Treatment of Phenolic Wastewaters in Microbial Fuel Cell Using Freely Suspended and Immobilized Cells

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ABSTRACT A microbial fuel cell (MFC) is a device that allows the oxidation of organic and inorganic substrates while simultaneously generating electrical energy. In this work, MFC-type bioreactor was used to degrade phenol, a toxic aromatic pollutant commonly found in industrial wastewaters. Biodegradation of phenol was carried out using Pseudomonas putida as biocatalyst in dual chamber MFCs. Sterile McKinney’s medium and phenol were placed in the anode while potassium ferricyanide was used in the cathode as the electron acceptor. Single graphite rod electrodes were used in experiments with freely suspended cells, while granular graphite electrodes were employed in MFCs to immobilize bacterial cells. Phenol concentrations ranging from 100-1000 mg L⁻¹ were tested under batch mode of operation. Results showed that biodegradation of phenol was accompanied by increase in biomass concentration and circuit potential and concomitant generation of current. Biodegradation rates as well as power and current produced in MFCs with granular graphite electrode were significantly higher than those obtained in the MFCs with single rod electrode. Finally, biodegradation of phenol was also achieved effectively.
in MFCs operated continuously and that both biodegradation rates and power generation in these systems were higher than MFCs operated batchwise. These results served as basis for expanding the application of MFCs to biodegradation of pollutants from livestock and domestic wastewaters.

**Keywords:** renewable energy, microbial fuel cell, wastewater, biodegradation, phenol

**INTRODUCTION** A microbial fuel cell (MFC) is a device that is capable of oxidizing organic and inorganic substrates while simultaneously generating electrical energy directly without any intermediary step. This promising technology has the potential to treat pollutants and wastes and utilize them as source of usable energy. This ability of MFCs makes them well suited for treatment of wastewaters. Wastewaters produced from domestic, agricultural, and industrial operations contain different types of organic compounds including proteins, carbohydrates, fatty acids, and aromatics (Tchobanoglous et al., 2003). One of the most common aromatic compound found in wastewaters is phenol. Phenols are commonly found in wastewaters generated from various industrial operations such those from the processing of coal tar, plastic, textile, varnish, gas liquor and petroleum (Kumar et al., 2005; Christen et al., 2012). Many phenolic compounds are considered priority pollutants by the US Environmental Protection Agency (EPA, 2011) and have been shown to be water-soluble and highly mobile (Collins and Daugulis, 1997) which in significant amounts could seriously contaminate streams and other bodies of water. Preventing high levels of phenol in surface waters is essential since it is toxic to fish and gives an objectionable taste to drinking waters even at low concentrations (Hill and Robinson, 1975; Seker et al., 1997; Kumar et al., 2005).

Removal strategies for waters contaminated with phenol include physical, chemical and biological techniques (Christen et al., 2012; Antony et al., 2013). Preference for employment of biological techniques is due to its ability to completely mineralize phenolic compounds and for being environmentally friendly and low cost (Agarry et al., 2008; Al-Khalid and El-Naas, 2012; Christen et al., 2012). The use of MFCs can be an effective bioremediation strategy with the ability to degrade phenol and at the same time produce direct energy. Biodegradation of phenol in MFCs can be achieved through the use of microorganisms that can utilize phenol as their energy source and are able to release electrons to an electrode. Selection of microorganisms as biocatalyst in an MFC is vital since phenol is known to cause inhibition of microbial activity even at relatively low concentrations (100 mg L$^{-1}$) (Hill and Robinson, 1975). In this work, *Pseudomonas putida* was used in batch and continuously operated MFCs for removal of phenol. Biodegradation of phenol was conducted using bacterial cell suspension in MFCs with single rod electrode and with immobilized cells in MFCs with granular electrode. MFC performance in terms of phenol biodegradation and current and power output was assessed.

