumarate, and coenzyme M) were mixed and dissolved in distilled water, and this mixture was dispensed into the culture bottles. The sealed bottle was autoclaved at 15 psi, 121°C for 30 minutes. After autoclaving the medium was cooled to 60°C, then the trace mineral supplement and vitamin solution were aseptically added to the sterilized medium. Thereafter, the sodium fumarate was added from a syringe filter-sterilized and pure 99% nitrogen-sparged stock solution and $NaHCO_3$ was added from a sterile stock solution prepared under a N_2 and CO_2 (80%:20%) gas mixture. Finally, the medium was flushed with N_2/CO_2 (4:1, v/v) for 15 minutes to remove oxygen. All the stock medium solutions were kept at 4°C in a cold room until use. Resazurin, a blue dye, was included in the media (1 mg/L) to monitor the redox potential of the media. On reduction its colour changed from blue to pink and then from pink to colourless. The resazurin was reduced within 5 minutes after the addition of the reducing agent. The initial pH value of the medium was adjusted to 6.8-7.0 by adding a 1N NaOH or 1N HCL solution.

2.3. Inoculum preparation

The cells were initially revived and re-cultured from a frozen stock culture under anaerobic conditions. The batch cultivation was carried out in a 100 mL anaerobic serum bottle (VWR International, LLC, Radnor, Pennsylvania, USA) suggested by ATCC. The stock medium was transferred into serum bottles using a pipette (Capp ecopipette, C5000-1, Capp ApS, Odense, Denmark). To reduce the medium before inoculation, 1 mL of a reducing agent (e.g. 5% conenzyme M) was added into each 100 mL of the medium. The bottles were tightly sealed with a 1.5 mm thick butyl rubber septum and 13 mm aluminium crimp caps. The medium was flushed with a N_2/CO_2 gas mixture (4:1, v/v) for 15 min prior to inoculation to develop an anaerobic condition. Inoculum size of 10% (v/v) of Geobacter sulfurreducens PCA strain was transferred anaerobically in the late-exponential phase by using a sterile hypodermic disposable syringe and all incubations were done in a water bath (EWB-10, Protech, Malaysia) at a constant temperature of 30°C for 5~6 days. The growth of Geobacter sulfurreducens PCA was monitored by measuring an optical density (OD) at 680 nm using a spectrophotometer (DR-2800 HACH, USA).

2.4. MEC start-up and operation

Prior to the experiments, the bacterium used in the MECs (Geobacter sulfurreducens PCA) was pre-acclimated using 1g/L sodium acetate as carbon sources or electron donor. This procedure has been shown to reduce start-up time and improve subsequent performance [21]. The basic procedure for the acclimatization of Geobacter sulfurreducens PCA was that once the repeatable maximum current (I_{max}) was obtained for at least three batch cycles, the anode biofilms was considered matured enough [13] and vortex-mixed (Press-to-Mix 34524, Gemini BV, Netherlands) for 25s. Three to four days were needed for the acclimatization of Geobacter sulfurreducens PCA for each set of different experiments.

Before the inoculation, the MEC was sterilized with an autoclave at 121°C, 15 psi for 30 minutes, and then washed carefully in MilliQ water. Afterwards, the MEC was filled with stock medium, which had the same composition as the medium used for reviving stock culture and batch cultivation except that no electron acceptor (sodium fumarate, $Na_2C_4H_2O_4$). In the meantime, the MECs were purged with 80/20% (v/v) mixture of N_2 and CO_2 for 30 minutes. Thereafter, the MEC was inoculated with 35 mL (10%, v/v) late exponential phase (4 days) cultures of Geobacter sulfurreducens PCA in the medium solution (working volume of the MEC: 350 mL). After inoculating the MEC, a fixed applied voltage ranging from E_{ap} = 0.6 V to E_{ap} = 1.1 V by step of 0.1 V was employed to the MEC system in each fed-batch cycle. The voltage was added using a programmable power supply (M10-OPP3205, Shanghai MCP Corp. China) by connecting the positive pole of the power source to the anodes while the negative led to a high precision resistor (10 Ω) and the cathodes. The voltages (V) produced by the MEC across the external resistor (R_{ex}) were recorded every 20 minutes using a bench-top digital multimeter (MT8145, Shanghai MCP Corp., China) connected to a personal computer. The current (I) was calculated using Ohm's law (I = V/R, R_{ex} = 10 Ω). The MEC operation and growth of bacterium were monitored by measuring the current produced in MEC regularly (every 20 minutes). In order to eliminate the possibility of less substrate affecting the growth of Geobacter sulfurreducens PCA and the hydrogen production in MEC, fresh medium (100 mL) were added into the solutions in MEC using a sterile syringe without opening the MEC inlets. When the current was decreased to < 0.15 mA the substrate was about to be completely depleted [22]. The current of 0.15 mA was defined as the end point of a fed-batch cycle. The MECs were running at a constant temperature of 30ºC throughout all the experiments and the pressure was assumed equal to 1atm. The initial pH of the MEC electrolyte solutions was adjusted to 6.8.

