Mineralization of Pentachlorophenol With Enhanced Degradation and Power Generation From Air Cathode Microbial Fuel Cells

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ABSTRACT: The combined anaerobic–aerobic conditions in air-cathode single-chamber MFCs were used to completely mineralize pentachlorophenol (PCP; 5 mg/L), in the presence of acetate or glucose. Degradation rates of 0.140 ± 0.011 mg/L-h (acetate) and 0.117 ± 0.009 mg/L-h (glucose) were obtained with maximum power densities of 7.7 ± 1.1 W/m³ (264 ± 39 W/m², acetate) and 5.1 ± 0.1 W/m³ (175 ± 5 W/m², glucose). At a higher PCP concentration of 15 mg/L, PCP degradation rates increased to 0.171 ± 0.01 mg/L-h (acetate) and 0.159 ± 0.011 mg/L-h (glucose). However, power was inversely proportional to initial PCP concentration, with decreases of 0.255 W/mg PCP (acetate) and 0.184 W/mg PCP (glucose). High pH (9.0, acetate; 8.0, glucose) was beneficial to exoelectrogenic activities and power generation, whereas an acidic pH (¼ 5.0) decreased power but increased PCP degradation rates (0.195 ± 0.002 mg/L-h, acetate; 0.173 ± 0.005 mg/L-h, glucose). Increasing temperature from 22 to 35 °C enhanced power production by 37% (glucose) to 70% (acetate), and PCP degradation rates (0.188 ± 0.01 mg/L-h, acetate; 0.172 ± 0.009 mg/L-h, glucose). Dominant exoelectrogens of Pseudomonas (acetate) and Klebsiella (glucose) were identified in the biofilms. These results demonstrate that PCP degradation using air-cathode single-chamber MFCs may be a promising process for remediation of water contaminated with PCP as well as for power generation.


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KEYWORDS: microbial fuel cell; PCP degradation rate; power production; mineralization

Introduction

Pentachlorophenol (PCP) is one of many recalcitrant and toxic compounds found in water that are used for various purposes, such as herbicides, insecticides, fungicides, wood preservatives, resins, and lubricants. The existence of these chemicals in groundwater, industrial wastewater effluents, sediments, and surface soils poses great challenges for treatment and remediation (Field and Sierra-Alvarez, 2008). Microbial co-metabolism is considered to be an effective route of PCP degradation, in which PCP is used as a second substrate and may serve as a carbon source for the growth of microorganisms (Field and Sierra-Alvarez, 2008; Shen et al., 2005). As the degradation of PCP occurs through co-metabolism, the type of carbon source can affect the effectiveness of this approach. Different carbon sources have been used in conventional biological processes for PCP degradation, including lactate (Yang et al., 2005), glucose (Banerji and Bajpai, 1994; Shen et al., 2005; Visvanathan et al., 2005), acetate (Stuart and Woods, 1998), sucrose (Shen et al., 2005), mixtures of glucose, acetic acid, formic acid, and yeast extract (Damianovic et al., 2009), as well as a combination of peptone, sucrose and meat extract (Ye et al., 2004). It has been found that creating combined anaerobic–aerobic conditions is a particularly efficient strategy for PCP degradation.
mineralization (Chen et al., 2010; Field and Sierra-Alvarez, 2008). However, there are additional remaining challenges for using this approach, such as increasing PCP degradation rates, reducing sludge generation, and minimizing energy demands of treatment processes.

