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A NEW DESIGN ENHANCES HYDROGEN PRODUCTION BY *G. SULFURREDUCENS PCA* STRAIN IN A SINGLE-CHAMBER MICROBIAL ELECTROLYSIS CELL (MEC)

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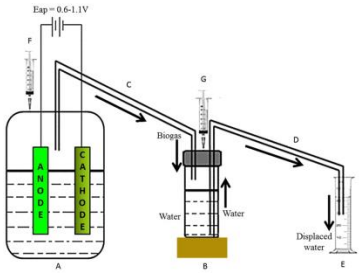
Graphical abstract



Abstract

Microbial electrolysis cell (MEC) is an innovative and green technology to generate hydrogen from a wide range of renewable energy sources and wastewater. At current stage, the performance of these systems is still far from real-world applications. The most likely limiting factors for successful commercialization of this technology are the large internal resistance, high fabrication and operational costs. The aim of the present study was to enhance hydrogen production, reduce the construction and operational costs in MECs via development of a novel MEC design. A single-chamber membrane-free MEC was designed and successfully produced hydrogen from organic substrate using a pure culture: *Geobacter sulfurreducens PCA*. The MEC system was operated with Platinum (*Pt*) cathode at applied voltage range of 0.6 V to 1.1 V. *Geobacter sulfurreducens PCA* strain and sodium acetate used as inoculum and a fuel sources, respectively. The conductivity of electrolyte solution in the MEC was 4.5 mS/cm. Due to an improved the MEC reactor architecture, the maximum hydrogen production rate (HPR) of 3.67 ± 0.03 m^3 H_2/m^3 d with volumetric current density (I_V) of 293.73 ± 1.18 A/m^3 was achieved under an external applied voltage (E_{ap}): 1.1 V. The highest overall hydrogen recovery (r_{H_2}) and overall energy efficiency (η_{E+S}) were 91.80 ± 1.06% and 66.97 ± 0.09%, respectively.

Keywords: Microbial electrolysis cell (MEC); *G. sulfurreducens* PCA strain; hydrogen production rate (HPR); applied voltage (E_{ap}); hydrogen recovery

1.0 INTRODUCTION

The climate changes and energy crisis are two of the most important issues in today's our modern world. Nowadays, fossil fuels (FFs) such as oil, coal, and natural gas account more than 86% of total world energy consumption, and transportation is completely reliant (95%) on oil [1]. However, there are some drawbacks of using FFs. Firstly, the FFs are limited resources that do not renew themself, this means they will eventually run out. An inconvenient truth is that proven petroleum reserves are projected to be depleted in less than 50 years at the current energy consumption rates [2, 3]. Moreover, the combustion of FFs is directly or indirectly related to global warming as carbon dioxide is the main compositions greenhouse gases (GHGs) [4, 5]. This phenomenon has led the scientists or engineers in energy sector to find a renewable and considered to be environmentally friendly and sustainable energy sources to replace the FFs [6].

In the recent years, hydrogen gas is considered perhaps the best candidate as an alternative energy carrier and source to FFs. H_2 has several advantages over other alternative energy sources. Firstly, burning H_2 does not link to GHG emissions, because the product of H_2 combustion is only water and no other byproducts [7-9]. Furthermore, it has the highest energy density per unit weight among the known gaseous fuels. H_2: 120-142 MJ/kg, CH_4: 50 MJ/kg or ethanol: 26.8 MJ/kg and 44 MJ/kg for gasoline [10-12]. Lastly, H_2 can be derived from a wide range of renewable biomass and wastewaters. So, it can be inexpensive, renewable and sustainable [13, 14].

Microbial electrolysis cell (MEC) is an emerging and promising biochemical tool for H_2 production from various types of organic matter, including wastewater and other biomass-based resources [15]. In an MEC, electrochemically-active bacteria (EAB) are employed to decompose substrate into carbon dioxide, electrons (e^-), and protons (H^+). The microorganisms transfer the e^- to the electron acceptor (anode) and the H^+ are released directly into the MEC electrolyte solutions. The e^- then travel with the help of external power sources to the cathode, and combine with the free H^+ in electrolyte generates hydrogen gas [16]. However, this reaction doesn't take place spontaneously. To generate H_2 at the cathode of MECs, a cathode potential of at least > -0.414V vs NHE is required. Anode, cathode, and overall reactions are written as below when sodium acetate used as a substrate:

Anode half reaction:

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

Cathode half reaction:

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

Total reaction in an MEC:

$$CH_3COO^- + 4H_2O \rightarrow 2HCO^{3-} + H^+ + 4H_2 \quad (3)$$

In comparison to other H_2 production processes, the MEC has some major advantages. Firstly, low energy consumption over water electrolysis (WE) [17]. Secondly, no expensive metals were required on the anode of the MEC system, due to it has self-sustaining microbial biocatalysts. Thirdly, the MEC has multiple superiorities over the dark fermentation (DF), such as higher hydrogen recovery [18], and more diverse substrate can be used [15, 19]. Lastly, the higher purity of H_2 is produced and therefore expensive gas purification process is not required [20, 21]. The overall performance of MECs may be affected by several process parameters: (i) microbiological factors, such as the type and source of inoculums used in MECs [22, 23]. (ii) Anode, cathode, membrane materials, and their properties; different electrode materials contribute differently in internal resistance [18, 21, 24]. (iii) External and internal resistances [25, 26]. (iv) MEC Electrolyte solution [27-29]. (v) Electron donors or substrate type, concentration and feeding rate [15]. (vi) Mode of operation; batch mode, fed-batch or continuous flow modes [21, 24, 30]. (vii) Anode surface area [18, 31]. (viii) Reactor architecture [32-35]. Among all abovementioned effective parameters on performance of MEC, the reactor design having huge impact on operational cost and hydrogen production rate (HPR) of MECs. The main goal of this work was to enhance hydrogen production, while reduce the construction and operation costs of a MEC through development of an innovative MEC design.

2.0 METHODOLOGY

2.1 MEC Configuration

A single-chamber membrane-free MEC was fabricated with a graduated Duran laboratory glass bottle (working volume: 350 mL), with 10.1 cm of Diameter and 15.2 cm of Height. Photograph of an MEC and it's components used in this study are shown in Figure 1.