**MATERIALS AND METHODS**

**Experimental setup** Microbial fuel cell type bioreactors in H-type configuration were used in all tests (Figure 1). The MFC was constructed with two identical glass cylindrical chambers, serving as the anode and cathode. Each chamber (approximately 285 mL volume) was fabricated with inlet and outlet ports and a flanged glass extension in the lower part which allowed the coupling of the chambers by an adjustable clamp. A 0.09 mm thick Nafion high exchange capacity proton exchange membrane (HEPEM, NE-1035 Alfa Aesar, Ward Hill, MA, USA) was used to separate the anodic and cathodic chambers. Graphite electrodes were used in the MFC either in rod or granular form. Single graphite rod electrode was used in tests with freely suspended cells while granular
A pure strain of *Pseudomonas putida* (ATCC 23973) was used in all tests. In MFCs operated batchwise, phenol concentrations of 100, 250, 500 and 1000 mg L⁻¹ were tested. After preparation of MFC setup, designated amounts of phenol was added to the anodic chamber and inoculated immediately. Approximately 1 mL of a concentrated suspension of *P. putida* cells was injected into the anodic solution as inoculum resulting in initial biomass concentration of 29-64 mg cell L⁻¹. The content in both chambers were constantly mixed using a magnetic stirrer. Samples of solution from the anodic chamber were regularly collected to determine phenol and biomass concentrations. Moreover, continuous monitoring of circuit potential was done, while current and power were measured periodically. After completion of batch tests, the MFC was switched to
continuous mode of operation by using a peristaltic pump to supply feed solution into the anodic chamber. The MFC was fed with modified McKinney’s medium containing 100 mg L⁻¹ phenol. Prior to use, the feed solution was sterilized in an autoclave, cooled down to room temperature and then purged with nitrogen gas. Feed was pumped at an initial flowrate of 1.2 mL h⁻¹. After establishment of steady-state conditions, the MFC was operated at the same flow rate for at least three additional residence times before flow rate was increased. The MFC was continuously operated at increasing feed flow rate up to a level where MFC performance was observed to have deteriorated (i.e., decrease in phenol biodegradation rate).

The experiments in MFC with granular graphite electrode were conducted essentially similar to the tests in MFCs with single rod electrode. Granular electrode was used with the aim of increasing the surface area of electrode to improve electron transfer and to provide a matrix with large surface area for cell immobilization and formation of biofilm. Prior to use, graphite granules were disinfected with bleach and rinsed thoroughly with water. Since stirring was not possible with granular electrode, a recirculation loop was devised in the anodic chamber where liquid content was withdrawn from the top of the anodic chamber and reintroduced into the bottom using a separate peristaltic pump. The recirculation flow rate was approximately 3000 mL h⁻¹. Batch tests were conducted in the same MFC with different concentrations of phenol, specifically 100, 250, 500 and 1000 mg L⁻¹ added sequentially starting with the lowest concentration. Following completion of batch tests, MFC was switched to continuous operation starting at a similar flowrate of 1.2 mL h⁻¹ and subsequently increased up to a level wherein MFC performance deteriorated. Concentration of phenol and circuit potential was constantly monitored similar to previous tests. In MFC tests with granular electrode, biomass concentration was not measured since optical density readings were not reliable due to added turbidity by particles from the granules.

**Analyses**

Determination of biomass concentration (in tests with freely suspended cells) was done by measuring the optical density (OD) of samples at 620 nm and then converting the readings to biomass concentration (mg cell-dry weight L⁻¹) using a predetermined calibration curve. Concentration of phenol was determined using an HPLC (Agilent Technologies 1200), equipped with a Di-Array detector (DAD) and Eclipse Plus C-18 column (150 mm x 4.6 mm dia.). To measure current and power generated from the MFC, external resistors in the range 50-6000 ohms (You et al., 2006) were applied during the biodegradation of phenol. Calculations of power and current were done following Ohm’s law. Current and power densities were calculated using the surface area of the single rod electrode (submerged portion) and in terms of working volume of the anodic chamber when granular electrode was used.

**RESULTS AND DISCUSSION**

Experiments were initially conducted in MFC with single rod electrode and freely suspended cells applied with an initial phenol concentration of 100 mg L⁻¹. After inoculation, it was observed that biomass concentration increased as phenol concentration was reduced, indicating the progress of the biodegradation process. Degradation of phenol continued and conversely biomass increased. As phenol became depleted, biomass growth was observed to level off and subsequently decreased as phenol was exhausted. The pattern observed in the biodegradation process was also reflected in the profile of the open circuit potential (OCP). The OCP started to increase as phenol degradation commenced and continued to increase as phenol degradation and biomass growth proceeded. The OCP reached its peak when biomass growth slowed down prior to complete depletion of phenol. The same observed patterns were also detected in similarly operated MFCs fed with 250, 500 and 1000 mg L⁻¹ phenol. However, as initial concentration was increased to 500 mg L⁻¹ phenol, a lag phase in biomass growth was observed which was significantly prolonged when 1000 mg L⁻¹ phenol was utilized. This finding reflects the known inhibitory effects of phenol especially at higher concentrations. Moreover, results showed that longer lag phase was encountered with higher phenol concentration similar to those observed
by Dapaals and Hill (1992) in conventional bioreactors where the increase in length of lag phase in growth of \textit{P. putida} was found to be exponential as phenol concentration was increased up to 600 mg L$^{-1}$.