One new promising method for more efficient and cost-effective PCP degradation is the use of microbial fuel cells (MFCs) (Huang et al., 2011a,b). An MFC is a device that uses microbes to convert the chemical energy stored in organic and inorganic compounds into electricity (Logan and Regan, 2006; Logan, 2009; Pant et al., 2010). It has been shown that PCP can be degraded in the anaerobic bioanode of a two-chamber MFC (Huang et al., 2011c), although the PCP degradation rate was slow (0.061 gPCP/gVSS-day) in a two-chamber MFC (Huang et al., 2011c). Although the PCP degradation rate was slow (0.061 gPCP/gVSS-day) in a two-chamber MFC (Huang et al., 2011c), it has been shown that PCP can be degraded in the anaerobic bioanode of a two-chamber MFC (Huang et al., 2011c), although the PCP degradation rate was slow (0.061 gPCP/gVSS-day) in a two-chamber MFC (Huang et al., 2011c). In addition, there was incomplete de-chlorination of PCP and the accumulation of undesirable degradation products such as 2,3,4,5-tetrachlorophenol and tetrachlorohydroquinone (Huang et al., 2011c). The main disadvantage of using a two-chamber MFC for PCP degradation is that the anode chamber is anaerobic, and thus the benefit of a combined anaerobic-aerobic condition for PCP degradation is not produced. In addition, it is well known that power production in two-chamber MFCs is low due to its high internal resistance, and that the water in the cathode must be aerated, which is an energy-demanding process (Logan, 2010; Ren et al., 2007).

Single-chamber, air-cathode MFCs can be used to avoid the need to aerate water, and generally have lower internal resistances and higher power densities than two-chamber MFCs (Liu et al., 2005a; Logan et al., 2007; Logan, 2009, 2010). One of the disadvantages of an air-cathode MFC for power generation is oxygen crossover from the cathode into the liquid anode chamber due to diffusion of oxygen through the cathode. Oxygen crossover leads to growth of aerobic bacteria on the cathode, which can lead to a decrease in power production (Kiely et al., 2011; Watson et al., 2011). The use of diffusion layers, such as carbon/polytetrafluoroethylene on the air-side of the cathode, or a separator between the anode and cathode, can reduce oxygen diffusion into the anode chamber and improve power production (Cheng et al., 2006; Watson et al., 2011; Zhang et al., 2011b).

In the case of PCP degradation, however, the micro-aerobic environment near the cathode may provide improved conditions for PCP mineralization due to the combination of anaerobic and aerobic conditions in the same chamber (Chen et al., 2010; Field and Sierra-Alvarez, 2008). In this study, PCP degradation in single-chamber, air-cathode MFCs was investigated in the presence of either a nonfermentable (acetate) or fermentable substrate (glucose). The degradation of PCP in the anode chamber was examined as a function of the number of diffusion layers (in order to alter oxygen transfer rates into the anode chamber), initial COD, initial PCP concentration, pH, and temperature. The performance of the system was evaluated in terms of PCP degradation rate, power production, and recovery of electrons in the substrates in terms of coulomic efficiency (CE). The microbial community on the anode was also analyzed.

Materials and Methods

Fuel Cell Assembly

Single-chamber air-cathode MFCs were similar to those used by Cheng et al. (2006). Graphite felt (2.0 cm × 2.0 cm × 3.0 cm) (Sanye Co., Beijing, China) was used as the anode instead of carbon paper to increase available anode surface area. A graphite rod was inserted into the graphite felt anode to conduct electrons to the external circuit. Cathodes were made by applying platinum (0.5 mg Pt/cm²) to the water side of the cathode, and diffusion layers to the air side of 30 wt% wet-proofed carbon cloth (type B-1B, E-TEK) as described by Cheng et al. (2006). The number of cathode diffusion layers was varied (1, 3, 5, or 7) in order to vary oxygen crossover and examine the effect of this on PCP degradation rate and power generation. Diffusion layers were made by brushing a polytetrafluoroethylene (PTFE) solution (60 wt%) onto one side of the cathode, followed by drying at room temperature and heating at 370 °C for 10 min (Cheng et al., 2006).

The net working volume of the cell was 24 mL, and the electrode packing was around 5,000 m²/m³-graphite felt volume. A reference electrode (Ag/AgCl electrode, 195 mV vs. standard hydrogen electrode, SHE) was used to obtain cathode and anode potentials, with all voltages reported here versus SHE. Two controls (duplicate reactors) were also operated: one was used as an abiotic control (no inoculum); the other was run in open circuit mode to examine changes in PCP and co-substrates in the absence of current generation. All of the reactors were wrapped with aluminum foil to exclude light.