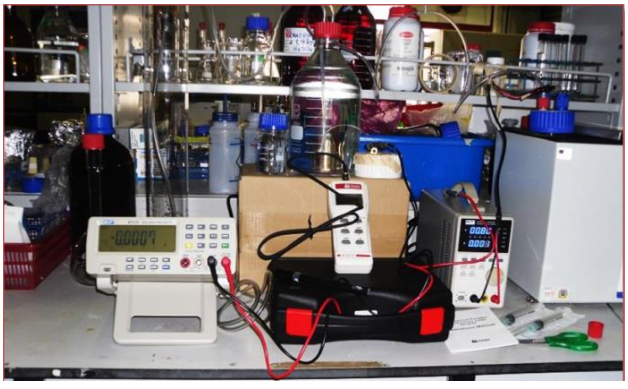


Figure 1 Photograph of MEC system used in this study, with power sources, benchtop multi-meter, water bath, water displacement gas collecting system, conductivity meter

The anode of MEC was graphite plates (GP) with a thickness; 0.64 cm, (GM-10, GraphiteStore.com Inc., Illinois, USA). For removing the impurities on the anode surface, all the anodes were polished via sandpaper, and soaked in 1N HCl and 1N NaOH for at least 2 hours. The cathode of MECs was type B carbon cloth containing 0.5 mg/cm^2 Pt catalyst (CC/Pt) (www.fuelcell.com, USA). The projected surface area of cathode was 78 cm^2. The anode and cathode were held and connected together via plastic screws with distanced 1.5 cm apart. Titanium wire (Alfa Aesar, USA) was utilized to connect the anode and cathode to the electrical circuit in the MEC system.

2.2 Enrichment of Microorganism

The cells used in this study were initially re-cultured from the frozen stock culture (ATCC 51573) under strictly anaerobic conditions. The batch cultivation of the cells was performed in a anaerobic serum bottle (100 mL, VWR International, Pennsylvania, USA) suggested by the ATCC. The prepared medium was transferred into the serum bottles via a pipette, and flushed with a N_2 and CO_2 gas mixture (80%:20%, v/v) for 15 min to make anaerobic condition before inoculation process. The culture bottles were tightly sealed. Inoculum size of 10% (v/v) of *cell* was transferred into culture bottles anaerobically in the late-exponential phase with a sterilized syringe. All the incubations were done in a water bath (EWB-10, Protech, Malaysia) at a maintained temperature of 30°C for 5~6 days (ATCC instruction). The growth of *G. sulfurreducens* PCA was monitored and determined through measuring an optical density (OD) at wavelength of 680 nm via spectrophotometer (DR-2800 HACH, USA).

2.3 Experimental Set-up and MEC Operation

The bacterium used in the MECs was pre-acclimated. Based on literature review, it has been shown that pre-acclimation of EAB to reduce start-up time, and improve subsequent performance of MECs [36]. The main procedure for the acclimatization of *G. sulfurreducens* PCA is that once the repeatable and stable maximum voltage or current was obtained for at least three batch cycles, then the anode biofilms were regarded matured enough to inoculate an MEC[6]. Before the MEC inoculation, the MEC systems were sterilized by autoclave at the temperature of 121°C, P =15 ps for 45 minutes. The MECs were filled with stock medium, which had the exactly same chemical ingredients as the medium used for batch cultivation except that exclude the electron acceptor; Sodium fumarate ($Na_2C_4H_2O_4$). Meantime, the MECs were flushed with 4/1 (v/v) of N_2 and CO_2 for at least 30 minutes. Thereafter, the MECs were inoculated with 35 mL (10%, v/v) of late exponential phase (4th days) cultures of *G. sulfurreducens* PCA.

After inoculating the MECs, an external applied voltage (E_{ap}) range of 0.6 V ≤ Eap ≤ 1.1 V by step of 0.1 V was supplied to the MEC system in each fed-batch cycle experiment. The E_{ap} was employed via an external power source (M10-OPP3205, Shanghai MCP Corp. China) through connecting the positive pole of the power supply to the anode and negative pole to an external resistor (R_{ex}) of 10 Ω and the cathode electrode. The system voltages (*V*) produced by a MEC system across the R_{ex} were recorded every 20 minutes time interval via a bench-top professional digital multimeter (MT8145, Shanghai MCP Corp. China). The current was calculated according to the Ohm's law ($I = V/R_{ex}$).

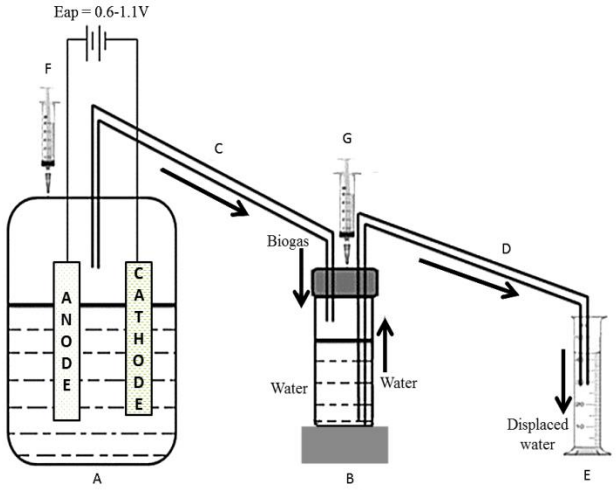


Figure 2 Schematic images of an MEC set-up and experimental procedures performed in current study

The MEC system operation and microbial activity of *G. sulfurreducens* PCA were monitored and inspected through measurement of the current produced (*I*) in the MEC system regularly. To eliminate the possibility of less fuel sources or substrate influencing the growth activity of *biofilm* and the performance of MECs, the 100 mL of fresh medium were transferred into the MEC systems with a syringe without exposing the MEC system medium inlets. The meaning of current was decreased to < 0.15 mA (0.00015 A) is that the carbon sources was about to be fully depleted [37]. The current of 0.00015 A was as an indicator for the end point of a fed-batch cycle experiment. Throughout all the experiments, the MEC systems were running at the maintained temperature; 30°C. The initial pH of electrolyte solutions in MECs was adjusted to 6.80. The MEC system set-up and experimental procedures for the present study are described in Figure 2. (*A*) Single-chamber MEC was connected to an external power sources (E_{ap} = 0.6 V to 1.1 V). Gas produced in the MEC system gets collected into an air-tight gas collecting container (*B*) filled with 95% saturated NaCl, pH = 0.5 through silicone tube (*C*). Produced H_2 and CO_2 being insoluble or less soluble in H_2O pushes it through another silicone tube (*D*) into a graduated measuring cylinder (*E*). The total volume or mass of displaced solutions in the graduated measuring cylinder is equal to H_2 and CO_2 collected on top of gas collecting tank or container (*B*). Feeding of fresh medium and gas sampling was performed from ports *F* and *G*, respectively.