2.5. Experimental set-up design

The setup MEC and experimental procedures for

this study are described in Fig. 2. A single-chamber membrane-less MEC (A) with graphite felt anodes was connected to an external power supply of 0.6-1.1 V. Biogas produced in the MEC were collected into an air tight gas collecting tank (B) filled with water (acidified 95% saturated NaCl, pH:0.5) via a silicone tube (C). Hydrogen and carbon dioxide, being insoluble in water, pushed it through another silicone tube (D) into a measuring cylinder (E). The volume of displaced water in the measuring cylinder was equal

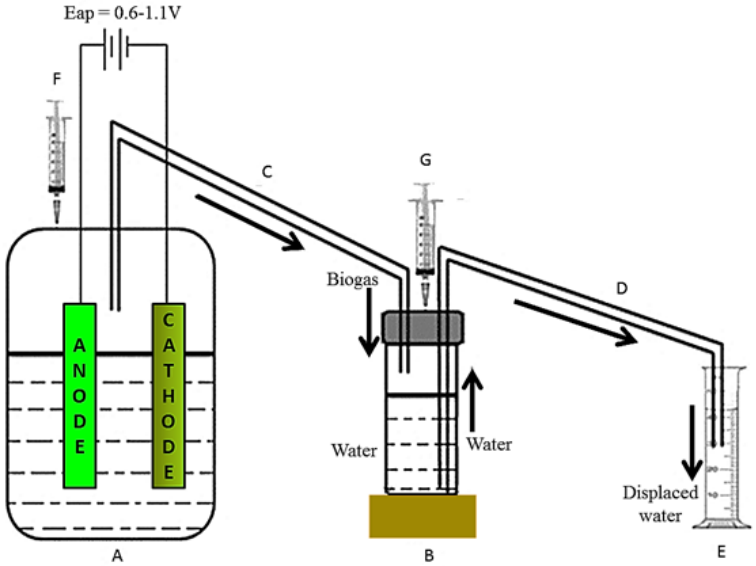

Fig. 2. Schematic of set up MEC and experimental procedures for this study (A) Single chambered membrane-less MEC (B) An air tight chamber filled water (acidified 95% saturated NaCl, pH:0.5) (C, D) Gas tight silicone tube (E) A measuring cylinder (F, G) sampling port.

to H_2 and CO_2 collected at the top of gas collecting tank B. Addition of fresh medium and gas sampling can be done from ports F and G, respectively.

2.6. Analytical methods and measurements

After each fed-batch cycle, the total volume of biogas produced in the MECs was measured using the water replacement method by connecting a gas tight gradual cylinder with the MEC with silicone tubing (Fig. 2). Gas sample bags (Tedlar Gas Sampling Bag, CEL Scientific Corp. CA, USA) were employed to collect the produced gas. During each fed-batch cycle, volumetric factional percentages of H_2, CH_4 and CO_2 were analyzed using a gas chromatograph (GC, model SRI 8600C, SRI Instruments, USA), equipped with a helium ionization detector (HID) and thermal conductivity detector (TCD). High purity helium (MOX 99.99%) gas was utilized as the carrier gas for the GC and run at a flow rate of 25 mL/min. Biogas was sampled regularly using a gas-tight syringe (250µL, Hamilton Samplelock Syringe, Hamilton Co., Reno, NV, USA) in duplicate from the MEC head space, and in triplicate for the gas sampling bags. The cumulative volume for a specific gas (Vi) such as H_2, CO_2 was calculated as below:

$$V_i = \left(V_t + V_h\right) * X_i \quad (3)$$

Where V_t is the measured gas volume (mL), V_h is the headspace volume of the reactor (mL) and gas collection tube at sample time (t), and X_i is the specific gas fraction (%). The MEC reactors and gas bags were sparged with Ultra High Purity (UHP) N_2 for 30 minutes to remove O_2 from the reactor. Gas bags were emptied using a vacuum pump (GAST DOA-P504-BN, USA) before being used.