Inoculation and Operation

Domestic wastewater was collected from the primary sedimentation tank of the Lingshui Wastewater Treatment Plant in Dalian, China, and used to inoculate the anode. For the initial acclimation, wastewater was added into a solution (V/V: 50/50) containing (per liter) (NH4)2SO4 0.386 g, K2SO4 0.149 g, NaH2PO4·2H2O (3.31 g), Na2HPO4·12H2O (10.31 g), vitamins (12.5 mL/L), and minerals (12.5 mL/L) (pH 7.0, conductivity 6.5 mS/cm) (Huang and Logan, 2008). Acetate or glucose was added at identical concentrations on a chemical oxygen demand (COD) basis. After the formation of stable and repeatable peaks in power, analytical grade PCP (Sigma, St. Louis, MO, 99.8%) dissolved in 0.2 M NaOH, together with acetate or glucose in the nutrient solution, was used as the medium. The replacement of anodic solution was done at the end of each fed-batch cycle (<20 mV). All reactors were operated at room temperature (22 ± 2 °C) except as indicated, where the temperature was varied to be 4, 35, and 50 °C. In the investigation of the
effects of pH, the medium pH was adjusted using 0.2 N H₂SO₄ or NaOH to the indicated values while the solution conductivity was kept the same at different pH values (conductivity change <5%).

Analyses

PCP was analyzed using a high performance liquid chromatograph (HPLC Agilent 1100) (Supporting Materials, SM), with the intermediates quantitatively determined by HPLC after a qualitative analysis using an APCI (−) ion trap mass spectrometer coupled to an LC (Agilent HPLC-MS/MS 6410).

The voltage across an external resistor was recorded (30 min intervals) using a data acquisition board (PISO813, Taiwan). Power density was normalized by the volume of the liquid medium (24 mL) and the projected surface area of the cathode (7 cm²). These parameters, as well as CE, were calculated as previously reported (Huang and Logan, 2008). The maximum power densities were calculated from polarization curves obtained by varying the external resistance (20 kΩ–100 Ω) for 60–90 min (single-cycle method). The bioelectrochemical behavior of anodic biofilms was examined using cyclic voltammetry (CV) and a three-electrode configuration with a potentiostat (CHI 650A, Chenhua, Shanghai). The scanned potential was between −0.6 and +0.6 V (vs. SHE), at a scan rate of 1.0 mV/s (Huang et al., 2011d).

Community analysis was performed using a polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE). Samples were collected from MFCs at the end of a cycle when the PCP (15 mg/L) was fully depleted (pH 7.0 and 22°C). Electrodes were fragmented using sterile scissors. Cells attached on the electrodes were removed by rinsing three times with sterile water, and concentrated by centrifugation. Genomic DNA extraction, PCR amplification, and DGGE analyses were performed as described in Supplementary Materials.

Results and Discussion

Effect of the Number of Diffusion Layers

The number of diffusion layers affected both PCP degradation rates and power densities. Three diffusion layers produced the highest PCP degradation rates (0.140 ± 0.011 mg/L-h, acetate; 0.117 ± 0.009 mg/L-h, glucose) and power densities (6.0 ± 0.9 W/m², 206 ± 30 mW/m², acetate; 5.1 ± 0.9 W/m³, 173 ± 32 mW/m³, glucose), with CEs of 57.6 ± 3.9% (acetate) and 40.4 ± 3.7% (glucose) for MFCs fed 5 mg/L of PCP (SM; Table S1). These PCP degradation rates were similar to results with five diffusion layers, but power densities were slightly lower. PCP degradation rates and power densities were reduced using one or seven diffusion layers. The application of additional diffusion layers is known to reduce the oxygen flux into the anode chamber (Cheng et al., 2006). This is consistent with results here, as shown by an increase in the CEs for both substrates (SM, Table S1). Based on these results, it appears that the use of three to five diffusion layers provided oxygen transfer into the anode chamber that was optimal for maximizing PCP degradation by anaerobic and aerobic pathways. As a result of these findings, three diffusion layers were used in MFCs in all subsequent tests.

