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Organic Removal Efficiencies and Power Production Capabilities of Microbial Fuel Cells with Pure Cultures and Mixed Culture

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Abstract

The voltage and the power production of four pure cultures and a mixed culture in five identical continuous-flow microbial fuel cells (MFCs) were evaluated and compared in the present study. Each microbial fuel cell was operated at four different flow rates with the external load resistance of 1000Ω. The result shows that good linear relationship between COD degradation rate and flow rate was obtained on the MFCs inoculated with *Pseudomonas putida*, *Corynebacterium glutamicum* and *Comamonas testosteroni*, not on the one inoculated with *Arthrobacter polychromogenes*. However, the relationship between COD degradation rate and flow rate on the MFC inoculated with mixed culture could be expressed as a binary quadratic equation. A lower influent pH favored the organic removal efficiency and degradation rate in the anode chamber of MFCs. The result also shows that the voltage outputs increased with the flow rate (shorter retention time) of anode chambers. However, good linear relationships between voltage and flow rate were only seen on the MFCs inoculated with *Comamonas testosteroni* and mixed culture. Among the five MFCs, the one inoculated with mixed culture displayed the highest voltage output at the same COD loading than others.

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1. Introduction

Microbial fuel cells (MFCs) have been considered a promising technology for power generation [1]. In MFCs, the microorganisms are able to convert the organic matter in wastewater into electricity. In a biological wastewater treatment system, if an anaerobic tank that acts as an anodic chamber is provided, the bacteria will use the organic matter in this tank to produce electrons and protons. The electrons will

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then be diverted toward an anode and subsequently flow across a conductive material containing a resistor, and finally be released in a cathodic chamber. The protons will also be transported to the cathodic chamber through the proton exchange membrane (PEM). Both electrons and protons will finally be depleted in the cathodic chamber, coupled with the reduction of oxygen to water [2].

Many researchers have used different species of pure culture in MFCs to generate electricity using this technique. Bond and Lovely [3] studied the power generation performance of *Geobactor sulfurreducens* and reported that production of current in MFC (65 mA/m^2 of electrode surface) or poised-potential (163–1143 mV/m^2) mode was greater than that reported in previous studies. Rabaey et al. [4] also claimed that the average power densities were 23.3±6.2 W/m^2 of anode for *Pseudomonas aeruginosa*, 4.9±1.8 W/m^2 of anode for isolate KRA1, and 28.4±2.3 W/m^2 of anode for isolate KRA3. They also concluded that the pure cultures yield lower power outputs than the mixed consortium. Min et al. [5] compared the power generation performance between a pure culture, *Geobactor metallireducens*, and a mixed culture, and reported power output of 40±1mW/m^2 for the former and 38±1mW/m^2 for the latter. Sun et al. [6] also found that higher power density can be obtained more easily by a mixed culture than a pure culture in MFCs. Rodrigo et al. [7] used an anaerobic pretreatment of the activated sludge of an urban wastewater treatment plant to test the electricity generation in a MFC. The power density generated in that study was found to depend mainly on the organic matter contain but not on the wastewater flow rate. They obtained a maximum power density of 25 mW/m^2.

In the present study, four pure cultures and one mixed culture (activated sludge) were individually inoculated into five identical MFCs and operated under the same conditions. Their organic removal efficiencies and power generation capabilities (voltage outputs) were then compared.

2. Materials and methods

2.1. Microorganism and artificial wastewater

One mixed culture and four pure cultures, *Pseudomonas putida*, *Comamonas testosteroni*, *Corynebacterium gultamicum* and *Arthrobacter polychromogenes*, were selected in the present study. These pure cultures were purchased from the Bioresource Collection and Research Center in Taiwan, and were incubated in our laboratory. Each pure culture was grown in 250 mL of disinfected artificial wastewater without aeration, and was allowed to acclimatize for two weeks. All species rapidly grew, and were then transferred to the anode chambers of four MFCs. *P. putida* was placed in MFC 1, *Corynebacterium gultamicum* in MFC 2, *Comamonas testosteroni* in MFC3, and *A. polychromogenes* in MFC 4. However, activated sludge was used as a mixed culture in MFC 5. Original sludge was obtained from the oxidation ditch of a wastewater treatment plant in Ping Tung, Taiwan and was grown with artificial wastewater anaerobically in our laboratory for about one month. The sludge liquid was then transferred to the anode chambers of MFC 5.

