High Electric Production by Membraneless Microbial Fuel Cell with Up Flow Operation using Acetate Wastewater

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ABSTRACT

Microbial fuel cell (MFC) is a new proposed technology reported to generate renewable energy while simultaneously treating wastewater. Membraneless microbial fuel cell (ML-MFC) system was developed to eliminate the requirement of membrane which is expensive and prone to clogging while enhancing electricity generation and wastewater treatment efficiency. For this purpose, a reactor was designed in two chambers and connected via three pipes (1 cm in diameter) to enhance fluid diffusion. Influent flowrate was maintained by adjusting peristaltic pump at the base of anaerobic chamber. Carbon cloth (235 cm²) was used as anode and paired with gas diffusion layer (GDL) carbon-Pt as cathode. Anaerobic sludge was filtered and used as starter feed for the anaerobic chamber. The experiment was carried out by feeding synthetic wastewater to anaerobic chamber; while current response and potential were recorded. Performance of reactor was evaluated in terms of chemical oxygen demand (COD). Electroactive microbe was inoculated from anaerobic sludge and showed current response (0.55-0.65 mA) at 0.35 V, range of diameter 1.5-2 µm. The result of microscopics can showed three different species. The microbial performance was increased by adding ferric oxide 1 mM addition as acceptor electron. The reactor was able to generate current, voltage, and electricity power of 0.36 mA, 110 mV, and 40 mWatt (1.5 Watt/m²), respectively, while reaching COD removal and maximum coulomb efficiency (EC) of 16% and 10.18%, respectively.

Keywords:
ML-MFC
Carbon cloth
GDL carbon-Pt
Acetate
Renewable energy

1. INTRODUCTION

Microbial Fuel Cells (MFC) is a combination of biological redox activities and electrochemical reaction in one cell system. MFC has received increasing attention and becomes an interesting issue over the past decade (Santoro, Arbizzani, Erable, & Ieropoulos, 2017). The most important factor in an MFC system is the simultaneous process of degradation of organic wastewater and electricity generation (Fornero, Rosenbaum, & Angenent, 2010; Wang, 2014). These two main benefits will boost the research towards application on full scale as a solution to the limitation of non-renewable energy as well as prevention of environmental pollution. Several studies have applied MFC on the treatment of various wastewater such as dyes (Thung et al., 2015), palm oil (Baranitharan et al., 2015), starch (Pant, Bogaert, Diels, & Vanbroekhoven, 2010), brewery (Feng, Wang, Logan, & Lee, 2008), and sewage (Ahn & Logan, 2010).

Researchers are usually focused on the material of electrode, microorganisms used and reactor designs. Noble metal catalysts, such as Pt and Au, are commonly used because of their inertness. However, other materials are also considered more feasible and widely used as anode-cathode, i.e., steel-graphite (Jadhav & Ghangrekar, 2009), paper-paper (Roma, 2008), graphite-graphite (Liu & Li, 2007; Reimers et al., 2007; Tartakovsky & Guiot, 2006), graphite granula-graphite granula (Zhuwei, Qinghai, Meng, &
Shaohua, 2008), carbon-carbon cloth (Feng et al., 2008) and carbon cloth-platinized titanium mesh (Larrosa-guerrero et al., 2010). Carbon-based materials now proposed as the attachment media of microbial cells to facilitate electron transfer directly via cytochrome protein and conductive pili (Santoro et al., 2017). Power production is significantly determined by the electrode material as well as the distance between electrodes, shape and dimension of electrodes.

Double-chamber MFC is the most investigated model of MFC reactor, in which the electrode chambers are separated by proton exchange membranes (Ali, Gomaa, Fathey, Abd, & Kareem, 2015; Baranitharan et al., 2015; Greenman, Gálvez, Giusti, & Ieropoulos, 2009; Larrosa-guerrero et al., 2010; Li, Zhang, Lin, Han, & Lei, 2010; Sun, Li, Li, Hu, & Zhang, 2013). However, this model has its limitation to a full-scale plant application, due to the high cost of membranes used and high possibility of clogging thus inhibiting the hydrogen transfer. In order to overcome this obstacle, single chamber air cathode MFC is then designed and resulting in a reduced total volume, simplified design and improved power output (Feng et al., 2008; Larrosa-guerrero et al., 2010; Wardana & Effendi, 2019; Wei, Han, & Shen, 2012; Zhu, Wang, Zhang, & Tao, 2011). MFC-MBR models as single chamber has been review to potential applied in the wastewater treatment (Bhargavi, Venu, & Renganathan, 2018). Single chamber system utilizes atmospheric air directly in their reaction at the electrode surface. The cathodes are encased by proton exchange membranes, thus proton migration is still obstructed and competition of substrate utilization will occur (Barbara & Pawel, 2020; Kim, Kim, An, Lee, & Chang, 2016).