PCP Degradation Pathways

PCP was successively dechlorinated, with the accumulation of intermediates during the fed-batch cycle, consistent with the literature on aerobic PCP degradation (Field and Sierra-Alvarez, 2008), with the specific intermediate concentrations affected by the co-substrate. Tetrachlorohydroquinone (2,3,5,6-tetrachloro-1,4-hydroquinone, or 2,3,5,6-TeCHQ), formed via hydroxylation of the para position during aerobic PCP degradation (Field and Sierra-Alvarez, 2008), accumulated to 0.73 ± 0.11 μM in PCP–glucose reactors at 12 h, compared to 0.24 ± 0.04 μM in PCP–acetate MFCs. At the same point in time, 2,3,6-trichloro-1,4-hydroquinone (2,3,6-TCHQ) reached 0.09 ± 0.02 μM (acetate) (Fig. 1A) and 0.37 ± 0.08 μM (glucose) (Fig. 1B) due to its formation through sequential dechlorination of 2,3,5,6-TeCHQ. 2,6-Dichloro-1,4-hydroquinone (2,6-DCHQ), produced from...
2,3,6-TCHQ, increased to 0.35 ± 0.11 μM (acetate) at 36 h and 0.50 ± 0.08 μM (glucose) at 24 h, and then gradually decreased in concentration.

2,3,4,5-Tetrachlorophenol, which is anaerobically produced through the removal of ortho-chlorines, reached maximum concentrations of 0.69 ± 0.11 μM (acetate) (Fig. 1A) and 0.78 ± 0.08 μM (glucose) (Fig. 1B) at 24 h. Successive dechlorination of these compounds was shown by the production of expected intermediates (3,4,5-TCP, 3,5-DCP, and phenol) (Chen et al., 2010; Field and Sierra-Alvarez, 2008). Total aromatics decreased from an initial concentration of 18.8 ± 1.6 μM to a concentration of 2.67 ± 1.6 μM (acetate) (Fig. 1A) and 5.46 ± 1.8 μM (glucose) at 36 h (Fig. 1B). Around 98% of all the intermediates were removed at 48 h, implying the effective degradation and mineralization of PCP in this reactor.

PCP was completely mineralized in the air-cathode single-chamber MFCs, overcoming the accumulation of partial de-chlorination products of 2,3,4,5-tetrachlorophenol and tetrachlorohydroquinone observed in previous two-chamber, hexacyanoferrate-cathode MFCs (Huang et al., 2011c). Here oxygen was transferred into the anode chamber through the cathode, in contrast to the two-chamber conditions where the anode is kept separated from the hexacyanoferrate solution and thus there is little oxygen crossover. The oxygen permeation through the cathode in single-chamber MFCs created a micro-aerobic environment that allowed a combination of anaerobic–aerobic processes in the same chamber (Cheng et al., 2006; Cheng and Logan, 2011), and thus led to more efficient PCP degradation. Based on the combination of aerobic and anaerobic PCP degradation, possible PCP degradation pathways were proposed as outlined in the supporting materials (SM Fig. S1).

**Effect of Initial COD Concentration on Power Production and PCP Degradation**

Power increased from 3.1 to 6.8 W/m³ (105–231 mW/m², acetate) and from 2.2 to 5.4 W/m³ (74–185 mW/m², glucose) with an increase in the initial COD from 390 to 780 mg/L in the presence of 5 mg/L of PCP (Fig. 2A and B). Higher initial CODs ranging from 1,170 to 1,560 mg/L generated similar powers of around 18.0 W/m³ (617 mW/m², acetate) and 9.0 W/m³ (315 mW/m², glucose). However, power generation was adversely affected by the presence of PCP in the medium, as power was higher in the controls lacking PCP (19.4 W/m³, 665 mW/m² with acetate; 15.0 W/m³, 513 mW/m² with glucose; COD = 780 mg/L) (Fig. 2A and B). CEs decreased with an increase in COD, 74 ± 3% to 24 ± 4% (acetate) and 51 ± 3% to 17 ± 3% (glucose), due to the increase in the length of a fed-batch cycle (Fig. 2C). These substrate concentrations are equivalent to 226 C (780 mg/L COD) and 452 C (1,560 mg/L COD), compared to only 0.4 C for PCP (5 mg/L), assuming complete dechlorination. Thus, the coulombs provided in the substrates were much higher than that in PCP.