2.4 Analytical and Measurements

The total volume of gas produced in the MECs was measured and determined through liquid or water displacement method (Figure 2). Gas sampling bags (GSB) (Tedlar GSB, CEL Scientific Corporation, USA) were used to collect produced gas sample. Volumetric factional percentages of H_2, CH_4 and CO_2 were determined by using a gas chromatograph (SRI 8600C, SRI Instruments, USA), which equipped with the helium ionization detector (HID) & the thermal conductivity detector (TCD). All biogases were sampled using a gas-tight syringe (250µL, Syringe, Hamilton Co, USA) regularly, in duplicate from head space of the MEC, and in triplicate from the GSBs. The volume of a specific gas (V_n); H_2, CO_2 was calculated as following:

$$V_n = (V_t + V_{head}) * X_n \quad (4)$$

Where V_t is released gas volume (mL) and V_{head} is the volume of the MEC headspace (mL) and gas collection tube, t is sample time, and X_n is the specific gas percentages known by GC (%).

The conductivity of electrolyte was measured via a digital conductivity meter (HC3010, Singapore). The anode and cathode potentials in the MEC system were determined via a Ag/AgCl reference electrode (RE-5B, BASi, USA) during each batch cycle. The initial and final pHs of electrolyte solutions in the MECs were determined by using a pH meter (827 pH Lab Meter, USA). The chemical oxygen demand (COD) analysis was performed at the beginning and end of each cycle experiment by using a 0.2µm filter and according to a standard method (HACH Company, USA).

2.5 Calculations

The overall performances of the MEC system were characterized in the following terms and formulas as previously described [5, 18, 31, 38, 39].

2.5.1 Hydrogen Yield and H_2 Production Rate

Hydrogen yield (Y_{H2}) is the theoretical number of moles H_2 produced based on substrate or carbon sources utilization (ΔCOD).

$$Y_{H2} = n_{th(H_2)} = \frac{\frac{b_{H_2}}{s} V_{MEC}\, \Delta COD}{M_s} \quad (5)$$

Where b_{H_2}/s is the maximum stoichiometric production of hydrogen for each the substrate or the number of moles of e^- produced each mol of substrate, V_{MEC} is the working volume the MEC reactor (m^3), ΔCOD is the change in fuel source concentration over a batch cycle experiment, and M_s is molecular weight of the fuel source. The COD removal efficiency (%) can be calculated as following formula:

$$\Delta COD = \frac{COD_f - COD_{in}}{COD_{in}} \times 100\% \quad (6)$$

Where COD_{in} is the initial COD concentration of the electrolyte solutions, COD_f is the final COD concentration. The number of moles of H_2 that can be recovered based on the current produced in one batch cycle n_{CE} calculated as follow:

$$n_{C_E} = \frac{\int_{t_0}^{t_F} I\, dt}{2F} \quad (7)$$

Where t_0 is the initial, t_F is final times of a batch cycle experiment. the current (I) was calculated using Ohm's law (I =V/Rex), where V is the measured voltage, Rex is external resistance, *dt* is the time interval between two data collection points, F is Faraday's constant (96,485 C/m e)

The coulombic hydrogen recovery (r_{C_E}) or the coulombic efficiency (C_E) is the number of electrons or H_2 recovered in the whole electrical circuit over the number of electrons or H_2 theoretically available from carbon source, and it can be calculated as follow:

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

The cathodic hydrogen recovery $r_{cat(H_2)}$ can be calculated by:

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

Where n_{H_2} is the mole (n) of hydrogen produced by the system during each cycles. The hydrogen recovery (r_{H_2}) is defined as the ratio of the hydrogen recovered (n_{H_2}) and ($n_{th(H_2)}$):

$$r_{H_2} = r_{C_E} \times r_{cat\,(H_2)} = \frac{n_{H_2}}{n_{th(H_2)}} \times 100\% \quad (10)$$

The maximum volumetric hydrogen production rate (HPR), (Q_{H_2}, m^3 H_2/ m^3 d) can be calculated as below:

$$Q_{H_2} = 3.68 \times 10^{-5} I_V T\, r_{cat} \quad (11)$$

Where I_V is the volumetric current density (A/ m^3), it is obtained through averaging the top 10 current densities. The 3.68×10^{-5} is the constant. *T* is temperature in Kelvin. r_{cat} is the cathodic hydrogen recovery.

2.5.2 Energy Recovery and Efficiency of the MEC system

The electrical energy (W_E) that contributed to the MEC system by the power supply, accounting for losses in the R as following:

$$W_E = \sum_n^1 (I E_{ap}\, \Delta t - I^2 R_{ex} \Delta t) \quad (12)$$

where I is the current (mA or A) calculated, E_{ap} is the external voltage applied (V) via the power supply, Δt is the time increment (s) for n data points measured during each cycle. The amount of energy contributed by the substrate or carbon source (W_s) can be determined as follow:

$$W_s = -\Delta H_s * n_s \quad (13)$$

Where ΔH_s is the heat combustion of the substrate or fuel sources and the number of moles of substrate consumed in each batch cycles (n_s):

$$n_s = \frac{\Delta COD\, V_{MEC}}{M_s \times 0.78 gCOD.g^{-1}} \quad (14)$$

Where M_s= 82 g $mol^{-1}$is molecular weight of substrate and 0.78 g COD/g is the conversion factor of CH_3COONa (64/82.4). The amount of energy recovered as hydrogen (W_{H_2}) calculated by:

$$W_{H_2} = \Delta H_{H_2} * n_{H_2} \quad (15)$$

Where ΔH_{H_2} is the heat combustion of hydrogen gas (285.84 kJ/mol). The energy efficiency based on electrical input (η_E) can be calculated as follows:

$$\eta_E = \frac{W_{H_2}}{W_E} \times 100\% \quad (16)$$

The energy efficiency based on utilized substrate or carbon source (η_s) can be calculated as follow:

$$\eta_S = \frac{W_{H_2}}{W_s} \times 100\% \quad (17)$$

The overall energy efficiency (η_{E+S}) which takes into account both the electrical and the substrate energy input can be calculated by formula:

$$\eta_{E+S} = \frac{W_{H_2}}{W_E + W_s} \times 100\% \quad (18)$$

The percentages or fractions of total energy added by the power source or electricity (e_E) as following:

$$e_E = \frac{W_E}{W_E + W_s} \times 100\% \quad (19)$$

And the percentages or fractions of total energy provided by substrate or fuel sources (e_S) is determined as follow:

$$e_S = \frac{W_S}{W_E + W_s} \times 100\% \quad (20)$$

3.0 RESULTS AND DISCUSSION

Characterization of H_2 production in a newly developed single-chamber MEC was performed with Pt cathode under various applied voltages (0.6–1.1 V). Theoretically, the voltage needed for the MEC based on sodium acetate is ~0.114 V compared to voltage 1.23 V for WE [32], but in practice voltage > 0.2 V is needed for the MEC, with > 0.5 V typically applied [40-42], while avoiding high voltages that could result water electrolysis. That was the reason why the performance of new MEC system was evaluated with E_{ap} of 0.6 V to 1.1 V. At each range of applied voltages, at least three cycle experiments were conducted before switching to another E_{ap}. The experimental results presented are the mean values ± Standard Deviation (SD) of the triplicate MEC experiments.

