



Phenol degradation in microbial fuel cells

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ABSTRACT

Microbial fuel cell (MFC) has gained a great attention attributable to its ability in generating electricity directly from and potentially enhancing biodegradation of contaminants. In this study, MFCs using phenol or glucose–phenol mixture as the substrate (fuel) were designed to investigate the biodegradation of phenol. In an aqueous air cathode MFC using phenol (400 mg/L) as the sole fuel, electricity was generated during the phenol degradation. The degradation rates of phenol in the MFC increased about 15% as compared to the open-circuit control. Further experiments were conducted by using a graphite-packed MFC with a ferricyanide cathode. When phenol served as the sole fuel, the peak voltage output was obtained when 90% of phenol was depleted. A unique pattern of twin voltage peaks was observed when phenol–glucose mixture was used as the fuel. At the occurrence of the first and second voltage peaks, phenol was degraded by 20% and 90%, respectively, suggesting a preferential sequence in substrate consumption. The maximal power densities were 9.1 and 28.3 W/m³ for MFCs using phenol and glucose–phenol mixture as the fuel, respectively. Co-occurring with electricity generation, the degradation efficiencies of phenol in all the MFCs reached above 95% within 60 h. The results indicate that the MFC can enhance biodegradation of recalcitrant contaminants such as phenol in practical applications.

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1. Introduction

Microbial fuel cells (MFCs) have been operated successfully by using a variety of readily degradable compounds, such as glucose, acetate, monosaccharides, and complex carbohydrates (e.g., starch and biodegradable organics in food wastewater, swine wastewater, and domestic wastewater), as substrates (the fuel) [1–5]. In a few cases, some biorefractory organics, such as cellulose and petroleum contaminants, were also used as the fuel in MFCs [6,7]. The near-term application for MFCs was presumed to generate power from wastewater [8]. The amount of power produced from the MFCs varies on the specific sources of the fuel. For example, with similar designs of the MFC, 506 mW/m² was produced with acetate [3], but 261 mW/m² with swine wastewater [2], and 146 mW/m² with domestic wastewater [9]. Toxic and biorefractory organics, which were found frequently in the wastewater, have a great influence on the wastewater treatment and should be concerned in the related MFC research. However, the development of MFCs using recalcitrant contaminants as fuels is still in its infancy and warrants further research.

Phenol has been detected in effluents from industries, including coal gasification, pharmacy, and productions of pesticides, fertilizers, dyes, and other chemicals. Although phenol is biodegradable both aerobically and anaerobically, it can be growth inhibitory to microorganisms at elevated concentrations, even to those species that can use it as a substrate [10]. Degradation of phenol was also found incomplete for concentrations higher than 400 mg/L, and the residual phenol might inhibit the removal of N and P in wastewater treatment [11].

In the anaerobic environment, phenol was degraded by methanogens, denitrifying, iron bacteria, and sulfate-reducing bacteria [12–14]. However, methane-producing processes have not been widely used due to low energy recovery from phenol and high operational costs [15]. In the MFC, electricity can be produced directly from the degradation of organic matter and high energy recovery can be obtained [16]. While under the denitrifying, iron, and sulfate-reducing conditions, the exhaustion of these electron acceptors may prevent the complete degradation of phenol, and the anaerobic degradation rates are usually lower than that under aerobic conditions. In the MFC, electrons released from the substrate oxidation in the anode are transferred via the external circuit to the cathode, where the electrons are eventually consumed by the terminal electron acceptors. The terminal electron acceptors can be easily replaced or even non-exhausted (e.g., using oxygen in ambient air as the electron acceptor) [7]. Combining with the benefit