The PCP degradation rate increased with initial COD concentrations (Fig. 2D), reaching the maximum of 0.140 ± 0.011 mg/L-h (acetate) and 0.117 ± 0.009 mg/L-h (glucose). These PCP degradation rates were both higher than that previously achieved in two-chamber MFCs, with a 35% increase for acetate (0.104 mg/L-h) and a 92% increase for glucose (0.061 mg/L-h) at the same COD and PCP concentrations (Huang et al., 2011c).

Current generation enhanced PCP degradation rates, demonstrating the importance of exoelectrogenic activity on PCP removal. In the open circuit controls, PCP degradation rates were reduced by up to 31–54% with acetate (0.062 ± 0.006 to 0.104 ± 0.005 mg/L-h), and 27–43% with glucose (0.052 ± 0.006 to 0.082 ± 0.007 mg/L-h) for CODs ranging from 390 to 1,560 mg/L (Fig. 2D). It has similarly been found that the degradation rates of other pollutants (chloroethane and diesel) are improved with current generation (Morris et al., 2009; Pham et al., 2009). In addition, these PCP degradation rates were higher than those achieved in the conventional biological processes, with range from 0.038 to 0.051 mg/L-h for PCP concentrations of 5–13 mg/L (Li et al., 2010; Mun et al., 2008; Szewczyk and Długonksi, 2009).

PCP degradation in air-cathode single-chamber MFCs is more complex than that occurring in a single chamber system, as a result of additional oxygen diffusion into the system. PCP is thought to be primarily degraded through chemical reduction using electrons from the cathode, similar to that reported by others (Sun et al., 2009), or co-metabolized by microorganisms on the anode and elsewhere in the reactor using acetate or glucose. When using an MFC, the enhanced growth of anaerobic and exoelectrogenic microorganisms in the reactor may help to increase the microbial population capable of PCP degradation compared to systems lacking current generation (Luo et al., 2010; Morris et al., 2009). These positive effects from both the anode and cathode, and the addition of some oxygen into the system through the air-cathode, may explain the enhanced PCP degradation rates with current generation in these MFCs compared to open circuit conditions in MFCs and in conventional biological processes.

**Polarization Curves**

Polarization data were obtained to determine the maximum power production in these reactors in the presence and absence of PCP. The maximum power densities were 7.7 ± 1.1 W/m³ (264 ± 39 mW/m², acetate) and 5.1 ± 0.1 W/m³ (175 ± 5 mW/m², glucose) at 780 mg/L of COD and 5 mg/L of PCP (SM, Fig. S2). Higher power (17.7 ± 0.9 W/m³, 605 ± 32 mW/m², acetate; 8.6 ± 0.4 W/m³, 293 ± 12 mW/m², glucose) was obtained at a higher initial COD concentration of 1,560 mg/L, while a lower substrate concentration of 390 mg/L reduced maximum power (4.7 ± 0.6 W/m³).
161 ± 19 mW/m², acetate; 4.0 ± 0.6 W/m³, 137 ± 19 mW/m², glucose) (SM, Fig. S2). These power densities were all lower than those produced by the controls with 780 mg/L COD and lacking PCP (19.4 W/m³, 665 mW/m², for acetate; and 15.0 W/m³, 513 mW/m² for glucose) (SM Fig. S2), demonstrating an adverse effect of PCP on system performance. These power densities using the graphite felt anodes in the controls (19.4 W/m³, 665 mW/m², with acetate) are lower than those previously reported (27 W/m³, 1,100 mW/m²) using a similar reactor with ammonia-treated graphite fiber brush anodes (Cheng and Logan, 2007; Cheng et al., 2011). This suggests that power densities could be further increased. However, these maximum power densities in the presence of PCP were four times (acetate) and three times (glucose) as much as those previously obtained in two-chamber MFCs (Huang et al., 2011c), illustrating the importance of reactor architecture for improving power generation and PCP degradation rates.

