PRELIMINARY COMPARISON OF 2-CHLOROPHENOL BIODEGRADATION USING MICROBIAL FUEL CELL AND ANAEROBIC SYSTEMS

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Abstract. 2-chlorophenol (2CP) is a typical contaminant found in industrial effluent that is both hazardous and persistent in the environment. The bioelectrochemical degradation of 2CP has been approved as a preferred method for removing the abrasive 2CP from wastewater. In this work, a microbial fuel cell (MFC) system inoculated with palm oil mill effluent (POME) sludge was used to degrade 2CP. The changes of morphology of the anode biofilm were observed under a light microscope and scanning electron microscope (SEM) for 2CP-fed MFC compared with the biofilm inoculated in an anaerobic chamber (AC). Maximum current density generated by the MFC was 97.30 mA/m^2 while degrading 75% 2CP. Lower 2CP degradation of 60% was observed using the AC. Also, the abundance of negatively stained bacteria is reduced in the AC biofilm. This research shows that bioelectrochemical 2CP degradation is more efficient than conventional AC degradation. POME has the potential to be a high-value substrate for bacteria that can generate electricity in the MFC while also degrading harmful 2CP.

Keywords: microbial fuel cell, POME, 2-chlorophenol

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Introduction

Microbial fuel cell (MFC) has emerged as one of the promising tools in wastewater treatment while generating electricity. A typical MFC is made up of an anode and cathode chamber separated by a proton exchange membrane (PEM), in which the oxidation of organic wastes and reduction of oxygen are continually occurred for stable current generation [1]. The ability of MFC to convert chemical energy into electricity makes MFC a potential source of green energy [2]. MFC has been approved to offer several advantages in terms of energy conversion efficiency, environmental-friendly and high wastewater treatment efficiency [3]. MFC is also believed to effectively treat hazardous contaminants typically found in industrial wastewater, for instance, heavy metals, dyes, synthetic chemicals and aromatic hydrocarbons, by electroactive bacteria.

Chlorophenol (CP) is a type of recalcitrant aromatic hydrocarbon heavily present in industrial wastewater [4]. It is often characterized by high toxicity, persistent to natural biodegradation, mutagenicity and carcinogenicity [5]. CP could easily pass conventional treatment and even advanced oxidation techniques due to its stable chemical structure of p-π conjugate, that yields high generation of toxic byproducts and reduces degradation efficiency [6]. To convert CP into a more naturally degradable compound, it is vital to cleave the C-Cl bonds via reductive dehalogenation. A preferable and more environmental-friendly way to perform reductive dehalogenation of CP is through microbial biodegradation. *Pseudomonas*, *Rhodococcus*, *Azotobacter*, *Bacillus*, and *Sphingomonas* appear to be excellent CP-degrading bacteria which utilize CP as their carbon sources [4]. *Bacillus subtilis* has recently been discovered to biodegrade 4-chlorophenol (4CP) in a batch and continuous packed-bed reactor with the highest degradation removal of 45% [7]. Hence, it is worthwhile to examine the CP biodegradation in an MFC system because the electrochemical mechanism used by the electroactive bacteria could result in a higher degrading rate.

In this study, the performance of palm oil mill effluent (POME)-inoculated MFC to biodegrade 2CP was compared with anaerobic systems. The bacterial biofilms formed on the carbon electrode were microscopically observed and compared with those in an anaerobic chamber (AC). This research could assist future study on the identification of the proper microbial strain responsible for 2CP breakdown while yielding optimal current generation.

Materials and Methods

MFC setup and operation. A double chamber MFC was constructed using two 500-mL glass bottles separated by a cation exchange membrane (CEM). Palm oil mill effluent (POME) sludge obtained from Malpom Industries Sdn. Bhd., Nibong Tebal, Penang, Malaysia was used as the inoculum in the anode chamber. The POME sludge was filtered through filter paper (0.45 μm) before feeding into MFC reactor. The anode chamber consisted of a growth medium (1 g/L NaCl, 0.53 g/L KH_2PO_4, 1.07 g/L K_2HPO_4, 0.3 g/L $CaCl_2$, and 0.2 g/L $MgSO_4$), glucose and 2CP (30 mg/L). The glucose was fed at a daily basis to maintain the growth of microbes throughout the MFC operation [8].