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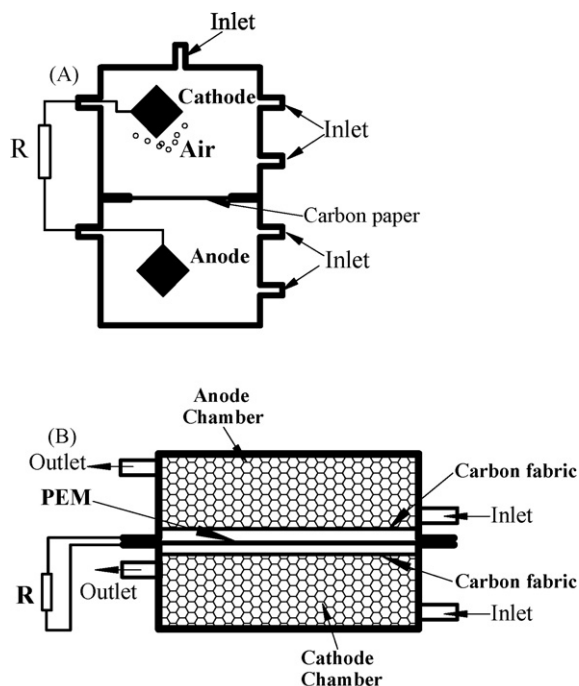


Fig. 1. Schematic diagrams of MFCs using an aqueous air cathode (A) and a ferricyanide cathode (B).

of power generation in offsetting the treatment cost, the MFC may offer a new technique in enhancing biodegradation of recalcitrant contaminants such as phenol in practical applications.

This study determined the degradation of phenol and power generation in an MFC with aqueous air cathode and a packing-type MFC using ferricyanide as the terminal electron acceptor. In some of the experimental treatments, co-substrate of glucose was used as fuel in the MFCs to reduce potential toxicity from phenol during shock load or temperature changes [17,18]. In addition, the use of co-substrates better represented field situations of mixed substrates. To our knowledge, the sequential utilization of substrates by the anodic bacteria in the MFC during power generation has not been investigated, when both recalcitrant (e.g., phenol) and readily degradable (e.g., glucose) substrates are mixed.

2. Materials and methods

2.1. MFC setup

Double chamber MFCs are often used in examining power production from using different substrates, or microbial communities that arise during the degradation of specific compounds [8]. Similarly, in this study a dual-chamber MFC with aqueous air cathode was constructed as shown in Fig. 1A. The diameter of the chambers was 7.0 cm. The electrodes were made of carbon paper (TGP-H-060, Toray Co., Japan) of the same size (5.0 cm × 5.0 cm) and the cathode was coated with a platinum (Pt) catalyst (0.40 mg Pt/cm²) on one side. The anode chamber was filled with substrate solution (pH 7.0). The cathode chamber was filled with the phosphate buffer solution (PBS) (pH 7.0) and continuously sparged with air. Both anodic and cathodic compartments have the same volume of 440.0 mL. The anode and cathode chambers were separated by a piece of carbon paper.

In follow-up tests, a packing-type MFC was constructed using granular graphite (#16–20, porosity 10%) as the packing material in both anode and cathode chambers (Fig. 1B). Both electrodes

were made of carbon cloth (UT70–20, Toray Co., Japan) of the same size (2.0 cm × 14.0 cm). The anode and cathode were separated by a proton exchange membrane (PEM, Nafion 212, Dupont Co., USA). The total volume of the anodic compartment was 58.0 mL with the packing and non-packing net volumes of 25.4 and 32.6 mL, respectively. The anode chamber was filled with substrate (pH 7.0). For the cathode, a 100 mM PBS was prepared and enriched with 50 mM of potassium hexacyanoferrate to optimize mass transfer efficiency between the cathode and terminal electron acceptor, and to avoid the loss of substrate (i.e., phenol) due to dissolved oxygen, which might leak through the PEM membrane and used by bacteria as the electron acceptor [19,20]. The substrate and ferricyanide solutions were cycled using a peristaltic pump with a flow rate of 20 mL/min.

A copper wire was used to connect the circuit containing a resistance of 1000 Ω (unless stated otherwise). All exposed metal surfaces were sealed with nonconductive epoxy resin. When the voltage was lower than 50 mV and the phenol concentration was less than 50 mg/L, the chambers were refilled with the same materials as those at the initial stage.