3.1 Volumetric Gas Production and Composition

Figure 2a demonstrates the variation of cumulative gas and H_2 production with applied voltage. An increment in the E_{ap} ranges of 0.6 V ≤ Eap ≤ 1.1 V drastically increased cumulative gas and H_2 volume. Only 200.72 ± 3.08 mL of total gas (hydrogen, and carbon dioxide) containing an average of 92 ± 0.28% (184.66 ± 1.83 mL) of H_2 was produced at E_{ap} = 0.6 V. However, total gas volume was increased to 267.59 ± 1.79 mL and 94 ± 0.40% of H_2 at E_{ap} = 1.1 V.

In terms of gas composition, H_2 concentrations in gas phase were greater than 92 ± 0.28% at each applied voltage (0.6 -1.1 V) (Figure 2b). The highest H_2 concentration of 96 ± 1.07% was achieved at E_{ap} = 0.9 V, while the lowest proportion of CO_2 was 4 ± 0.27%. In this study, CO_2 was also accumulated but no CH_4 was detected throughout the experiment and this might be due to the use of a pure culture. This result suggested that CH_4 production in MECs can be avoided by using pure culture. This finding is consistent with previous studies that have demonstrated pure culture MEC is one of the methods to avoid hydrogen losses to methanogens [43].

3.2 Volumetric Hydrogen Production Rate (HPR) and Current Density

The obvious variations were observed in hydrogen production rate (*HPR*) along with the elevation of applied voltage from E_{ap} = 0.6 V to E_{ap} = 1.1 V (Figure 3). An increase in applied voltages led to a dramatically rise in volumetric HPR from 1.55 ± 0.13 m^3 H_2/m^3 d (E_{ap} = 0.6 V) to 3.67 ± 0.55 m^3 H_2/m^3 d (E_{ap} = 1.1 V). The lowest volumetric HPR of 1.55 m^3 H_2/m^3 d was achieved at E_{ap} = 0.6 V and pH = 6.8. This value is 62 times higher than that (0.025 m^3 H_2/m^3 d) of using a single-chamber MEC with *Shewanella oneidensis* MR-1 (pure culture) and lactic acid ($C_2H_4OHCOOH$) as a fuel source at E_{ap} = 0.6 V [44]. Moreover, at E_{ap} = 0.7 V, the HPR of 2.01 ± 0.44 m^3 H_2/m^3 d was achieved, which is considerably higher than that (1.9 m^3 H_2 /m^3 d) of using a single-chamber MEC with a pure culture of *G. sulfurreducens* PCA and sodium acetate (CH_3COONa) as a carbon source at E_{ap} = 0.7 V [43].

However, the maximum volumetric HPR of 3.67 ± 0.55 m^3 H_2 /m^3 d is four times lower than that achieved in an acetate-fed and two chamber MEC reactor with mix culture at E_{ap} = 1.0 V [6]. The maximum volumetric HPR of 3.67 ± 0.55 m^3 H_2 /m^3 d is slightly higher than that (3.43 ± 0.12 m^3 H_2 /m^3 d) of using single chamber membrane-less MECs at pH = 7.0 and E_{ap} of 0.8 V ([45].

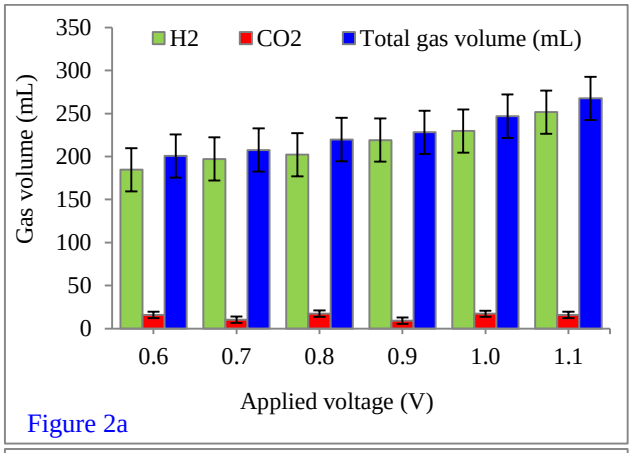


Figure 2a

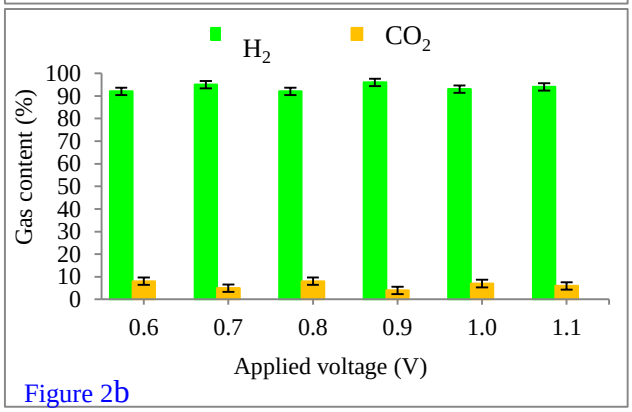


Figure 2b

Figure 2 Effect of E_{ap} on (a) gas volume (mL) and (b) gas contents (%) with different applied voltages using pure culture at pH = 6.8

The volumetric current density (I_V) increased linearly with E_{ap} for all the MECs (Figure 3). The reason may be that an increase in E_{ap} increased anode potential, resulting in a raise in current density from 152.68 ± 2.68 A/m^3 at E_{ap} = 0.6V to 293.73 ± 2.81 A/m^3 at E_{ap} = 1.1 V. The highest current density of 293.73 ± 2.81 A/m^3 is slightly higher than that previously reported (292 A/m^3) by Call and Logan [39]. At E_{ap} = 0.7 V, the current density of 188.02 ± 1.47 A/m^3 achieved here is 449% higher than that previously reported 34.2 A/m^3 at E_{ap} of 0.7 V in a tubular two-chamber MEC [46]. These results showed that there was little correlation between the maximum HPR and volumetric current density.