Finally, membrane less microbial fuel cell (ML-MFC) has been developed as a response to all previous challenges. The absence of membrane will simplify the system configuration and reduce both the cost of maintenance and reactor (Kim et al., 2016; Thung et al., 2015). However, the absence of barrier between anaerobic (anode) and aerobic (cathode) chambers will lead to uncontrolled mixing of electrolyte in both chambers via migration, convection, and diffusion. This study has applied an up-flow system to indirectly separate anode and cathode chambers while facilitating hydrogen transfer to the cathode. This design reactor and operation system can reduce a potential influx of oxygen from cathodic to anodic chamber and lower the cost investment. Carbon cloth and Pt-coated carbon GDL (gas diffusion layer) were used as anode and cathode, respectively, to observe their ability to remove synthetic wastewater as organic pollutant. Acetate was chosen as synthetic waste based on a simplicity of structure that have convenience to oxidation. This condition will facilitate evaluation of a ML-MFC that have designed in this research. The performance of ML-MFC was investigated on varied up flow rate and electricity generation were chosen as an indicator based on potential and current response.

2. EXPERIMENTAL SECTION

2.1. Inoculum and substrates

The ML-MFC was inoculated with mixed cultures anaerobic sludge. 1 L of phosphate buffer that consisted of 0.1 g KCl; 0.2 g NH₄Cl; 0.6 g NaH₂PO₄; 10 mL of trace element; 2 g NaHCO₃; 2.722 CH₃COONa and 15 g ferric oxide, was added to 400 mL of anaerobic sludge. It was then mixed and filtered using a vacuum pump. Filtrate obtained was then pumped into ML-MFC reactor at a fixed flow rate of 8 mL/min using a peristaltic pump. 4 L of nutrient that consisted of 0.4 g KCl; 0.8 g NH₄Cl; 2.4 g NaH₂PO₄; 40 mL of trace element; 8 g NaHCO₃, 60 g ferric oxide, and 10.8 g CH₃COONa was further fed to the reactor at a fixed flow rate of 11mL/min under anaerobic condition (The nitrogen gas given during the filling process). DC potential was set (DY 2030 POTENTIOSTAT DIGI-IVY) at anode and cathode at 0.3 V. The anode chamber was operated in a circulated system. All the chemicals used, except for ferric oxide, were of analytical grade. Biofilm formed at anode surface was observed using Scanning Electron Microscopy (SEM) and identified using a digital microscope and Gram staining.
2.2. Configuration and operation of ML-MFC reactor

The configuration of ML-MFC reactor depicted in Fig. 1. Carbon cloth with MPL (Microporous Layer) and Gas Diffusion Electrode (GDE) cloth with 20% Platinum were used as anode and cathode, respectively, and obtained from Fuel Cell Store, U.S.

Soon after biofilm formed on anode surface, synthetic wastewater was then fed to the reactor. The constituent in the synthetic wastewater was 1000 mg/L CH₃COONa. It was fed to the reactor using a peristaltic pump at fixed flow rate of 12 mL/min. Current response and potential obtained during experiment were monitored (the frequency record is 30 s) using GWINSTEK GDM-396 with interface program Ver1.00 and HANTEK 365D. Water was sampled in the inlet and outlet points of reactor and analysed for COD. The performance of reactor in optimum power output generation was evaluated at varied flowrate of 12, 14, and 16 mL/min. This scale flow rate is
chosen based on HRT and specification of peristaltic pump. To obtain coulombic efficiency, a closed flow system was used with an initial COD concentration of 1000 mg/L, the flow has been setting at 12 mL/min. The value of COD in the next cycle is based on the measurement results of the sample taken (intake point and output cathodic chamber).