2.2. Microbial inoculum and medium

Bacteria that thrive in MFC biofilms are distributed across many phylogenetic subclasses, such as α-, β-, δ-, γ-subclass Proteobacteria [21–25]. For quick start-up of the MFC, the MFCs were inoculated with 200 mL of mixed aerobic activated sludge and anaerobic sludge (1:1, v/v), which were known to contain a greater diversity of electrochemical active bacteria. The sludge inocula were collected from the Liede Municipal Wastewater Treatment Plant of Guangzhou City, China. The packing-type MFCs were inoculated using pre-acclimated bacteria from the anode of an aqueous air cathode MFC that had been running in the fed batch mode for over 5 months. Substrates used in the experiments included glucose, glucose–phenol mixture, and phenol. The experiments with phenol (1000 mg/L) and glucose (500 mg/L) as the mixed fuels were conducted by the packing-type MFC. The anodic medium consisted of (per liter of deionized water): Na₂HPO₄ 4.0896 g, NaH₂PO₄ 2.544 g, NH₄Cl 0.31 g, KCl 0.13 g, trace metals solution 12.5 mL, vitamin solution 12.5 mL [26]. The initial pH of all solutions was adjusted to 7.0 and all MFCs were operated in a temperature-controlled lab at 30.0 ± 0.1 °C.

2.3. Analysis

Samples of the anode solutions were taken every 12 h for measurements of glucose and phenol concentrations. Glucose concentrations were analyzed by the anthrone method [27]. Phenol concentrations were analyzed using the 4-aminoantipyrine spectrophotometric method [28].

Voltages across the resistance were measured using a multimeter and data were automatically recorded by a data acquisition system. Area power density (P_A , W/m²) and volumetric power density (P_V , W/m³) are calculated as follows:

$$P_A = \frac{IU}{A} \quad (1)$$

$$P_V = \frac{IU}{V} \quad (2)$$

where I is the current (A), U is the voltage (V), A is the cross surface area of the anode or cathode (m²), and V is the non-packing volume of the anodic compartment (i.e., the volume of the liquid media) (m³). The volumetric power density indicates how much power is generated from unit volume of wastewater. The Coulombic

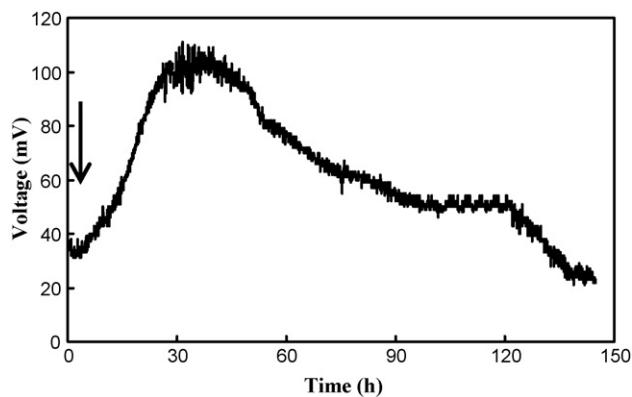


Fig. 2. Electricity voltage output of the aqueous air cathode MFC using phenol as sole fuel at a concentration of 400 mg/L. The arrow shows the time of anode solution replacement.

efficiencies (CEs) (%) are calculated by:

$$CE = \frac{\sum_{i=1}^n U_i t_i}{RFb\Delta SV} M \times 100\% \quad (3)$$

Here U_i is the output voltage of MFC at time t_i , R is external resistance, F is Faraday's constant (96 485 C/molelectrons), b is the number of moles of electrons produced per mol of COD (4 mol of e^- /mol of COD), ΔS is the removal of COD concentration (g/L), V is the liquid volume (L), M is the molecular weight of oxygen (32 g/mol) [3].

The maximum power density was determined by adding fresh substrate to the MFC and establishing constant power, changing the external resistances over a range of 50–5000 Ω , and recording the voltage (typically 5–10 min per resistance) [29]. The power was then calculated for each resistance as a function of the current.