Phosphate buffer solution (100 mM) was used as a catholyte in the cathode chamber. Nitrogen gas was sparged into the anode chamber for 10 minutes to provide an anaerobic condition while the cathode chamber was fully opened for aeration. A carbon cloth (5 cm × 5 cm) was used as the anode and cathode electrode. Both of the electrodes were connected to a

titanium wire through 1 kΩ resistor. A computer was used to monitor the voltage output generated by the MFC. The MFC system was operated at 30-35 °C on a magnetic plate and was operated over five days.

The 2CP degradation and biofilm morphology of AC were compared with MFC. The setup of MFC and AC are illustrated in Figure 1. Both MFC and AC experiments were carried out in duplicate.

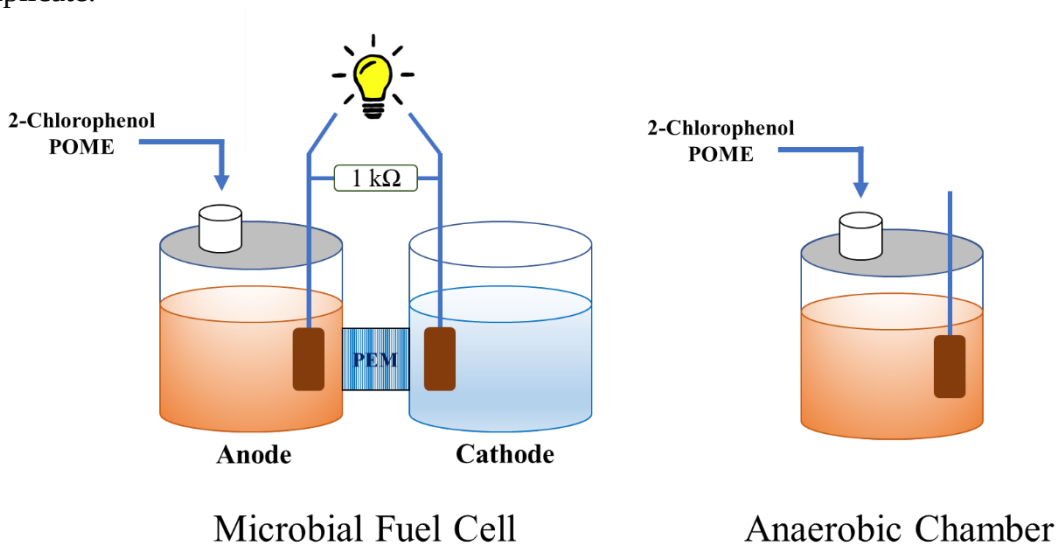

Figure 1. The experimental setup of MFC and AC.

Electrochemical analysis. Current density and voltage were recorded using a multimeter (ASAKI) over five days across 1 kΩ external resistance. The current and voltage values were used to obtain the power output. The power density (mW/m^2) and current density (mA/m^2) were determined by dividing power and current generated by anode surface area in m^2.

Phenolic degradation. The 2CP was fed into the anode chamber of the MFC and AC for microbial inoculum acclimatization. To determine the percentage degradation of 2CP, 5 mL samples were collected every 24 hours from both MFC and AC. The samples were centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was used to examine the degradation of 2CP. The degradation of 2CP was analyzed by using Folin Ciocalteu's method.

One mL of the supernatant was mixed with 5 mL of Folin Ciocalteau's reagent (diluted tenfold) and 4.0 mL of sodium carbonate solution (75 g/l). The absorbance was measured at 765 nm after 30 minutes incubation and compared to the 2CP standard calibration curve. Based on samples taken on the first and last days of MFC operation, the phenolic degradation was calculated based on Equation 1 where C_i and C_f are initial and final concentrations of 2CP, respectively.

$$Phenolic\ degradation\ \% = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

Biofilm morphology using scanning electron microscopy. Scanning Electron Microscope (SEM) was used to characterize the biofilms established on the electrodes in MFC and AC reactors. The electrodes with the attached biofilms from both MFC and AC were aseptically removed and washed gently with phosphate buffer saline solution. Then, the

samples were treated with 2.5 wt. % glutaraldehyde and washing agent of 50 mM phosphate buffer [9]. The electrode samples were dried at 80 °C for 24 h and were coated with a thin layer of gold before being viewed under high resolution SEM imaging.