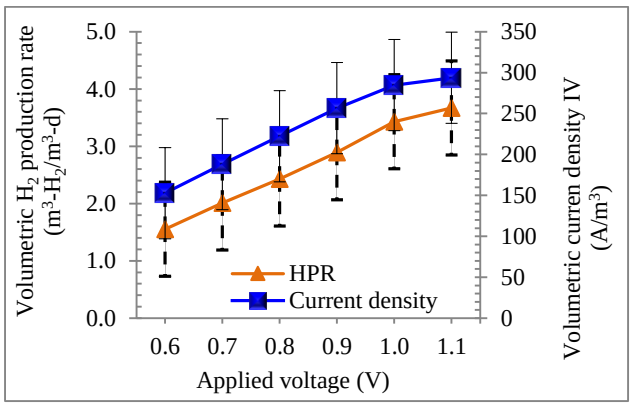


Figure 3 Effect of different applied voltages on volumetric hydrogen production rate (HPR) and volumetric current density (I_V) in single chamber and pure culture MEC

3.3 Hydrogen Recoveries

The overall hydrogen recovery (r_{H_2}), the cathodic hydrogen recovery ($r_{cat(H_2)}$), and the coulombic efficiency (C_E) variation with the different E_{ap} were shown in Figure 4. The lowest r_{H_2} and $r_{cat(H_2)}$ were 84.09 ± 1.05% and 91 ± 1.31%, respectively, which were obtained at E_{ap} of 0.6 V. It was found that $r_{cat(H_2)}$ remained high (>100%) at E_{ap} greater than 0.9 V. The highest r_{H_2} of 91.80 ± 0.14% and $r_{cat(H_2)}$ of 112 ± 2.12% were achieved at E_{ap}= 1.1 V. Of late, similar trend was observed by Cheng and Logan [18], the maximum overall hydrogen recoveries (r_{H_2}) of 91% using acetate and 71% using $C_6H_{12}O_6$. As can be seen in Figure 4, there was no significant difference between r_{H_2} at E_{ap} = 0.8 V and E_{ap} = 0.9 V (88.04 ± 1% vs. 88.89± 0.9%). The similar r_{H_2} were obtained by Liu *et al*. [47]. It was concluded that at lower applied voltages, lower r_{H_2} was primarily due to a low $r_{cat(H_2)}$, which was also resulted from the increased experiment cycle length. The (C_E) did not significantly vary with different applied voltages. There was a gradual drop in C_E from E_{ap} = 0.6 V to E_{ap} = 1.1 V (Figure 4). The C_E decreased inversely with the applied voltage. The C_E reached peak values of 92.05 ± 0.99% at the lowest E_{ap} of 0.6 V, this value is considerably higher than that of 78% (C_E) by Lu *et al*. [48] and 71 ± 2% (C_E) Li *et al*. [45]. The lowest C_E of 81.82 ± 0.94% was achieved at the maximum E_{ap} = 1.1 V. The C_E were generally over 81.82% at all applied voltages, which show a good efficiency of capturing electrons from the substrate and converting to current in the MEC system.

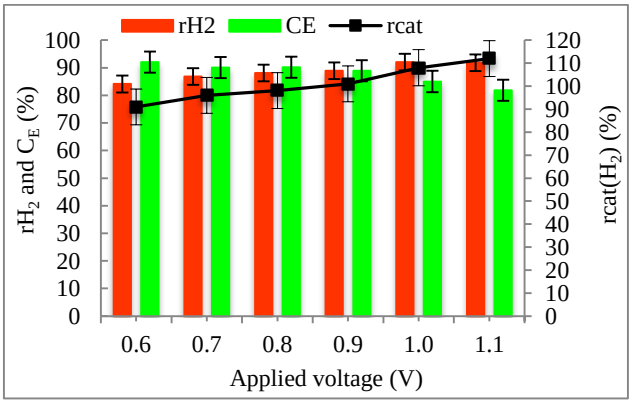


Figure 4 Hydrogen recoveries at different E_{ap}

3.4 Energy Efficiencies

Generally, in the MEC system, the energy inputs are electricity and substrates or fuels source consumed, while the energy outputs are H_2 produced. The energy efficiencies can be calculated based on both electrical and substrate inputs- overall energy efficiency or based on only electrical input - electrical energy efficiency, substrate - substrate energy efficiency. Oftentimes, the overall energy efficiency (η_{E+S}) based on both electricity and substrate is less than 100% [49]. In this this study, η_{E+S} varied with different applied voltages (Figure 5). The η_{E+S} were not remarkably correlated with E_{ap} over this range 0.6 -1.1 V. However, η_{E+S} was fairly constant at approximately 64.45 ± 1.19 % during the whole operational period. The lowest η_{E+S} of 61.31 ± 1.15 % was obtained at E_{ap} = 1.0 V, this value is slightly higher than that of 57 ± 3% by Tokash *et al*. [50].

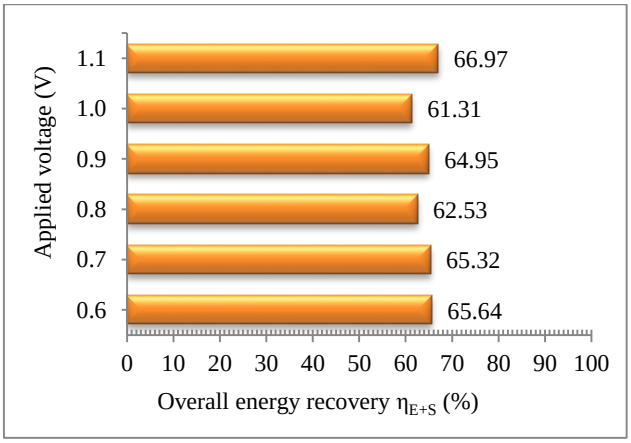


Figure 5 The overall energy efficiency (η_{E+S}) as function of applied voltage in single-chamber and pure culture MEC

Higher electrical energy efficiencies (η_E) were achieved at the lower E_{ap} (Figure 6). The η_E reached the peak value of 243.68 ± 3.09% at E_{ap} of 0.6 V, while the lowest value of 173.33 ± 2.23% was obtained at E_{ap} = 1.1 V. Lu *et al*. [48] achieved the electrical energy efficiencies ranged from 154 ± 9 to 200 ± 18% using an applied voltage of 0.6 V or 0.8 V. The (η_E) above 100% proves that the process is beneficial economically under the current conditions if high H_2 recovery can be maintained. A decrease in η_E were expected based on the larger amount of electrical energy input into the system at higher E_{ap} [38].