The artificial wastewater was prepared with 1000 mg/l glucose, 300 mg/l nutrient broth, 167 mg/l NH_4Cl, 25 mg/l K_2HPO_4, 25 mg/l NaH_2PO_4, 5 mg/l $FeCl_3$, 100 mg/l $MgCl_2$, 10 mg/l $MnSO_4$, and 133 mg/l $CaCl_2$. However, for mixed culture (sludge), *Comamonas testosteroni* and *A. polychromogenes*, 25 mg/l NaOH and 175 mg/l $NaHCO_3$ were added to adjust the different pH levels of the wastewater. For pure culture, the artificial wastewater was autoclaved, and the delivery tubes were disinfected with 95% ethyl alcohol before each use.

2.2. MFC design and operational conditions

All MFCs had the same size and configuration. For each MFC system, two similar-sized graphite carbon electrodes (CCM-400C; Central Carbon Co., Ltd., Taiwan) were used for both anode and cathode

(6.3 cm length × 4 cm width × 3 mm thickness). The electrodes were fixed in the respective anodic and cathodic chambers by fully inserting them into the liquids in both chambers. Both anodic and cathodic chambers were made of Plexiglas acrylic sheets, and each chamber had an effective water volume of 797 cm^3 (8.3 cm side water depth × 9.8 cm width × 9.8 cm length). All chambers were sealed with superglue. The covers and the connections of the anodic chamber were sealed with silicone to achieve anaerobicity in the chambers during operation. For MFCs 1-4, all chambers were disinfected with 95% ethyl alcohol before use.

The anodic chamber was sealed with superglue. Finally, the cover and all connections were sealed with silica gel to maintain an anaerobic condition in the chamber during operation. A peristaltic pump was used to pump fixed amount of artificial wastewater into each anodic chamber. The effluent of the anodic chamber first flowed into a water seal box, and then overflowed into an intermediate chamber so that anaerobicity was maintained in the anode chamber. The liquid in the intermediate chamber was periodically discharged by a peristaltic pump, which was controlled by a timer. This process ensured that continuous flow conditions at different flow rates were maintained by the MFCs. The fresh liquid from intermediate chamber was collected for analysis. Air was purged into each cathodic chamber by an air stone connected to a blower to supply oxygen needed for the electrochemical reaction. The dissolved oxygen concentration was maintained in the range of 7.1–7.5 mg/l. The cathodic chamber was filled with 30.8 mM of $KH_2PO_4 \cdot H_2O$ solution (adjusted to pH of approximately 7.0 by 1.0 N HNO_3) and aerated water as the cathodic electrolyte. The mixing in anodic chamber was very slow only to reduce the substrate gradient and to provide effective contact between the substrate and the culture; however, the mixing was only provided by aeration in cathodic chamber.

The anodic and the cathodic chambers were separated by a PEM (Nafion 117, Dupont Co., USA). The membrane was installed in a Plexiglas acrylic pipe (1.5 cm, inner diameter, and 23 cm, total length) connecting the anodic and the cathodic chambers. The anode and the cathode were connected externally with a concealed copper wire, and voltage was measured at an external load resistance 1000 Ω.

The MFCs were operated at an ambient temperature of 24–26 °C in our laboratory. All systems were run under identical continuous flow conditions at four different rates (0.50, 1.00, 1.44, and 2.88 l/day) to compare their power generation capabilities. Synthetic wastewater (influent) and effluents from the anodic chambers were collected for analysis. The performance of an MFC system was considered stable when both COD removal efficiency and power output were stable. The flow rate was set to 0.50 l/day in Phase 1. The voltage of each MFC was measured almost every day until the system was stable. When voltage readings became stable for a few days, influent and effluent water samples of MFCs and voltage readings during the last two days of Phase 1 were taken for COD analysis and power density calculation, respectively. The average COD concentration and the average voltage on both days were then used for the analysis. Similarly, MFC power production and water quality under flow rates of 1.00 (Phase 2), 1.44 (Phase 3), and 2.88 l/day (Phase 4) were evaluated.

2.3. Analyses of water quality and power generation

The influents (synthetic wastewater) and the effluents of the anodic chambers were collected for water quality analyses. All effluent samples were filtered through filter papers (Whatman grade 934AH) before COD analysis. COD was determined using the closed reflux method mentioned in the Standard Methods. pH, temperature, and DO (DO200; YSI, Inc., USA) of water in the cathodic chamber were also measured. The potentials were measured using a digital multimeter with data acquisition unit (U1253B, Agilent Technologies, Malaysia) and converted to power (mW) and power density (mW/m^3 of anodic chamber). All statistical data analyses were performed using the SPSS 13.0 software.