The electric conductivity was measured by using a digital professional conductivity meter (HC3010, Trans Instruments (S) Pte Ltd, Singapore). The anode and cathode potentials of the MECs were measured using an Ag/AgCl reference electrode (RE-5B, BASi, USA) during each batch cycle. The initial and final pH

values were measured using a pH meter (827 pH Lab Meter, Metrohm, USA). Chemical oxygen demand (COD) analysis of the MEC solution was performed at the beginning and end of each fed-batch using a 0.2 μm filter and following a standard method (TNT plus COD Reagent, HR, 150 tests; HACH Company, USA) described by Logan et al. [20].

2. 7. Calculations

MEC performance was characterized using calculations as described previously in Refs. [9, 11, 23] except as noted. Detailed explanations of the calculation methods for all parameters are provided as below:

Hydrogen yield is the theoretical number of moles H_2 produced based on substrate usage (ΔCOD). The theoretical hydrogen yield is calculated in moles hydrogen produced, [$n_{th(H_2)}$] as follows:

$$n_{th(H_2)} = \frac{b_{H_2}/s V_{MEC} \Delta COD}{M_s} \quad (4)$$

Where [b_{H_2}/s] is the maximum stoichiometric production of hydrogen based when the substrate is 4 mole H_2/mole acetate or the number of moles of electrons produced per mole of substrate (8 mole e^-/ mole acetate), V_{MEC} is the MEC operation volume or the volume of liquid (m^3) in the reactor (0.5 L), ΔCOD (g-COD/L) is the change in substrate concentration over a batch cycle, and M_s is the substrate (sodium acetate) molecular weight ($M_{CH3COONa}$ = 82g/mole). The COD removal (K) was calculated as follows:

$$\Delta COD = \frac{COD_f - COD_{in}}{COD_{in}} \times 100\% \quad (5)$$

Where COD_{in} was the initial COD concentration of the electrolyte; COD_f was the final COD concentration of the effluent after each batch cycle. The number of moles of hydrogen that can be recovered based on the current produced over one batch n_{CE} can be calculated by:

$$n_{c_E} = \frac{\int_{t_0}^{t_F} Idt}{2F} \quad (6)$$

Where t_0 and t_F are the initial and final times of the batch experiment. The current (amps; A) was calculated according to Ohm's law ($I = V/R_{ex}$), where V is the measured voltage and R_{ex} is the external resistance (10Ω), dt is the time interval between two data collection points (in this case, 15 minutes), 2 is the number of mole of electrons used per mole of hydrogen produced, and F=96,485 Coulombs/mole electron is the Faraday's constant. The coulombic hydrogen recovery [r_{C_E}] is the same as the coulombic efficiency [C_E], the number of electrons or H_2 recovered in the circuit over the number of electrons or H_2 theoretically available from the substrate, and is calculated by:

$$r_{C_E} = C_E = \frac{n_{c_E}}{n_{th(H_2)}} \times 100\% \quad (7)$$

The cathodic hydrogen recovery [$r_{cat(H_2)}$] was calculated by:

$$r_{cat(H_2)} = \frac{n_{H_2}}{n_{C_E}} \times 100\% \quad (8)$$

Where n_{H_2} is the number of moles of hydrogen produced by the system during each fed-batch cycle. The hydrogen recovery [r_{H_2}] is a significant index for MEC performance, which was defined as the ratio of the hydrogen recovered [n_{H_2}] and the maximum theoretical hydrogen produced based on substrate utilization [$n_{th(H_2)}$]:

$$r_{H_2} = r_{C_E} \times r_{cat(H_2)} = \frac{n_{H_2}}{n_{th(H_2)}} \times 100\% \quad (9)$$