Gram's staining. The Gram staining process distinguishes between Gram-positive and Gram-negative bacteria**.** A drop of distilled water was placed on a clean glass slide and one loop of bacteria from the biofilm was dispersed on distilled water of the glass slide to form smears. The slide was slightly flamed to fix the microbes to the slide. After that, the smear was flooded with Gram's crystal violet for 20 seconds and washed off by rinsing gently with water. Then the smear was flooded with Gram's iodine for 30 seconds and washed thoroughly with 95% ethanol for 3 seconds. The ethanol was washed gently with running tap water. The smear was covered with safranin for 30 seconds, washed with water for a few seconds and dried at room temperature prior to examination under a light microscope. A drop of immersion oil was added when using the high-power objective (100 X).

Results and Discussion

Electricity generation. The current density generated by the MFC was recorded for 5 days as shown in Figure 2. A low current generation is observed during the first 24 hours of MFC operation. The current started to increase gradually until 72 hours of MFC operations. The maximum current density produced was 95.3 mA/m^2 as achieved on day 3 of MFC operation. The current density produced in this MFC is slightly higher compared to the previous study that also used bacteria inoculum from POME that only produces 91.12 mA/m^2 [2]. The ability of bacteria to survive and degrade 2CP in MFC systems, despite its toxicity, may be demonstrated by the rising current output up to day 3. The current density generated further suggests that bacteria from POME could be classified as exoelectrogenic bacteria, capable of simultaneously producing current and degrading harmful pollutants.

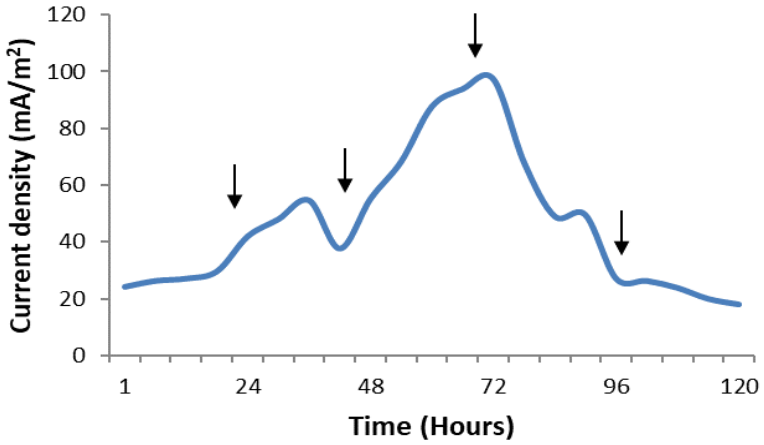

Figure 2. Current density generated by MFC fed with 2CP. The arrows indicate glucose feedings.

Phenolic degradation. The reduction of 2CP concentration in MFC and AC was observed over 5 days as depicted in Figure 3. Both MFC and AC were initially fed with 30 mg/L of 2CP. Figure 3 shows the degradation of 2CP by MFC and AC. Both MFC and AC reactors could degrade the 2CP. However, MFC showed a higher removal of 2CP compared to

AC which is 75% and 60% respectively. Different degree of abundance of biofilms and bacterial diversity could be one of the factors that contribute to the different performance between MFC and AC [6]. The degradation of 2CP in both MFC and AC proves that bacteria species from POME have excellent ability to adapt to the toxicity of the 2CP. Bacteria species from POME showed a good performance although the degradation rate is lower compared to the previous research that removed more than 90% of chlorophenol from the MFC [6, 10]. This is due to the fact that different types of bacteria from different sources of inoculum that develop biofilm on the anode have varied tolerances for hazardous compounds, thus require different metabolic adaptations.

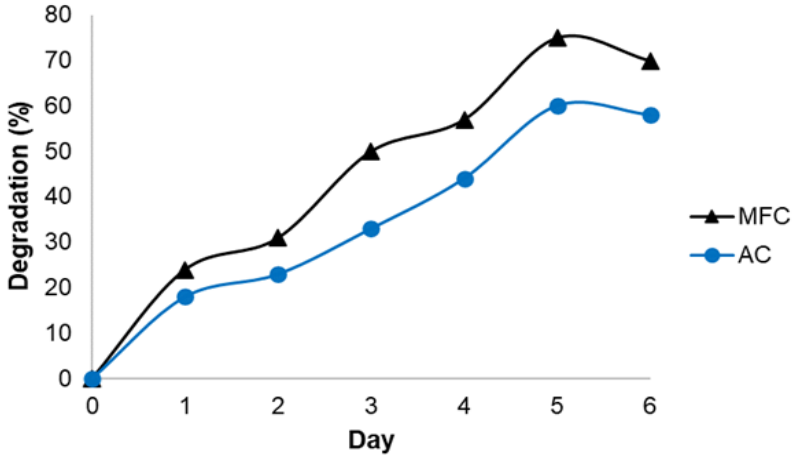

Figure 3. Degradation of 2CP by MFC and AC.