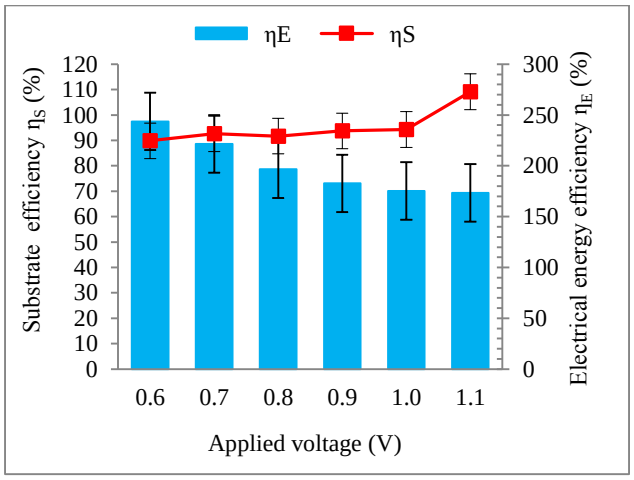


Figure 6 Electrical energy efficiency (η_E) and substrate energy efficiency(η_S) at different applied voltages

However, both the highest and lowest values were above 100% at all E_{ap}. The reason why η_E were higher than 100% was that the (η_E) only takes into account electrical energy input not including the substrate energy input as well. Additionally, theoretically the η_E will decrease to 100% when the E_{ap} increases to around 1.45 V [51]. On the contrary, the substrate energy efficiency (η_S) reached the highest value of 109.16 ± 1.12% at E_{ap} = 1.1 V, while the lowest value of η_S (89.83 ± 1.62%) was obtained at E_{ap} = 0.6 V. The η_S increased with increase of the E_{ap}. However, there was a marginal drop in (η_S) at E_{ap} of 0.8 V, that might be occurred due to current leakage in the MEC system.

3.5 Electrical and Substrate Energy Contribution

As shown in Figure 7, at lower applied voltages, a larger amount of energy was derived from substrate (CH_3COONa), while at the higher applied voltages the electricity (external power supply) had a greater contribution. For instance, at E_{ap} = 0.6 V approximately 73% of the energy was derived from the substrate, whereas only 27% of energy came from the power sources. Increasing the applied voltage to E_{ap} = 1.1 V decreased the contribution of the substrate to 61%, while the power sources contributed 39% of total energy. The key finding from this experiment is that when a higher voltage is employed to MEC system, MECs obtain more energy from electricity than from substrates. Similar trend was observed by [51] & Call and Logan [39].

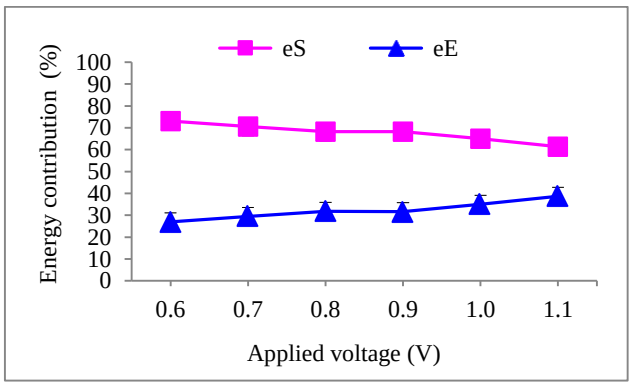


Figure 7 Electrical energy contribution (e_E) and substrate energy contribution (e_S) as function of applied in voltage single chamber and pure culture MEC.

4.0 CONCLUSIONS

In this study, a novel single-chamber MEC was designed and fabricated whose main features is to minimize the space between the anode and the cathode, while still using high surface area of electrodes (anode and cathode). The new design greatly reduced the internal resistance (R_{in}) and high cost associated with membranes. During MEC applied voltage test, E_{ap} showed significant influences on MEC's performance. The maximum hydrogen production rate (Q) of 3.67 ± 0.03 m^3 H_2 /m^3 d with volumetric current density (I_V) of 293.73 ± 1.18 A/m^3 was achieved at E_{ap} = 1.1 V in this newly developed MEC. Furthermore, the highest r_{H_2} and η_{E+S} of the MEC system were 91.80 ± 1.06% and 66.97 ± 0.09%, respectively. The observations indicated that the new MEC reactor design can reduce anode-cathode distance and lower total the internal resistance of MEC, thus enhance HPR.

It is worthy to mention that methanogenesis was completely avoided by using the pure bacterial culture of *G. sulfurreducens* PCA in this MEC, no CH_4 gas was detected throughout MEC operations. To sum up, these results clearly indicate that this innovative MEC configuration could be a feasible strategy for efficiently hydrogen production and treatment of wastewater in MECs.

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References

[1] Kadier, A., Kalil, M. S., Abdeshahian, P., Chandrasekhar, K., Mohamed, A., Azman, N. F., Logroño, W., Simayi, Y., Hamid, A. A. 2016. Recent Advances and Emerging Challenges Inmicrobial Electrolysis Cells (MECs) for Microbial Production of Hydrogen and Value-added Chemicals. *Renew. Sust. Energ. Rev.* 61: 501-525.

[2] Balat, M. and Balat, M. 2009. Political, Economic and Environmental Impacts of Biomass-based Hydrogen. *Int. J. Hydrogen Energy*. 34: 3589-3603.

[3] Lamb, R. 2010. When Will We Run Out of Oil, and What Happens Then? [Online]. From: http://science.howstuffworks.com/environmental/energy/run-out-of-oil.htm; [Accessed on 20 February 2016].

[4] Azman, N. F., Abdeshahian, P., Kadier, A., Shukor, H., Al-Shorgani, N. K. N., Hamid, A. A., Kalil, M. S. 2016. Utilization of Palm Kernel Cake as a Renewable Feedstock for Fermentative Hydrogen Production. *Renew. Energ*. 93: 700-708.