The maximum volumetric hydrogen production rate, Q in [m^3 H_2/ m^3d] is calculated as:

$$Q_{H_2} = 3.68 \times 10^{-5} I_V Tr_{cat(H_2)} \quad (10)$$

Where I_V is the current density (CD) (A/m^2 or A/m^3), it was the current produced in the batch cycle per unit

membrane surface area or unit liquid volume ($I_v = I_{max}/V = U_{max}/R_{ex}V$ is the volumetric current density, and Umax is the maximum voltage averaged over stable phase). 3.68×10^{-5} is the constant that includes Faraday's constant, 1 atm of pressure and unit conversions. The temperature (T) is in Kelvin and [$r_{cat(H_2)}$] is the cathodic hydrogen recovery.

3. Results and Discussion

It has been reported that volumetric HPR and current density can be used to evaluate the performance of the MEC system [9, 23]. In order to identify the most suitable experimental conditions for a single-chamber MEC system, three main parameters in MECs: current density (I_V), H_2 volume (V_{H2}), and HPR were used to evaluate the performance of the MEC. All the experiments were carried out in triplicate and mean values ± SD of triplicate experiments were reported.

3.1. Influence of applied voltages (E_{ap})

In the laboratory-scale of MEC, the extra voltage is generally applied by a DC power supply. To explore the effect of applied voltage on the MEC performance, different applied voltages (0.6 V ≤ E_{ap} ≤ 1.1 V) were employed in each cycle. At each applied voltage, at least three cycle experiments were carried out before switching to another applied voltage. The data presented are the average values ± SD of triplicate experiments. As shown in Fig. 3, the applied voltages exhibited a clear influence on hydrogen production in MECs. As can be seen, there was a constant increase in HPR from 1.55 ± 0.13 m^3 H_2/m^3 d to 3.67 ± 0.55 m^3 H_2/m^3 d at the applied voltage of 0.6 V to 1.1 V. Correspondingly, volumetric current density and H_2 volume were also increased with applied voltage from E_{ap} = 0.6 to E_{ap} = 1.1 V. The highest HPR (3.67 ± 0.55 m^3 H_2/m^3 d), volumetric current density (293.73 ± 2.81 A/m^3), and H_2 volume (251.53 ± 3.37 mL) were achieved at an applied voltage of 1.1 V. These results may be due to the fact that at the higher applied voltages, the rate of electrons production is higher

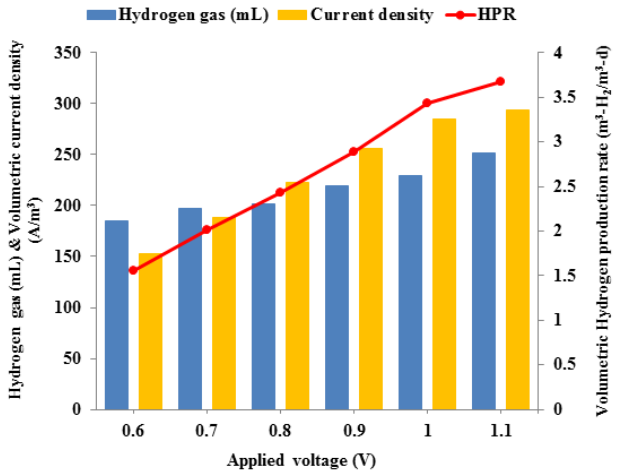

Fig. 3. Effect of applied voltages on hydrogen production by G. sulfurreducens PCA strain in single-chamber MEC (surface area of anode: 80.72 cm^2, electrode distance: 2 cm, N_2:CO_2 of 4:1 were kept constant).

[24].

All aforementioned observations suggested that applied voltages exerted considerable influences on the performance of the MECs in terms of HPR, current density, and H_2 volume. It was concluded that the most suitable applied voltage for enhancing hydrogen production in the MEC was 1.1 V.