3. Results

3.1. Power generation from phenol in the MFC with aqueous air cathode

Power was generated in the MFC with aqueous air cathode and phenol (400 mg/L) as the sole fuel. A lag time about 300 h was observed before the constant voltage output was established. The maximum output voltage measured was in the range of 111–140 mV (the external resistance $R = 1000 \Omega$). Constant and repeatable power cycles were obtained during six refills of the anode chamber. One of the representative cycles was presented in Fig. 2. The average maximum output voltage and the average maximum power density obtained from the MFC were 121 mV and 6 mW/m² (anode)

Table 1

Concentrations and removal rates of phenol in MFCs under closed and opened circuit conditions

Time (h)	Closed circuit		Opened circuit	
	Phenol (mg/L)	Phenol removal (%)	Phenol (mg/L)	Phenol removal (%)
0	400.0	0	400.0	0
24	271.3 ± 26.3 ^a	32.2 ± 6.6	326.4 ± 3.7	18.4 ± 0.9
48	198.3 ± 44.0	50.4 ± 11.0	237.9 ± 8.5	40.5 ± 2.1
72	130.7 ± 37.4	67.3 ± 9.3	163.8 ± 18.7	59.0 ± 4.7
96	76.3 ± 27.7	80.9 ± 6.9	105.9 ± 21.7	73.5 ± 5.4
120	39.6 ± 15.6	90.1 ± 3.9	64.7 ± 18.1	83.8 ± 4.5
144	18.2 ± 6.7	95.5 ± 1.7	46.6 ± 15.6	88.3 ± 3.9

^a The mean value and standard deviation of multiple cycles ($n = 3$).

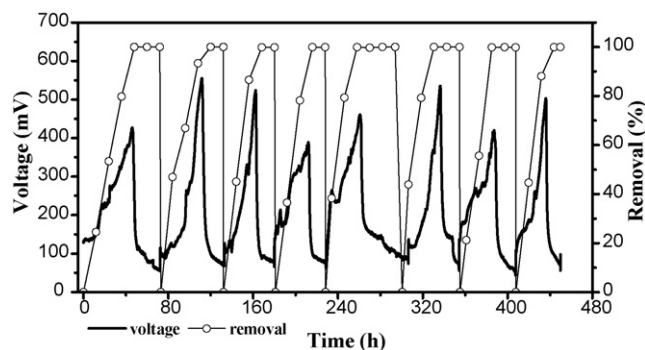


Fig. 3. Electricity voltage output and the phenol removal of the ferricyanide cathode MFC using phenol as sole fuel at a concentration of 1000 mg/L.

($R = 1000 \Omega$), respectively. At the end of each power cycle, the average removal of phenol was 85%.

Phenol degradation rates in MFCs with closed circuit were compared to those in MFCs with opened circuit. Phenol degradation rates in the closed circuit MFCs were 8–14% higher than those in the opened circuit MFCs, based on the mean and standard deviation values calculated from multiple runs (Table 1).

3.2. Power generation from phenol using ferricyanide cathode MFC

To pursue greater power output from phenol as the fuel, we tested a packing-type MFC using ferricyanide as the terminal electron acceptor. Shorter acclimation time was observed in the MFCs using ferricyanide cathode (about 80 h) than that in the MFCs using aqueous air cathode (about 300 h). During eight cycles of continuous electricity generation with 1000 mg/L phenol as the fuel, one peak voltage occurred corresponding to each cycle (Fig. 3). The maximum output voltage ranged from 387 to 540 mV ($R = 1000 \Omega$), presumably attributable to the metabolic fluctuations of microbial populations in the anode chamber.

Within 48 h of each electrical cycle, the removal of phenol in the anode chamber reached more than 90%. The maximal removal rate of phenol was usually at the point when the voltage output reached the peak (Fig. 3).

3.3. Influence of the supplemental glucose on the performance of ferricyanide cathode MFC

When phenol–glucose mixture was used as the fuel, the voltage output of the MFCs showed a distinctive twin-peak pattern on the voltage–time curves. After each fuel refill, the first peak voltage appeared within 10 h and the second peak emerged 28 h following the first peak. This twin-peak pattern remained consistent throughout the electrical cycles (Fig. 4), and the first peaks (>650 mV) were always higher than the second ones (about 600 mV).