[5] Kadier, A., Kalil, M. S., Mohamed, A., Hasan, H. A., Abdeshahian, P., Fooladi, T., Hamid, A. A. 2017. Microbial Electrolysis Cells (MECs) as Innovative Technology for Sustainable Hydrogen Production: Fundamentals and Perspective Applications. Hydrogen Production Technologies. Sankir, M. and Sankir N. D. (eds.). Wiley-Scrivener Publishing LLC, USA. 407-458.

[6] Cheng, S. and Logan, B. E. 2011. High Hydrogen Production Rate of Microbial Electrolysis Cell (MEC) with Reduced Electrode Spacing. *Bioresour. Technol.* 102: 3571-3574.

[7] Abdeshahian, P., Al-Shorgani, N. K. N., Salih, N. K. M., Shukor, H., Kadier, A., Hamid, A. A., Kalil, M. S. 2016. The Production of Biohydrogen by a Novel Strain Clostridium sp. YM1 in Dark Fermentation Process. *Int. J. Hydrogen Energy*. 39: 12524-12531.

[8] Kuppam, C., Pandit, S., Kadier, A., Dasagrandhi, C., Velpuri, J. 2017. Biohydrogen Production: Integrated Approaches to Improve the Process Efficiency. Microbial Applications Vol.1 - Bioremediation and Bioenergy. Kalia, V. C., Kumar, P. (Eds.). Springer: New York, London. 189-210.

[9] Kadier, A., Abdeshahian, P., Simayi, Y., Ismail, M., Hamid, A. A., Kalil, M. S. 2015. Grey Relational Analysis for Comparative Assessment of Different Cathode Materials in Microbial Electrolysis Cells. *Energy*. 90: 1556-1562.

[10] Schlapbach, L. and Züttel, A. 2001. Hydrogen-storage Materials for Mobile Applications. *Nature.* 414: 353-358.

[11] Lin, C. Y., Lay, C. H., Chu, C. Y., Sen, B., Kumar, G. and Chen, C. C. 2012. Fermentative Hydrogen Production from Wastewaters: A Review and Prognosis. *Int. J. Hydrogen Energy*. 37(20): 15632-15642.

[12] Kumar, G. and Lin, C. Y. 2014. Biogenic Hydrogen Conversion of De-oiled Jatropha Waste (DJW) via Anaerobic Sequencing Batch Reactor Operation: Process Performance, Microbial Insights and CO_2 Reduction Efficiency. *Sci. World. J.* 2014: 1-9.

[13] Azman, N. F., Abdeshahian, P., Kadier, A., Al-Shorgani, N. K. N., Salih, N. K. M., Lananan, I., Hamid, A. A., Kalil, M. S. 2016. Biohydrogen Production from De-oiled Rice Bran as Sustainable Feedstock in Fermentative Process. *Int. J. Hydrogen Energy*. 41: 145-156.

[14] Lai, Z., Zhu, M., Yang, X., Wang, J. and Li S. 2014. Optimization of Key Factors Affecting Hydrogen Production from Sugarcane Bagasse by a Thermophilic Anaerobic Pure Culture. *Biotechnol. Biofuels.* 7(119): 1-11.

[15] Kadier, A., Simayi, Y., Kalil, M. S., Abdeshahian, P. and Hamid, A. A. 2014. A Review of the Substrates Used in Microbial Electrolysis Cells (MECs) for Producing Sustainable and Clean Hydrogen Gas. *Renew. Energ*. 71: 466-472.

[16] Pant, D., Singh, A., Bogaert, G. V., Olsen, S. I., Nigam, P. S., Diels, L. and Vanbroekhoven, K. 2012. Bioelectrochemical Systems (BES) for Sustainable Energy Production and Product Recovery from Organic Wastes and Industrial Wastewaters. *RSC. Adv.* 2: 1248-1263.