3.2. Influence of anode surface area (cm^2)

The surface area of the anode is critical, as it is responsible for the generation of electrons. Hence, in this study the effect of anode surface area on MEC performance was investigated. In this experiment, the surface area of the anode was consecutive enlarged in the range of 37.6 to 94 (cm^2), while the surface area of the cathode was kept constant at 78 (cm^2). As shown in Fig. 4, the surface area of the anode had asignificant impact on the H_2 generation in MECs. The HPR, current density, and H_2 volume increased linearly with increases in the surface area of the anode. The highest current density reached 300.57 ± 12 A/m^3 and the corresponding HPR was 3.96 ± 0.11 m^3 H_2/m^3 d at E_{ap} = 1.1 V. Another important and interesting observation was that there was a sharp increase in HPR and current density between the anode surface

area of 68.44 and 80.72 (cm^2). All the parameters reached a peak at the anode surface area of 94 (cm^2). A possible explanation is that the high surface area of the anode facilitates the biofilm formation, which was exposed to a larger surface area to adhere and transfer large number of electrons to the anode, thus increasing the current density. It is known that a high current density implies a high potential of the MEC system to achieve high HPR [23].

The results obtained in this study were in line with previous studies [25-28]. It was reported the bacteria in the biofilm are responsible for electron generation and its transfer to the anode of the MEC [25-27]. Biofilms on the anode have been demonstrated to increase the current due to the direct transfer of electrons to the anode [28]. The results obtained with variation of anode surface area revealed that providing a large surface area for bacterial growth at the anode would be a key parameter to enhance the HPR in MECs.

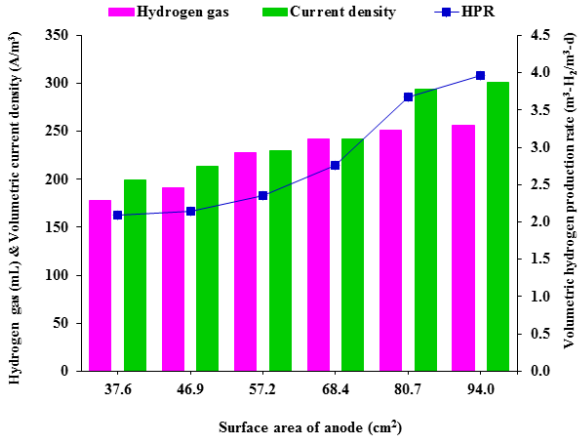

Fig. 4. Effect of anode surface area on hydrogen production by G. sulfurreducens PCA strain in single-chamber MEC (E_{ap} = 1.1 V, electrode distance: 2 cm, N_2:CO_2 of 4:1 were kept constant).

3.3. Influence of anode-cathode distances (cm)

The inter-electrode distance (from anode canter to cathode) is a key parameter for enhancing HPR in MECs. In order to study the effect of average anode-cathode distance on hydrogen production in MECs, a range of electrode distance, including 0.5, 1, 1.5, 2, 2.5, and 3 (cm) were tested. Fig. 5 depicts the performance of the MEC with different electrode distances. As can be seen in Fig. 5, the HPR, current density, and H_2 volume rapidly increased as the electrode distance decreased from 3 to 1.5 (cm) at E_{ap} = 1.1 V. Whereas, a further reduction of electrode distance from 1.5 to 0.5 (cm) decreased the HPR, current density, and H_2 volume remarkably. The highest HPR of 4.11 ± 0.47 m^3 H_2 /m^3 d was observed when the average electrode distance was 1.5 cm at E_{ap}=1.1 V. The corresponding current density and H_2 volume were 308.32 ±3.02 A/m^3 and 262.13 ±4.3 mL, respectively. As is evident, the volumetric current density and HPR underwent a sharp decrease when the electrode distance was decreased in a certain range

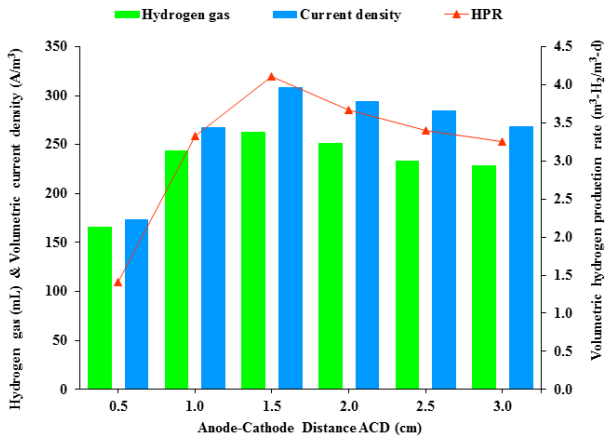

Fig. 5. Effect of electrode distance on hydrogen production by G. sulfurreducens PCA strain in single-chamber MEC (E_{ap} = 1.1 V, surface area of anode: 94 cm^2, N_2:CO_2 of 4:1 were kept constant).