2.3. Analysis and evaluation

Three anodes (Carbon Cloth with MPL) and one cathode (GDL Pt/C 20%) were connected. A 300 Ω resistor was used in the external circuit. Potential and current response were both recorded using a data logger (DC Voltage 60 mV, DC Current 6-600 mA, Capacitor 4-400 μF, Diode 0-2 V). Water samples taken from inlet (container before anodic chamber) and outlet points (overflow at cathodic chamber) were all preserved using sulfuric acid (Merck: pro analysis 98%) to obtain a condition pH<2. The chemical oxygen demand (COD) was tested by APHA AWWA method (SM 5220 C). Coulombic efficiency (CE) was calculated based on specific formula of 

$$CE = \frac{MI}{(F \times b \times q \times \Delta COD)}$$

where M is the atomic weight of acetate, I is current, F is the Faraday number (96485 C/mol), b is the number of electrons generated per 1 mol of acetate, q is the flowrate and ΔCOD is the difference of influent and effluent COD.

3. RESULT AND DISCUSSION

3.1. Microbial growth, attachment, and enrichment on anode surface

Electroactive microorganisms commonly grow on the surface of anode. Phosphate buffer was used to provide a suitable and optimum condition for microbial growth of anaerobic sludge (Zhao et al., 2016 and DSMZ, 2007). Disodium hydrogen phosphate and sodium dihydrogen phosphate were used as buffer in this experiment. pH value was adjusted to 6.8 to reduce the growth rate of methanogenic bacteria as competitor. It should be prevented due to their role in faster degradation of simple organic compounds to methane. The Gibbs free energy of methanation is higher thus making it more favourable than simple oxidation which would hinder the production of electron in MFC.

Electron donor on anode and supplement addition of 0.1 M ferric oxide was carried out to support microbial growth and enrichment. Ferric oxide plays an important role as electron acceptor via reduction of three-valent to two-valent iron (Wei et al., 2012). The role of electron acceptor was significant on the transfer of electron liberated by the activity of electroactive microorganisms in organic oxidation, especially acetate. The presence of electroactive microorganisms and the effect of ferric oxide on reactor performance were indicated by current response, as depicted in Fig. 2.

![Figure 2. Current response observed for 1, 2, and 3 days at 0.35 V vs Ag/AgCl reference electrode](image)

The presence of electroactive microorganisms was indicated by a gradual increase of current response. The increase was detected at 30 min interval for a few days. Current response was decreased after day 3 at 0.0006 A from 0.000625A for 3 hours. However, it was still higher than the response detected without ferric oxide (0.000029 A). Microbial growth at anode surface was also confirmed by SEM images of anode surface. As depicted in Fig. 3, biofilm has been shown to grow in virtual layer on anode surface. Thin long layer was clearly observed on carbon anode surface (Fig. 3b); 1.5 – 2 µm in diameter and 6 µm in length. In addition, there were also clumps of biofilm in the shape of irregular mass on anode surface. Further confirmation of microorganism on anode surface, or so-called biofilm, was also conducted using a microscope via Gram staining of bacterial colonies (Fig. 4).
Figure 3. Surface image of anode before (a) and after microbial growing (b)

Figure 4. Microbial identification by microscope method

Figure 5. Current production of 1000 mg/L synthetic acetate wastewater at varied flowrate and operational time

Figure 6. Current production to time process using ferric oxide as media with constant flowrate 12 mL/min
The Gram staining shows three distinct types of bacteria, i.e. *bacillus*, *coccus*, and *streptococcus*. *Bacillus* bacterium was identified as long, rod-shaped cell while *coccus* as spherical and round-shaped. *Streptococcus* bacterium also detected as strings of beads grouping in chains.

### 3.2. The production of current, voltage and power in reactor application

Up flow ML-MFC was able to convert energy contained in organic matter into electricity. Bioelectricity obtained was represented as current response (A), potential (V), and power (P). Current output obtained during experiment was monitored (Fig. 5 and 6). Current was generated when electricigens oxidized organic compounds in wastewater and produced electron, carbon dioxide, and hydrogen. The electron will be transferred to anode and further transmitted to external circuit. While hydrogen will be transported to anode in liquid phase and serve as electron acceptor and further converted into H2O. This closed cycle is the main mechanism of electricity generation.