3. Results and discussion

3.1. Wastewater treatment characteristics

The influent and effluent characteristics of the five MFC anode chambers are listed in Table 1. The operational data, treatment efficiencies, and substrate degradation rates are listed in Table 2. The COD removal efficiency and the substrate degradation rate in the anodic cells with lower influent pH (i.e., MFCs 1 and 2) were higher than those with higher influent pH (i.e., MFCs 3, 4 and 5). According to the data in Table 2, a lower influent pH apparently favored organic matter removal efficiency and degradation rate in the anode chamber, in accordance with the report of Behera and Ghangrekar [8]. As the flow rate increased the HRT decreased in the anode chamber. The time required by the microorganisms in the anodic chamber to degrade organic matter decreased with increased flow rate. Therefore, the average COD removal efficiency of each culture decreased. However, the COD degradation rate in the anodic chambers increased as the flow rates increased from 0.5 to 2.88 l/day. The exception was that of A. polychromogenes, which decreased when the flow rate reached 2.88 l/day. Rodrigo et al. [7] reported that a small part (about 0.25%) of wastewater COD was used for power generation. Therefore, the COD degradation rates of all MFCs at different flow rates were expected to affect the MFC power generation performance. This will further be discussed in the following section.

Table 1. Influent and effluent characteristics of the five MFC anode chambers

Systems	Inf. COD (mg/l) (Range)	Eff. COD (mg/l) (Range)	Inf. pH (Range)	Eff. pH (Range)
MFC 1 (*Pseudomonas putida*)	1335.8 (1172-1380)	589.5 (323.0-872.0)	6.05 (5.78-6.29)	3.87 (3.54-4.38)
MFC 2 (*Corynebacterium glutamicum*)	1335.8 (1172-1380)	540.6 (327.5-1020.0)	6.05 (5.78-6.29)	3.77 (3.30-4.94)
MFC 3 (*Comamonas testosteroni*)	1171.4 (1072-1268)	713.8 (564.0-912.0)	8.34 (8.11-8.52)	5.91 (5.07-6.36)
MFC 4 (*Arthrobacter polychromogenes*)	1171.4 (1072-1268)	733.0 (512.0-1068.0)	8.34 (8.11-8.52)	5.64 (5.01-6.20)
MFC 5 (mixed culture)	1129.5 (1034-1290)	733.0 (206.0-822.0)	7.24 (6.23-7.85)	5.64 (5.21-7.12)

Fig 1 shows that good linear relationships between COD degradation rate and flow rate could be found on MFCs 1, 2 and 3 with the coefficient of determination (R^2) = 0.9096 and p = 0.05, R^2 = 0.8048 and p = 0.013, R^2 = 0.9787 and p = 0.002, respectively. The relationship between COD degradation rate and flow rate on MFC 5 can be expressed well as a binary quadratic equation (R^2 = 1.000 and p = 0.005). However, neither linear nor binary quadratic equation could be used to express the relationship between COD degradation rate and flow rate on MFC 4 (R^2 = 0.0867 and p = 0.699).

Table 2. Operational data, treatment efficiencies and COD degradation rates of five MFCs

Systems	Flow Rate (l/day)			
	0.50 (Phase 1)	1.00 (Phase 2)	1.44 (Phase 3)	2.88 (Phase 4)
	Hydraulic Retention Time (hours) of Anodic Chamber			
	38.3	19.1	13.3	6.6
	COD Removal Efficiency (%) COD Degradation Rate (kg COD/m^3/day)			
MFC 1	74.7	65.4	42.9	38.0
(*Pseudomonas putida*)	0.72	1.09	0.94	1.88
MFC 2	78.5	71.7	58.8	30.8
(*Corynebacterium glutamicum*)	0.75	1.19	1.29	1.52
MFC 3	50.0	37.0	31.3	26.0
(*Comamonas testosteroni*)	0.39	0.50	0.58	1.16

MFC 4 (Arthrobacter polychromogenes)	52.2 0.31	43.5 0.58	52.9 1.04	13.4 0.60
MFC 5 (mixed culture)	82.3 0.60	55.7 0.73	46.2 0.98	30.3 1.31