of electrode distances: 3.32 ±0.54 m^3 H_2 /m^3 d with 0.5 (cm) and 1.41 ± 0.77 m^3 H_2 /m^3 d with 1 (cm). For electrochemical reactions, reducing the electrode distances should reduce ohmic resistance and increase current. However, the HPR, the current density, and H_2 volume did not increase when the electrode distance was reduced from 1.5 to 0.5 (cm). The reason for this was likely insufficient surface area on the anode for bacteria attachment because of the too shorten electrode space. It was observed that reducing the electrode distance was not as important as maintaining high anode surface area for bacteria in an MEC. This

finding is in agreement with previous findings reported by Cheng et al. [29]. It has been proven that the Geobacter sulfurreducens PCA strain was able to produce high cumulative H_2 and current with the most efficient electrode distance of 1.5 (cm). The most suitable electrode distance for hydrogen production in MEC might be affected by the type of the MEC designs, and electrode materials used.

3.4. Influence of N_2/CO_2 volume ratio

The effect of N_2/CO_2 volume ratio on hydrogen production in MECs was investigated using various proportions of N_2 and CO_2 gas (pure N_2, 1:4, 4:1, pure CO_2). From Fig. 6, it becomes clear that the HPR was varied from 2.74 ± 0.37 m^3-H_2/m^3-d with pure CO_2 to 4.25 ± 0.58 m^3 H_2 /m^3 d with proportion of 4:1 (N_2:CO_2). The highest HPR, current density, and H_2 volume observed in the present study were 4.25 ± 0.55 m^3 H_2 /m^3 d, 314.01 ± 2.81 A/m^3, and 270.09 mL

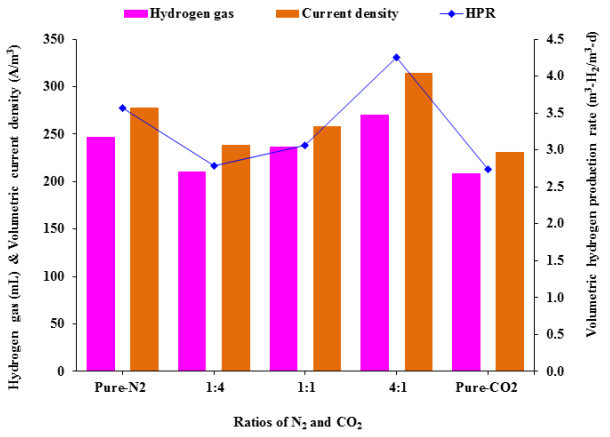

Fig. 6. Effect of volume ratios of N_2 and CO_2 mixture gas on hydrogen production by G. sulfurreducens PCA strain in single-chamber MEC (E_{ap} = 1.1 V, surface area of anode: 94cm², and electrode distance: 1.5 cm were kept constant).

respectively. It was found that HPR, current density, and H_2 volume increased significantly with pure N_2. In contrast, pure CO_2 was inhibitory for hydrogen production by Geobacter sulfurreducens PCA strain in MEC. The HPR of 2.74 ± 0.19 m^3 H_2 /m^3 d, current density of 230.55 ± 1.43 A/m^3, and H_2 volume of 208.63 ± 3.67 mL were only achieved with pure CO_2. The results here demonstrate that the N_2/CO_2 volume ratio significantly affected the hydrogen production efficiency in MECs. This finding might be explained by the following phenomena. In a sodium bicarbonate buffering system, carbon dioxide (CO_2) combines with water (H_2O) to form carbonic acid (H_2CO_3), which in turn rapidly dissociates to form hydrogen ions (H^+) and bicarbonate (HCO^{3-}) as shown in the reactions below:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO^{3-} + H^+ \quad (11)$$