Effect of Initial PCP Concentration

PCP degradation rate increased with its concentration over a range of 5–15 mg/L to maximum rates of 0.171 ± 0.002 mg/L-h (acetate) and 0.159 ± 0.011 mg/L-h (glucose), but the rate decreased slightly at 20 mg/L (Fig. 3A). PCP degradation rates with current generation were always larger than those of the open circuit controls (Fig. 3A).

Power production was inversely proportional to initial PCP concentration, with changes of 0.255 W/mg PCP (acetate, \( R^2 = 0.9977 \)) and 0.184 W/mg PCP (glucose, \( R^2 = 0.9934 \)) over the concentration range of 5–20 mg/L of PCP (Fig. 3B). This effect of initial PCP concentration on power production was primarily due to reduced anode performance, as shown by an increase in the anode potential with initial PCP concentration increase (Fig. 3C).

Effect of Initial pH

A lower pH was beneficial to PCP degradation, but not power production. PCP degradation rates with acetate decreased from a maximum of 0.195 ± 0.002 mg/L-h at pH 5 to 0.154 ± 0.003 mg/L-h at pH 10 (Fig. 4A). Glucose fed reactors followed the same trend, with PCP degradation rate decreasing between a pH of 5 (0.173 ± 0.005 mg/L-h) to 10 (0.136 ± 0.004 mg/L-h) (Fig. 4A). PCP degradation rates were lower in the open circuit controls (Fig. 4A), showing the benefit of current generation for PCP degradation at all pH values. These results are consistent with conventional biological processes, where it was found that a pH of 6–7 was beneficial to PCP degradation due to the requirement of five...
protons for PCP de-chlorination (Mun et al., 2008; Singh et al., 2009). Power production increased with pH, to a maximum of $5.5 \pm 0.3 \text{ W/m}^2$ ($189 \pm 10 \text{ mW/m}^2$) at pH 9 (acetate), and $3.3 \pm 0.3 \text{ W/m}^2$ ($113 \pm 10 \text{ mW/m}^2$) at pH 8 (glucose) (Fig. 4B). An optimal pH = 9 for acetate is consistent with previous reports using this substrate in the absence of PCP (He et al., 2008).

Changes in power production were due to changes in anode potentials. There was no appreciable change of cathode potential with the change of pH from 5 to 10 in either PCP–acetate or PCP–glucose MFCs. Anode potentials decreased inversely with pH over the range of 5 to 9 for acetate, and from 5 to 8 for glucose (Fig. 4C). More negative anode potentials accounted for the increase in power generation with pH, showing that the higher pH values were in general beneficial for exoelectrogenesis (Fig. 4C).

The effect of pH on the biocatalytic activities of the anode biofilms was further assessed using CV. Only a single set of oxidation–reduction peaks were observed across the pH range of 5–10 for all biofilms, with no peaks measured in abiotic controls (Fig. 5A and B). The peaks using acetate were in the range of $0.26$ to $0.32 \text{ V}$ (Fig. 5A), consistent with previous reports using this substrate at similar pH and in the absence of PCP (Liu et al., 2005b), indicating the less effect of PCP and pH on the potentials of the oxidation–reduction peaks. In contrast, the peaks using glucose appeared at the range of $0.36$ to $0.40 \text{ V}$ (Fig. 5B), more positive than the reported $0.01$ to $0.36 \text{ V}$ at similar pH in the absence of PCP (Rabaey et al., 2004), demonstrating the importance of PCP on the potentials of the oxidation–reduction peaks. These results show that substrate, much more than pH, had a substantial impact on the potentials of the oxidation–reduction peaks. The sizes of oxidation–reduction peaks were significantly affected by pH, with maxima at pH 9 (acetate) and 8 (glucose), consistent with the highest power densities at these pHs (Fig. 4B).
Effect of Temperature