Consumptions of phenol and glucose corresponded with the increase of output voltages. The average degradation of glucose reached 80% within 12 h of the MFC establishment. The degradation of phenol was close to 20% when the first peak voltages appeared and 90% at the second peak voltage. Within 60 h, the degradation of phenol was above 95% (Table 2).

Power density was obtained by measuring stabilized voltages at external resistances ranging from 50 to 5000 Ω . As shown in Fig. 5, when the first peak voltage (635 mV) appeared, the maximal volumetric power density was determined to be 28.3 W/m³ with a current density of 58.9 A/m³, and the corresponding max-

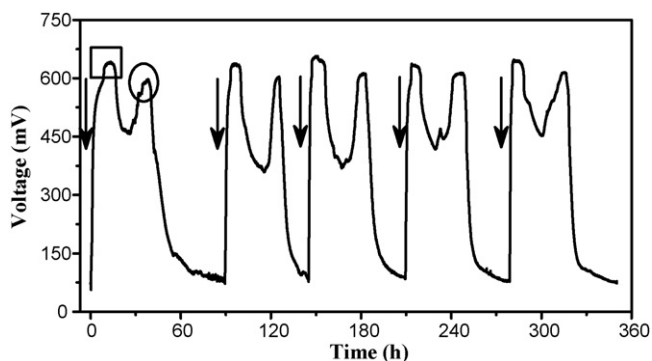


Fig. 4. Electricity voltage output of the MFC using phenol–glucose mixture as fuel. The arrows show the replacement time of fuels. The square and circle show the time of the first and second voltage peaks in each cycle, respectively.

imal area power density was 342.0 mW/m^2 (cathode). When the second peak voltage (599 mV) appeared, the maximal volumetric power density and area power density were 12.6 W/m^3 and 152.2 mW/m^2 (cathode), respectively, with a current density of 39.3 A/m^3 .

3.4. Substrate utilization in the ferricyanide cathode MFC

The amounts of coulomb recovery by the MFCs were calculated based on the electrical cycles shown in Fig. 6. The electrical charges obtained by the MFC were 92.0, 47.8, and 39.4 C when using phenol–glucose mixed, glucose, and phenol as the fuel, respectively. The Coulombic efficiencies of the MFC were 2.7%, 7.7%, and 1.5% when using phenol–glucose mixed, glucose, and phenol as the fuel, respectively.

Table 2
Concentrations of phenol and glucose in MFCs using phenol–glucose mixture as the fuel

Time (h)	Phenol (mg/L)	Glucose (mg/L)
0	1000	500
12	709.6 ± 29.4^a	86.2 ± 1.7
36	403.0 ± 63.1	3.4 ± 2.4
48	153.3 ± 28.5	0.9 ± 0.8
60	0.2 ± 0.3	ND ^b
72	ND	ND

^a The mean value and standard deviation of multiple cycles ($n = 3$).

^b Not detected.

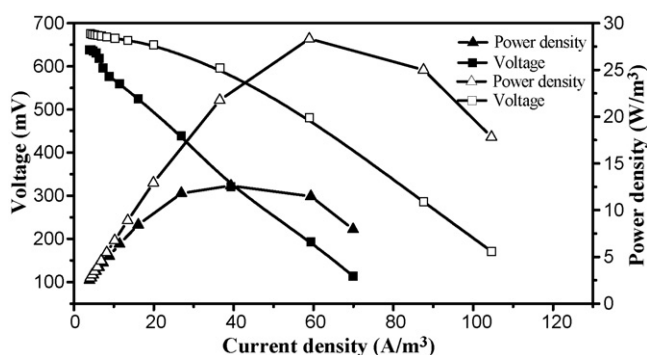


Fig. 5. Power density curves when the first (hollow symbols) and second (solid symbols) peak voltages appeared.