The pressure increase during the anoxic gas purge elevated the concentration of HCO^{3-} and H^+ ions in the electrolyte solution and resulted in the increase of pH and internal resistance. It is important to note that the pH value of the electrolyte solution is an imperative factor in the hydrogen production process in MECs, and decisively influences the activity of enzymes involved in the metabolic pathway of hydrogen generation and the growth of exoelectrogenic bacteria. As a consequence volumetric current density and hydrogen production rate were decreased. Similarly, Gadhe et al. [30] reported that a high pH value of the culture medium lessen the activity of the hydrogenase enzyme by changing the metabolic pathway from acidogenesis to solventogenesis resulting in low hydrogen production. Furthermore, numerous reports suggest that H_2 partial pressure is an extremely important factor for continuous hydrogen synthesis [31, 32]. When the pH value is controlled at 6.5-7.0 the anode biomembrane works normally; whereas, when the pH value is below 6.0 the H_2 production is decreased significantly [17]. Moreover, an experimental investigation carried out by Kyazze et al. [14] revealed that the pH value affects the H_2 production by restricting the applied voltage. As the applied voltage is kept at 0.6 V with pH of 5.0, the MEC could obtain higher HPR than both the pH values of 7.0 and 9.0. Therefore, pH control is a key factor for H_2 production in MECs. The results of the present study point out that the most suitable volume ratio of N_2 and CO_2 for hydrogen production by Geobacter

sulfurreducens PCA strain was 4:1.

4. Conclusions

An enhancement of the MEC performance was observed by investigating the influence of key process variables such as applied voltage, anode surface area, anode-cathode distance, and N_2/CO_2 volume ratio on current and hydrogen production in MECs. The observed experimental results showed that the most suitable experimental conditions for hydrogen production in single-chamber MECs are: applied voltage of 1.1 V, anode surface area of 94 (cm^2), electrode distance of 1.5 (cm) and N_2/CO_2 volume ratio of 4:1. Furthermore, the H_2 volume, current density and hydrogen production rate of the MEC were greatly improved under those optimum conditions, reaching 270.09 mL, 314.01 ± 2.81 A/m^3, and 4.25 ± 0.55 m^3 H_2 /m^3 d, respectively.

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Nomenclature

A_{an}	Surface area of the anode (cm^2)
b_{H_2}/s	The number of moles of electrons produced per mol of substrate (8 mol e^-/mol acetate)
COD_0	Initial COD concentration of the electrolyte (mg/L)
ΔCOD	COD removal efficiency (%)
C_E	The coulombic efficiency (%)
dt	The time interval between two data collection points (S)
e^-	Electron (e)
E_{ap}	Applied voltage (V)
F	Faraday's constant (96,485 Coulombs/ mole electron)
H^+	Proton
I_{max}	Maximum current (A)
I_V	The volumetric current density (A/ m^3)
$n_{th(H_2)}$	Theoretical number of moles H_2 produced based on substrate (mol)
n_{H_2}	The number of moles of H_2 produced during a batch cycle (mol)
M_S	Substrate molar weight (g/mol)
Pt/CC	Carbon cloth containing 0.5mg Pt/cm^2 cathode
Q_{H_2}	Volumetric hydrogen production rate (m^3 H_2 /m^3 d)
R	The universal gas constant (8.314 J/K/mol)
R_{ex}	External resistance (10Ω)
R_{in}	Internal resistance (Ω)
$r_{cat(H_2)}$	The cathodic hydrogen recovery (%)
r_{H_2}	The hydrogen recovery (%)
T	The absolute temperature (K)
t, t_0 and t_F	Time/Initial and final times of the batch experiments (S)
U_{max}	The measured voltage (V)
V_i	The cumulative volume for a specific gas (such as H_2, CO_2) (mL)
V_t	Measured gas volume (mL)
V_h	The headspace volume of the reactor (mL)
X_i	The specific gas fraction (%)

Acronyms

ATCC	American Type Culture Collection
COD	Chemical oxygen demand (mg/L)
GC	Gas chromatography
HID	Helium ionization detector
HPR	Hydrogen production rate (m^3 H_2 /m^3 d)
MAP	Microbial anode potential
MEC	Microbial electrolysis cell

NHE	Normal hydrogen electrode
OD	Optical density
PBS	Phosphate buffer solution
SD	Standard deviation
TCD	Thermal conductivity detector

Subscript

an	Anode
S	Substrate
in	Internal
ex	External
ap	Applied voltage (V)
max	Maximum
th(H_2)	Theoretical number of moles H_2
cat	Cathode
0/F	Initial/Final
i	The specific gas
h	Head space

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