In MFC with granular electrode, successful biodegradation of 100 mg L$^{-1}$ phenol was achieved and complete depletion of phenol was accomplished significantly faster (approximately 9 times) than in similarly operated MFC with single rod electrode. Faster biodegradation at the higher concentrations of 250, 500 and 1000 mg L$^{-1}$ phenol was also observed. Tests were repeated at each concentration by sequential addition of similar concentrations except at the highest tested concentration of 1000 mg L$^{-1}$ phenol since considerable time for complete degradation (>9 days) was required at this level. As stated earlier, biomass concentration could not be determined in MFC with granular electrode but concentration profiles of phenol indicated that practically no lag phase was detected in all tested concentrations. This was in contrast with the observed lag phase found in MFCs with single rod electrode especially at higher phenol concentration.

The biodegradation rates of phenol achieved in MFCs with single rod and granular electrodes fed with various concentrations of phenol are shown in Figure 2. The lowest biodegradation rate was attained when the lowest initial phenol concentration of 100 mg L$^{-1}$ was fed in MFC either with single rod or granular electrode. Increase in initial feed concentration to 250 mg L$^{-1}$ resulted to improvement in phenol biodegradation rate. However, further increase in initial concentration resulted to a decline in the biodegradation rate. In terms of power and current output, a similar trend was also observed at different initial phenol concentrations. The lowest power and current output was achieved when MFC with single rod electrode was fed with 100 mg L$^{-1}$ phenol. Significant improvement in power and current was observed as initial phenol concentration was increased. However, further increase in initial concentration to 1000 mg L$^{-1}$ resulted to substantial decrease in power and current output. The deterioration in performance of MFC as initial phenol concentration was increased could be attributed to the known inhibitory effects of phenol especially at higher concentrations.

Comparison of MFC performance between single rod and granular electrodes showed that phenol biodegradation rates were significantly higher in MFC with granular electrode than in similarly operated MFCs with single rod electrode. The improved performance was also reflected in current output from MFC with granular electrode which was almost 10 times higher than in similar MFC with single rod electrode. These findings show that the use of granular electrode in MFC enhances biodegradation of phenol and the corresponding current output. It can be speculated that the presence of more biomass in the granule matrix resulted to higher biodegradation rates. Accumulation of biomass around the surface of granules, especially in the vicinity around the entry port of the recirculation loop, was observed as the sequential tests progressed indicating immobilization of bacterial cells. Bioreactors with immobilized cells have been shown to have better biodegradation performance such as those in batch tests conducted by Annadurai et al (2011) in shake flasks where immobilized cells of \textit{P. putida} were found to be superior in the degradation of 100-400 mg L$^{-1}$ phenol than with freely suspended cells.
Figure 2. Biodegradation rates of phenol at different initial concentrations in MFCs with single rod or granular graphite electrode.

Continuous biodegradation of phenol in MFC with single rod electrode was found to be challenging with the system only capable of handling relatively low feed flowrates under 5 mg L\(^{-1}\) h\(^{-1}\) before deterioration in MFC performance was detected. Additionally, no substantial improvement in the biodegradation rate was observed as feed flowrate was incrementally increased. The biodegradation rates achieved from all applied flowrates covering a range of only 0.3 mg L\(^{-1}\) h\(^{-1}\) reflected a rapid decline in the efficiency of phenol removal from 94% to 39%. In MFC with single rod electrode, biodegradation rate and corresponding power and current output attained in the continuous system was comparable to those achieved in the batch system. In contrast, MFC with granular electrode was found to achieve improved performance when operated continuously with biodegradation rate more than 3 times higher than in the batch system. Subsequently, power and current output in MFC with granular electrode was significantly enhanced after switching to continuous operation. Overall, MFC performance in terms of phenol biodegradation and power and current output were substantially improved when granular electrode was used.

CONCLUSIONS In this study, the use of MFCs was shown to be effective in the biodegradation of phenol, a complex structured organic pollutant found in wastewaters. Results revealed that biodegradation of phenol was achieved with concomitant generation of energy. In MFC with single rod electrode and freely suspended cells, it was observed that biomass concentration increased as biodegradation of phenol proceeded. Moreover, biodegradation rate and corresponding power and current output improved with increase in initial phenol concentration but deteriorated when higher concentration (>500 mg L\(^{-1}\)) was applied, indicating possible inhibition effects of the substrate. When MFC was operated continuously, resulting current output was significantly higher than in the batch system although no significant improvement in the biodegradation rate was observed. With
the use of granular electrode, performance of MFC in terms of phenol biodegradation and energy output was significantly enhanced when switched to continuous operation. Furthermore, the use of granular electrode and immobilized cells in MFC resulted to significantly faster phenol biodegradation and higher power and current output compared to similarly operated MFC with single rod electrode. This work have shown that application of MFC, especially with immobilized cells, has the capability to effectively degrade pollutants, including complex structured organic compounds such as those from livestock and agricultural operations. Moreover, unlike other wastewater treatment strategies, the use of MFCs has the added benefit of generating direct and sustainable energy that could offset energy cost associated with the treatment process.

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REFERENCES