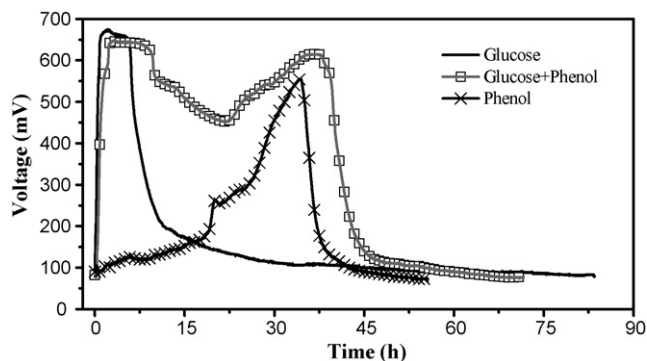


Fig. 6. Voltage–time curves for the MFCs using glucose, phenol, and phenol–glucose mixture as fuels.

4. Discussion

4.1. Power generation

The study was initially conducted using the MFC with oxygen as the terminal electron acceptor in the cathode. The maximum power density (6 mW/m^2 anode) obtained was substantially lower than that from MFC studies using readily degradable compounds, such as 494 mW/m^2 from glucose and 305 mW/m^2 from butyrate [9,3]. The recalcitrance of phenol and the inadequate population of capable microbes might have resulted in the slower phenol degradation and lower power output, as attested by the extended lag time (approximately 300 h). To improve the MFC efficiency in electricity generation, a graphite packing-type MFC was constructed to provide larger surface areas to enhance bacterial growth. Ferricyanide was used as the cathode electron acceptor due to its higher oxidizing potential than oxygen, which avoided the influence of oxygen to the phenol removal. When using 1000 mg/L phenol as the sole fuel, the power generated (9.1 W/m^3 with $R = 1000 \Omega$) was comparable to the maximum power densities obtained from oxygen cathode MFCs fed with acetate (12.7 W/m^3) or butyrate (7.6 W/m^3) [3]. More importantly, our results demonstrate that recalcitrant compounds such as phenol can be used as the fuel in the MFC for power generation. Although *Geobacter* species can use aromatic compounds as sole carbon sources and electron donors, the carbon sources that most pure cultures of various electricity-generating bacteria utilize are primarily limited to easily biodegradable organics, such as organic acids and fermentative products [30–32]. Results from this study agreed with others in the literature that mixed microbial populations appear to perform well in MFCs when complex organics are used as the fuel [2,5,33]. Efforts are currently attempted to use molecular techniques to characterize microbial communities on the anode and in the anodic chamber in the phenol-degrading MFCs.

The maximal voltage outputs obtained from the MFCs containing phenol–glucose mixture were obviously higher than that from the MFCs containing phenol only. The glucose co-substrate presumably stimulated the growth of whole populations of microbes in the anode chamber. In addition, more electrons might be generated in unit time from the synchronous degradation of phenol and glucose by the same or different consortia of microbes.

The observed twin-peak pattern of the voltage peaks in the MFCs using phenol–glucose mixed fuel has not been reported before. Although it appeared that there was a preferential order in substrate degradation for the mixed substrates, the correlation of the two peaks with the degradation sequence of glucose and phenol was difficult to determine, because of the possible formation of degradation intermediates.

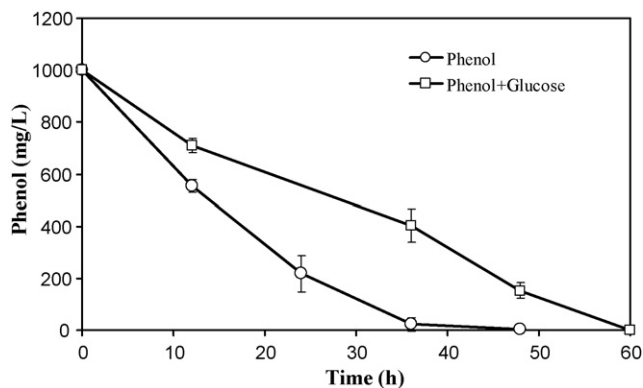


Fig. 7. Comparison of phenol degradation rates in the MFCs using phenol and phenol–glucose mixture as fuels.