Anode microbial biofilm morphology. The biofilm formation on the MFC and AC electrodes was microscopically observed using FESEM and light microscope. Figure 4 shows image of the original carbon cloth electrode as compared to the MFC and AC electrodes that had been attached with bacteria. The anode surface structure was conserved in the MFC system, which might be attributed to reduced sludge and cell debris accumulation on the anode surface [11]. The unbroken anode surface structure could maintain the electron flows for current generation. In contrast, the anode surface of the AC was observed to be somewhat broken, indicating that there was intense anaerobic degrading activity, resulting in increased sludge and cell debris deposition on the anode.

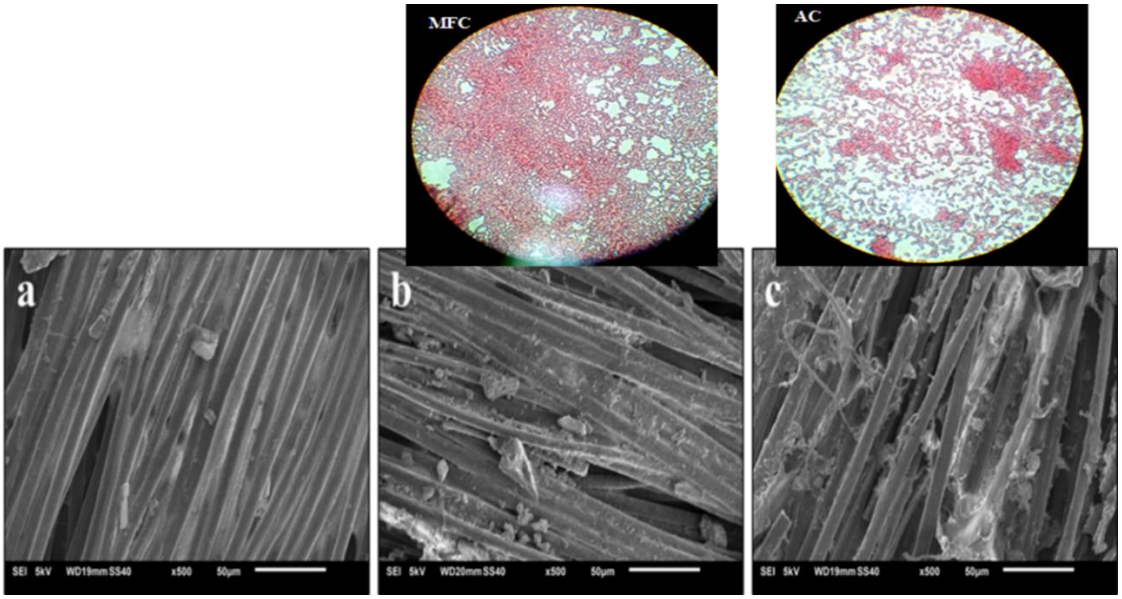

Figure 4. SEM and light microscope images of (a) original carbon cloth anode (b) anode biofilm in MFC and (c) anode biofilm in AC.

Figure 4 also shows Gram staining of the MFC and AC bacteria biofilm. After 5 days of operation, both MFC and AC biofilms contained slightly rod-shaped bacteria that were red in colour, indicating that the bacteria were gram-negative having a thin peptidoglycan wall. Based on the previous studies, most bacteria identified in MFC are belong to the class of *Proteobacteria* [12]. Under this bacterial class, *Geobacter* and *Shewanella* that are also gram-negative, mostly identified in MFC that can transfer electrons directly by using conductive pili [13]. Besides, it is clearly observed that the MFC biofilms are more abundant compared with that of AC, demonstrating a well-maintained biofilm formation for better 2CP degradation and current generation [14]. This also implies that bacteria in AC would not be able to survive until the end of operation, resulting in lower 2CP degradation efficiency.

Conclusion

A simultaneous current generation and high CP degradation by POME-based biofilm in MFC could be a good indicator of their electrogenicity. Compared with AC, MFC demonstrated better CP removal while maintaining good biofilm formation over 5 days of operation. Through microscopic characterization, MFC appears to be highly efficient for 2CP degradation and bio-electricity generation compared to AC systems that can only treat the contaminant with its low propensity for biofilm formation.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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