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Q1 **Sustainable green technology on wastewater treatment:**
 2 **The evaluation of enhanced single chambered up-flow**
 3 **membrane-less microbial fuel cell**

Q3 Q2 **Wei-Eng Thung¹, Soon-An Ong^{1,*}, Li-Ngee Ho², Yee-Shian Wong¹, Fahmi Ridwan¹,**
 5 **Yoong-Ling Oon¹, Yoong-Sin Oon¹, Harvinder Kaur Lehl¹**

6 1. Water Research Group (WAREG), School of Environmental Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

7 2. School of Materials Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia

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ABSTRACT

This study demonstrated the potential of single chamber up-flow membrane-less microbial 17
 fuel cell (UFML-MFC) in wastewater treatment and power generation. The purpose of this 18
 study was to evaluate and enhance the performance under different operational conditions 19
 which affect the chemical oxygen demand (COD) reduction and power generation, 20
 including the increase of KCl concentration (MFC1) and COD concentration (MFC2). The 21
 results showed that the increase of KCl concentration is an important factor in up-flow 22
 membrane-less MFC to enhance the ease of electron transfer from anode to cathode. The 23
 increase of COD concentration in MFC2 could led to the drop of voltage output due to the 24
 prompt of biofilm growth in MFC2 cathode which could increase the internal resistance. It 25
 also showed that the COD concentration is a vital issue in up-flow membrane-less MFC. 26
 Despite the COD reduction was up to 96%, the power output remained constrained. 27

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Introduction

43 Most of the wastewater treatment plants nowadays are using 44
 45 activated sludge process to treat the wastewater. This 46
 47 activated sludge process requires aeration which consumes 48
 49 large amount of energy and high capital costs of maintenance. 49
 50 The recent research about biotechnology is by using methane, 50
 51 hydrogen, and butanol to produce renewable bioenergy (Mehta 51
 52 et al., 2010; Montpart et al., 2015; Sangeetha et al., 2016). Microbial 52
 53 fuel cell (MFC) is one of the promising biotechnologies which 53
 54 uses wastewater as biofuel to generate bioelectrical power in

wastewater treatment plant. The advantages of MFC application 55
 in wastewater treatment industries are its ability to highly reduce 56
 polluted wastewater and simultaneously produce bioelectricity. 57
 The polluted wastewater includes domestic wastewater, food 58
 processing wastewater, dye-containing wastewater, brewery 59
 wastewater, swine wastewater, and paper mill wastewater 60
 (Feng et al., 2008; Mansoorian et al., 2013; Min et al., 2005; 61
 Sciarria et al., 2013). The general concept of MFC depends on the 62
 microbes to convert the chemical energy in biodegradable 63
 organic wastewater into bioelectricity. The microbes at anode 64
 electrode generate electrons and protons by oxidizing the 65

Q4 * Corresponding author. E-mail: ongsoonan@yahoo.com (Soon-An Ong).

biodegradable organic wastewater. Bioelectrical current is generated by using the electrons and protons that transfer from anode to cathode.

The efficiency of electricity generation can be improved because it can be affected by the MFC architecture, electrode properties, and operational condition. Extensive research was conducted to investigate the power generation from different MFC architectures, such as single chamber air-cathode MFC, double chamber MFC, up-flow tubular MFC, down flow tubular MFC and baffled MFC (Feng et al., 2010; Jayashree et al., 2016; Mansoorian et al., 2013; Min et al., 2005; Zhu et al., 2011). These types of MFC have several disadvantages, which are high cost, high maintenance and difficult to upscale in real application. Therefore, researchers are putting more attention on membrane-less MFC (Kim et al., 2015; Roustazadeh Sheikhyousefi et al., 2016; Santoro et al., 2014). Besides the design of the MFC, the electrode properties are one of the important factors which could affect the voltage output performance. The previous study included the material used as cathode that could affect the oxygen reduction process, which directly affects the electricity generation (Thung et al., 2015). Besides, the surface morphology of carbon material led to the growth rate of the biofilm, where the thick biofilm growth on the surface material could hamper the oxygen reduction process at cathode region (Thung et al., 2016). Salinity of the wastewater could be crucial for MFC. The increase of salt concentration could increase the conductivity, but high salinity could poison the microbes (Oh and Logan, 2006; Zhang et al., 2010). Zarei et al. (2012) found that the level of substitution of NaCl with KCl can be at least 25% without risking the microbiological safety (Boziaris et al., 2007; Zarei et al., 2012).