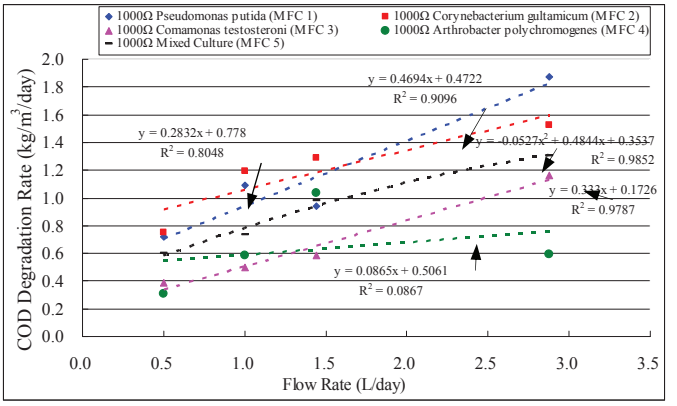

Fig. 1. Relationships between COD degradation rate and flow rate on all MFCs.

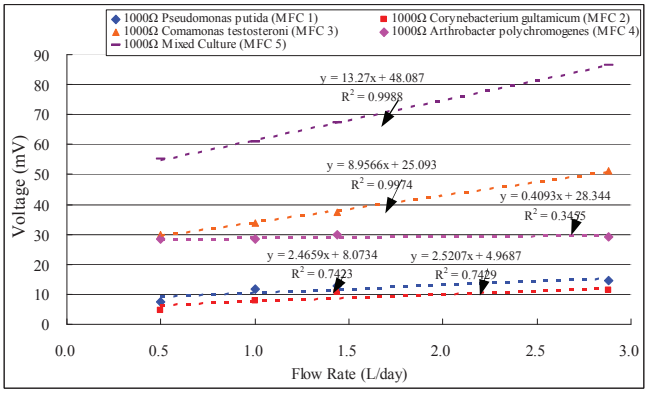

Fig. 2. Voltage production at different flow rates on all MFCs

3.2. Voltage outputs of MFCs at different flow rates

The voltage productions of five MFCs are shown in Fig. 2. The result shows that the voltage outputs increased with the flow rate (shorter retention time) in the anode chambers. As mentioned above, the COD degradation rate in the anodic chambers increased as the flow rates increased from 0.5 to 2.88 l/day. Therefore, more COD was possibly degraded and used for power generation at higher flow rates. The result also shows that MFC 5 produced higher voltage than others. The MFCs inoculated with *Comamonas testosteroni* and *A. polychromogenes* produced higher voltage than those inoculated with P. putida and *Corynebacterium gultamicum*. Therefore, the mixed culture displayed better power generation performance than the pure cultures selected in the present study. It is also speculated that lower pH in MFCs 1 and 2 might have inhibited the power generation activity of *P. putida* and *Corynebacterium glutamicum*. As mentioned above, the COD removal efficiency and the substrate degradation rate in the anodic cells were higher for *P. putida* and *Corynebacterium glutamicum* than for *Comamonas testosteroni*, *A. polychromogenes*, and mixed culture (see Table 2). Therefore, a lower pH may reduce the power

generation functions of both bacteria by partially inhibiting the conversion of degraded COD to electricity in MFCs 1 and 2. Similar results have also been reported by other researchers. Ren et al. [9] reported that power production significantly decreased when the pH in the anode chamber dropped to 5.2 due to the acidic products of fermentation. Behera and Ghangrekar [8] also concluded that a higher feeding pH (8.0) favored a more effective extracellular electron transfer and a higher power production. Jiang et al. [10] also revealed that a higher anodic pH favored a higher MFC power output. A low MFC pH apparently inhibited the activity of electrogenic bacteria, resulting in the severe inhibition of power generation.

Fig. 2 also shows that good linear relationship between voltage and flow rate was only seen on MFCs 3 and 5 (R^2 = 0.9974, p = 0.001 and R^2 = 0.9988, p = 0.001, respectively), which produced higher power output than other MFCs. The R^2 was 0.7423 for *P. putida* (p = 0.139) and 0.7429 for *Corynebacterium glutamicum* (p = 0.138). The linear relationship between power density and COD loading rate was low on *A. polychromogenes*, with R^2 values of only 0.3455 and p = 0.412. The voltage generation capability of *A. polychromogenes* might not be affected by flow rate. Based on the voltage outputs of the four pure cultures, we also suspected that the Gram-negative bacteria (*P. putida* and *Comamonas testosteroni*) can generate more power than the Gram-positive bacteria (*Corynebacterium glutamicum* and *A. polychromogenes*) under the same operational conditions and wastewater characteristics. However, further studies need to be undertaken to confirm these assumptions.

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