[17] Rozendal, R. A., Hamelers, H. V. M., Molenkamp, R. J. and Buisman, C. J. N. 2007. Performance of Single Chamber Biocatalyzed Electrolysis with Different Types of Ion Exchange Membrances. *Water Res.* 41: 1984-1994.
[18] Cheng, S. and Logan, B. E. 2007. Sustainable and Efficient Biohydrogen Production Via Electrohydrogenesis. *Proc. Natl. Acad. Sci. USA.* 104: 18871-18873.
[19] Li, C. and Fang, H. H. P. 2007. Fermentative Hydrogen Production from Wastewater and Solid Wastes by Mixed Cultures. *Crit. Rev. Env. Sci. Tec.* 37: 1-39.
[20] Selembo, P. A., Perez, J. M., Lloyd, W. A. and Logan, B. E. 2009. High Hydrogen Production from Glycerol or Glucose by Electrohydrogenesis Using Microbial Electrolysis Cells. *Int. J. Hydrogen Energy* 34: 5373-5381.
[21] Lee, H. S. and Rittmann, B. E. 2010. Significance of Biological Hydrogen Oxidation in a Continuous Single-chamber Microbial Electrolysis Cell. *Environ. Sci. Technol.* 44: 948-954.
[22] Picioreanu, C., Head, I. M., Katuri, K. P., van Loosdrecht, M. C. M. and Scott, K. 2007. A Computational Model for Biofilm-based Microbial Fuel Cells. *Water Res.* 41(13): 2921-2940.
[23] Zhang, Y. and Angelidaki, I. 2014. Microbial Electrolysis Cells Turning to be Versatile Technology: Recent Advances and Future Challenges. *Water Res.* 56: 11-25.
[24] Selembo, P. A., Merrill, M. D. and Logan, B. E. 2009. The Use of Stainless Steel and Nickel Alloys as Low-cost Cathodes in Microbial Electrolysis Cells. *J. Power Sources.* 190: 271-278.
[25] Wang, A., Liu, W., Ren, N., Zhou, J. and Cheng, S. 2010. Key Factors Affecting Microbial Anode Potential in a Microbial Electrolysis Cell for H_2 Production. *Int. J. Hydrogen Energy* 35: 13481-13487.
[26] Jung, S. and Regan, J. M. 2011. Influence of External Resistance on Electrogenesis, Methanogenesis, and Anode Prokaryotic Communities In Microbial Fuel Cells. *Appl Environ Microbiol.* 77(2): 564-571.
[27] Merrill, M. D. and Logan, B. E. 2009. Electrolyte Effects On Hydrogen Evolution and Solution Resistance in Microbial Electrolysis Cells. *J. Power Sources.* 191: 203-208.
[28] Nam, J. Y. and Logan, B. E. 2012. Optimization of Catholyte Concentration and Anolyte pHs in Two Chamber Microbial Electrolysis Cells. *Int. J. Hydrogen Energy.* 37: 18622-18628.
[29] Yossan, S., Xiao, L., Prasertsan, P. and He, Z. 2013. Hydrogen Production in Microbial Electrolysis Cells: Choice of Catholyte. *Int. J. Hydrogen Energy.* 38: 9619-9624.
[30] Tartakovsky, B., Manuel, M. F., Wang, H. and Guiot, S. R. 2009. High Rate Membrane-Less Microbial Electrolysis Cell For Continuous Hydrogen Production. *Int. J. Hydrogen Energy.* 34: 672-677.
[31] Kadier, A., Simayi, Y., Logroño, W. and Kalil, M. S. 2015. The Significance of Key Operational Variables to the Enhancement of Hydrogen Production in a Single-chamber Microbial Electrolysis Cell (MEC). Iran. J. Hydrogen Fuel Cell. 2(2): 85-97.
[32] Liu, H., Grot, S., and Logan, B. E. 2005. Electrochemically Assisted Production of Hydrogen from Acetate. *Environ. Sci. Technol.* 39: 4317-4320.
[33] Rozendal, R. A., Hamelers, H. V. M., Euverink, G. J. W., Metz, S. J. and Buisman, C. J. N. 2006. Principle and Perspectives of Hydrogen Production through Biocatalyzed Electrolysis. *Int. J. Hydrogen Energy.* 31: 1632-1640.
[34] Kadier, A., Simay,i Y., Abdeshahian, P., Azman, N. F., Chandrasekhar, K., Kalil, M. S. 2016. A Comprehensive Review of Microbial Electrolysis Cells (MEC) Reactor Designs and Configurations for Sustainable Hydrogen Gas Production. *Alexandria. Eng. J.* 55: 427-443.
[35] Jeremiasse, A. W., Hamelers, H. V. M., Saakes, M. and Buisman, C. J. N. 2010b. Ni Foam Cathode Enables High Volumetric H_2 Production in a Microbial Electrolysis Cell. *Int. J. Hydrogen Energy.* 35: 12716-12723.
[36] Ren, L., Siegert, M., Ivanov, I., Pisciotta, J. M. and Logan, B. E. 2013. Treatability Studies on Different Refinery Wastewater Samples Using High Throughput Microbial Electrolysis Cells (MECs). *Bioresour. Technol.* 136: 322-328.
[37] Ren, L., Tokash, J. C., Regan, J. M. and Logan, B. E. 2012. Current Generation in Microbial Electrolysis Cells with Addition of Amorphous Ferric Hydroxide, Tween 80, or DNA. *Int. J. Hydrogen Energy.* 37: 16943-16950.
[38] Logan, B. E., Cheng, S., Watson, V. and Estadt, G. 2008. Graphite Fiber Brush Anodes for Increased Power Production in Air Cathode Microbial Fuel Cells. *Environ. Sci. Technol.* 41: 3341-3346.
[39] Call, D. F. and Logan, B. E. 2008. Hydrogen Production in a Single Chamber Microbial Electrolysis Cell (MEC) Lacking a Membrane. *Environ. Sci. Technol.* 42: 3401-3406.
[40] Escapa, A., Gil-Carrera, L., García, V. and Morán, A. 2012. Performance of a Continuous Flow Microbial Electrolysis Cell (MEC) Fed with Domestic Wastewater. *Bioresour. Technol.* 117: 55-62.
[41] Nam, J. Y, Tokash, J. C. and Logan, B. E. 2011. Comparison of Microbial Electrolysis Cells Operated with Added Voltage or by Setting the Anode Potential. *Int. J. Hydrogen Energy* 2011. 36: 10550-10556.
[42] Lu, L., Xing, D. F. and Ren, N. Q. 2012. Bioreactor Performance and Quantitative Analysis of Methanogenic and Bacterial Community Dynamics In Microbial Electrolysis Cells During Large Temperature Fluctuations. *Environ. Sci. Technol.* 46: 6874-81.
[43] Call, D. F., Wagner, R. C. and Logan, B. E. 2009. Hydrogen Production by Geobacter Species and a Mixed Consortium in a Microbial Electrolysis Cell. *Appl. Environ. Microbiol.* 75(24): 7579-7587.
[44] Hu, H., Fan, Y. and Liu, H. 2008. Hydrogen Production Using Single-chamber Membrane-free Microbial Electrolysis Cells. *Water. Res.* 42: 4172-4178.
[45] Li, X. H., Liang, D. W., Bai, Y. X., Fan, Y. T. and Hou, H. W. 2014. Enhanced H_2 Production from Corn Stalk by Integrating Dark Fermentation and Single Chamber Microbial Electrolysis Cells With Double Anode Arrangement. *Int. J. Hydrogen Energy.* 39(17): 8977-8982.
[46] Huang, L., Jiang, L., Wang, Q., Quan, X., Yang, J. and Chen, L. 2014. Cobalt Recovery with Simultaneous Methane and Acetate Production in Biocathode Microbial Electrolysis cells. *Chem. Eng. J.* 253: 281-290.
[47] Liu, W. Z., Huang, S. C., Zhou, A. J., Zhou, G.Y., Ren, N. Q., Wang, A. J. and Zhuang G. Q. 2012. Hydrogen Generation in Microbial Electrolysis Cell Feeding with Fermentation Liquid of Waste Activated Sludge. *Int J Hydrogen Energy.* 37: 13859-13864.
[48] Lu, L., Ren, N. Q., Zhao, X., Wang, H., Wu, D. and Xing, D. F. 2011. Hydrogen Production, Methanogen Inhibition and Microbial Community Structures in Psychrophilic Single Chamber Microbial Electrolysis Cells. *Energy. Environ. Sci* 4: 1329-1336.
[49] Lalaurette, E., Thammannagowd, S., Mohagheghi, A., Maness, P. and Logan, B. E. 2009. Hydrogen Production from Cellulose in a Two-stage Process Combining Fermentation and Electrohydrogenesis. *Int. J. Hydrogen Energy.* 34(15): 6201-6210.
[50] Tokash, J. C. and Logan, B. E. 2011. Electrochemical Evaluation of Molybdenum Disulfide as a Catalyst for Hydrogen Evolution in Microbial Electrolysis Cells. *Int. J. Hydrogen Energy.* 36: 9439-9445.
[51] Logan, B. E. 2008. *Microbial Fuel Cells*. John Wiley & Sons: New Jersey.