Q5 In this study, the operational condition includes the increase of chemical oxygen demand (COD) concentration and KCl concentration with single chamber up-flow membrane-less MFC to identify the effect of COD reduction and power generation. The information of this study may be useful in the optimization of single chamber up-flow membrane-less microbial fuel cell (UFML MFC) technology in the future.

1. Materials and methods

1.1. Inoculum and substrates

Mixed culture activated sludge was collected from a rubber glove industry wastewater treatment plant, Shorubber (Malaysia) Sdn. Bhd. The anode electrode was inoculated with activated sludge in a closed container to cultivate anaerobic microorganism. The UFML MFC reactors were fed with synthetic wastewater which contained sodium acetate (1.569 g/L) as the main organic carbon substrates. The synthetic wastewater contained nutrient solution (pH 6.55) which includes (per liter): NH_4Cl 0.31; KCl 0.13; K_2HPO_4 3.4; KH_2PO_4 4.4; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 0.1; and NaCl 0.116 g.

1.2. Design of UFML MFC bioreactor and operational conditions

The single chambered laboratory scale UFML MFC was fabricated with acrylic material whereas described in previous study (Thung et al., 2015). The size of these reactors is 4.5 cm

diameter and height of 25 cm. The working volume of the reactor is 0.11 L. The size of the gravel is 6 mm in diameter, and it filled into the anode region of UFML MFC. The gravel and the material used as electrode was inoculated with the mixed culture activated sludge for more than a month. This could immobilize the microorganism on the surface of gravel and electrode. Total of 2 UFML MFCs were used in this operational study whereas MFC1 and MFC2. MFC1 contained of carbon felt ($3 \times 2.5 \times 2$ cm, 36 cm^2 , SG-222; provided by Maido Corporation, Japan) as anode electrode and cathode electrode. MFC2 anode used the same material as MFC1, but different cathode electrode, which used Pt-loaded carbon paper (2×5 cm, 20 cm^2) instead. Anode and cathode were connected with copper wire. MFC1 was used to study the effect of KCl concentration on the power generation and COD reduction. MFC2 was used to evaluate the effect of the organic loading rate on power generation and also the effect of alternating from closed circuit to open circuit on COD reduction.

A peristaltic pump (BT100-100M, Longer Precision Pump) was used to pump in the synthetic wastewater from the bottom anodic region upward to cathodic region of MFC1 and MFC2. The synthetic wastewater was allowed to flow continuously at flow rate of 0.152 mL/min for 1 hr. intervals per day. Air pump was supplied at the cathodic region at room temperature $29 \pm 2^\circ\text{C}$.

1.3. Analytical methods

After the change of parameters, all voltage output and COD reduction data were collected after a week for stability. A digital data logger (Graphtec GL820, USA) was connected to the MFC to monitor continuously the voltage output at 1 hr intervals. Polarization curves were collected from varying resistors from 50 to 20,000 Ω . The internal resistance was collected from the highest power density value in polarization curves. Current (I) was calculated from Ohm's law ($V = IR$) where V and R represent the voltage and resistance respectively. Current density (A/m^2) and power density (W/m^2) were calculated by the geometrical surface area of anode electrode. The Columbic Efficiency (CE) was calculated by the ratio of experimental Coulombs to theoretical Coulombs transferred. $\text{CE} = MI (F bq\Delta\text{COD})^{-1}$ where M is the molecular weight of substrate, I is the stable current, F is the Faraday's constant (96,485 C/mol), b is the number of moles of electrons produced per mol of substrate, q is the volumetric influent flow rate and ΔCOD is the difference in the influent and effluent COD (Logan et al., 2006). The collected samples at anode region and cathode region were centrifuged (CENCE L500, China) before analysis. COD was determined using spectrophotometer (DR 2800, Hach).

2. Result and discussion

2.1. Power generation and COD reduction of MFC1 at different KCl concentrations

The maximum power density obtained from the polarization curve was $5.8 \pm 0.4 \text{ mW}/\text{m}^2$ at current density of $39.6 \pm 1.2 \text{ mA}/\text{m}^2$ with concentration of KCl $\times 1$ (0.13 g/L) (Fig. 1b). When the concentration of KCl increased to $\times 5$ (0.65 g/L) 175

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