Fig. 5 shows current trends at varied operational time and flowrate. It tended to decrease at flowrate 12 and 16. Interestingly, the opposite increasing trend was observed at flowrate 14, though a decrease was observed after 20 h. Compared to operational time, flowrate is more influential towards current. Current production at flowrate 12 mL/min was lower than higher flowrate (14 and 16 mL/min). It was possibly attributed to low electron and hydrogen transport to anode in anaerobic chamber (Zhu et al., 2011) and hydrogen to cathode in aerobic chamber, respectively, as those transfer were mostly affected by diffusion. It was further confirmed by current response data at flowrate 14 mL/min. Generally, higher flowrate means shorter retention time of wastewater inside the reactor and leads to lower electron production. However, current response observed at flowrate 14 mL/min was 64% higher (0.094 mA) than 12 mL/min (0.058 mA). This trend indicated that the impact of electron and ion (hydrogen) transport was more dominant than electron production from the oxidation by microorganisms. Lorenzo et al. 2009 (Di, Curtis, Head, & Scott, 2009) mentioned that electrochemical activity of microorganisms will be enhanced at higher mass transfer due to increasing flowrate. Higher flowrate will also decrease internal resistance of fluid (Wang, 2014) and leads to an increasing electricity production. However, this trend was not observed at flowrate 16 mL/min, in which the current response was lower than flowrate 14 mL/min. It was possibly due to electron production as a limiting factor and resulted in negative correlation between electron/ion transfer and electrical production.

Low current response observed was possibly due to slow electron transfer. However, it can be controlled by using electron acceptor, as clearly revealed in Fig. 6. Ferric oxide addition to anodic chamber as electron acceptor will
facilitate biofilm growth at anode surface. This trend was in accordance with the finding of Bond dan Lovley, 2003 (Bond, Lovley, Bond, & Lovley, 2003). Ferric oxide addition was able to increase current response from 0.058 mA to 0.35 mA or six times higher, at flow rate 12 mL/min.

The positive effect of electron acceptor (ferric oxide) on energy production of ML-MFC reactor was also supported by potential and power density as shown in Fig. 7. The specific design and operational of reactor used in this experiment resulted into high potential of 88-110 mV and power production of 32-40 mW at flowrate 12 mL/min. Power density obtained was about 1.5 W/m², in which anode surface area was 235 cm². It was higher than the findings of other studies by Ahn and Logan 0.422 W/m² (Ahn & Logan, 2010), Ali et al 0.209 W/m² (Ali et al., 2015), Du et al 0.536 W/m² (Zhuwei et al., 2008) and Kim et al 0.46 W/m² (Kim et al., 2016)).

The maximum production of energy was likely because of the high effectivity of electron and hydrogen transfer in anaerobic and aerobic chamber, respectively. Electron transport was facilitated by high contribution of fluid diffusion and electron acceptor (ferric oxide). While cation transport relied on diffusion and migration processes that went unhindered by materials (membrane).

3.3. COD removal and Coulombic efficiency

Another essential feature of up flow ML-MFC is the organic compound’s degradation. Fig. 8 shows that reactor was able to achieve high efficiency of organic compounds degradation. Synthetic wastewater was treated and reached 13% efficiency at retention time of 6.9 h. This tendency was linear at initial COD of 500 – 1000 mg/L and increased to 16% at initial COD of 500 – 125 mg/L. COD removal was lower at initial COD less than 125 mg/L (9% lower). Low COD removal (13 – 16%) indicated that anaerobic microorganism growth at anode was still unsatisfactory. It was in accordance with relatively low efficiency at high COD (>521 mg/L) and increased rate of efficiency at COD 521-125 mg/L. Significantly poor performance at low initial COD (<125 mg/L) was likely because of the declining probability of contact between pollutant and microorganisms.

Power was generated from degradation of substrate via biological redox activities and measured as coulombic efficiency (CE). It was calculated based on COD removal and output voltage. Initial COD significantly affected coulombic efficiency, ranging from 0.42%, 1.1%, 6.27%, and 10.18% at initial COD 500-1000, 125-500, 112-125 and less than 112 mg/L respectively. Higher coulombic efficiency observed at low initial COD implied positive correlation between substrate concentration and microorganisms at anode surface.

4. CONCLUSION

Up flow ML-MFC was able to avoid uncontrolled mixing of electrolytes in anaerobic and aerobic chamber and ensure unhindered hydrogen transfer. Carbon cloth was a potential material to be used as anode as it effectively supported microbial growth. Addition of electron acceptor (ferric oxide) gave a significant effect on reactor performance. Reactor was able to generate current, potential, and electrical power of 0.36 mA, 110 mV, and 40 mWatt (1.5 Watt/m²), respectively, from synthetic wastewater treatment (1000 mg/L acetate) at 6.9 h of hydraulic retention time (HRT). Coulombic efficiency (EC) and COD removal was significantly determined by substrate load, 10.18% (<112 mg/L) dan 0.42% (>500 mg/L).

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