4.2. Degradation of organic compounds

Results from this study demonstrate that the MFC can enhance phenol degradation as compared to the open-circuit controls, in which the normal anaerobic metabolism prevailed. We attributed this enhancement to the transfer of electrons to the terminal electron acceptor of oxygen in the cathode, instead of other electron acceptors such as sulfate and metals in the anaerobic anode chamber, realizing an indirect aerobic degradation [7]. Therefore, the MFC technology may be applied in phenol treatment in the anaerobic environment such as groundwater, which is frequently depleted of indigenous terminal electron acceptors (e.g., nitrate or Fe^{3+}).

Tay et al. [18] indicated that glucose supplement at the concentrations of 500–4000 mg/L promoted biodegradation of phenol at concentrations of 420–2100 mg/L. As shown in Fig. 7, a supplement of glucose at 500 mg/L initially delayed the phenol (1000 mg/L) degradation in the MFC. Microorganisms in the anode chamber of the MFCs might prefer glucose as the initial substrate when phenol–glucose mixture was the fuel, rendering a tentative delay in phenol degradation. When microbial populations increased through glucose metabolism, the degradation of phenol was resumed and substantially enhanced.

4.3. Coulombic efficiency and substrate utilization

The power generation using phenol as the sole fuel was lower than that using glucose, although the theoretical amount of coulombs contained in phenol was three times higher than glucose (calculations based on the concentrations of phenol and glucose containing in the substrates). Results of CEs indicated that the degradability of substrates had a great influence on the MFC power generation, which was consistent with other studies [3,8]. The amount of electrical charges obtained by MFCs fueled by the phenol–glucose mixture was 4.8 C higher than the sum of power from the two MFCs fueled with the same amount of phenol and glucose individually. The presence of the glucose co-substrate apparently enhanced the phenol degradation and subsequent electricity generation.

The Coulombic efficiency calculated based on the total substrate was less than 10% in the MFCs, indicating a substantial loss of electrons in the system. Coulombic efficiencies reported by others vary widely from 0.04% to 97% [23,31,34,35]. Many factors could attribute to the electron consumption in MFCs: (1) Mineralization of phenol was incomplete in the anode chamber, based on that the COD removals were in the range of 83.9–96.4%. (2) The substrate was oxidized by other anaerobic microbes such as methanogenic archaea. The comparison of phenol removals in the closed circuit

MFC and the open-circuit control indicated that other respiratory manners, such as methane-production, were carried out simultaneously with the electron transfer to the electrode. (3) Electrons transferred from substrate to other non-electrode electron acceptors in the solution, such as sulfate that came in with trace metals and oxygen [9]. (4) System internal resistance may also account for a significant portion of the reduction in the CE.

5. Conclusions

Electricity was successfully generated by using phenol as the fuel in double chamber MFCs inoculated with sludge that contained a mixed populations of bacteria. In an aqueous air cathode MFC using phenol (400 mg/L) as the sole fuel, electricity was generated during the phenol degradation; and the degradation rates of phenol in the MFC were increased by approximately 15% as compared to the open-circuit controls. Further experiments were conducted using a graphite-packed MFC with a ferricyanide cathode. When phenol served as the sole fuel, the peak voltage output was obtained when 90% of phenol was depleted. A unique pattern of twin voltage peaks was observed when phenol–glucose mixture was used as the fuel. At the occurrence of the first and second voltage peaks, phenol was depleted by 20% and 90%, respectively, suggesting a preferential sequence in the substrate consumption. The maximal power densities were 9.1 and 28.3 W/m³ for MFCs using phenol and glucose–phenol mixture as the fuel, respectively. Co-occurring with electricity generation, the degradation efficiencies of phenol in all the MFCs reached above 95% within 60 h. The results indicate that MFC may be a novel method in enhancing biodegradation of recalcitrant contaminants such as phenol in practical applications.

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