Temperature affected all aspects of the MFC, including maximum power densities, open circuit potentials, CEs, and PCP degradation rates. The maximum power density was obtained at 35°C, with 6.3 ± 0.3 W/m² (216 ± 10 mW/m², acetate) (Fig. 6A) and 4.1 ± 0.4 W/m² (141 ± 15 mW/m², glucose) (Fig. 6C), with lower power densities at the other temperatures in the order: 22°C > 4°C > 50°C. This general trend with performance was similar with the changes in open circuit potentials, with the highest open circuit potentials achieved at 35°C (0.73 ± 0.001 V, acetate; 0.65 ± 0.002 V, glucose) (Fig. 6A and C). These results of power versus temperature are consistent with those obtained in MFCs in the absence of PCP, where it was shown that lower temperatures reduced performance (Cheng et al., 2011). Here it was also observed that a much higher temperature of 50°C also substantially reduced performance (1.0 ± 0.04 W/m², 34 ± 2 mW/m², acetate; 0.8 ± 0.1 W/m², 27 ± 5 mW/m², glucose), implying the absence of thermophilic exoelectrogenic bacteria (Liu et al., 2011; Mathis et al., 2008).

The changes in the electrode potentials (Fig. 6B and D) indicated that the anode potential was more affected by temperature than the cathode. Anode potentials for MFCs fed glucose changed more than those with acetate (Fig. 6B and D). It has been agreed that, in the absence of PCP and using acetate as a fuel, reducing temperature from 30 to 4°C led to an increase in the anode potential by 34% and a decrease of the cathode potential by 37% due to the decrease of cathodic reaction rates and exoelectrogenic bacteria activities, although the solubility of dissolved oxygen in water increased (Cheng et al., 2011; Oh et al., 2004). Thus, the presence of PCP here mainly contributed to the greater sensitivity of anodic exoelectrogenic bacteria to temperature, which resulted in greater changes in anode potential with temperature.

CEs increased with a temperature change from 22 to 35°C, resulting in CE = 38.3 ± 1.5% (acetate) and 31.3 ± 1.7% (glucose) at 35°C (SM Fig. S3). These CEs are lower than those obtained in the absence of PCP (Liu et al., 2008; Logan et al., 2007), mainly due to the increased oxygen transfer into the reactor with less diffusion layers here, as well as the longer cycles due to the adverse effect of PCP on exoelectrogenic bacteria. At 4 or 50°C, the CEs were substantially reduced. For example, for acetate the CEs decreased to 4.2 ± 2.1% at 50°C and 10.8 ± 1.3% at 4°C. These values were much lower than a CE = 31% at 4°C in the absence of PCP (Cheng et al., 2011), suggesting that the low CE was resulted from effects of PCP on the anodic biofilm.

PCP degradation rates were consistent with other changes in performance, with the maximum degradation rates obtained at 35°C (0.188 ± 0.01 mg/L-h, acetate; 0.172 ± 0.01 mg/L-h, glucose) (SM, Fig. S3). Temperatures of 50 or 4°C substantially decreased PCP degradation rates (SM, Fig. S3). In all the cases, PCP degradation rates under closed circuit conditions were higher than those under open circuit conditions, demonstrating that current generation increased PCP degradation rates.

While PCP mineralization under combined anaerobic–aerobic conditions in air-cathode single-chamber MFCs provides a promising and efficient process for remediation of water contaminated with PCP, there are still many challenges to enable practical applications. The platinum catalyst used here on the cathode should be replaced by either nonprecious metal catalysts (Logan, 2010) or bio-cathodes (Huang et al., 2011a; Logan, 2010) to reduce capital costs. In addition, oxygen transport through the cathode could be adjusted by modifying the cathode material or using different diffusion layers on the outside of the cathode (Cheng et al., 2006; Luo et al., 2011; Zhang et al., 2011a) in order to better stimulate PCP degradation. Further investigations in these directions are warranted.

Bacterial Morphologies and Community

Both PCP–acetate (Fig. 7A) and PCP–glucose (Fig. 7C) MFCs had anodes sparsely populated with bacteria. This was in contrast to anodes in acetate (Fig. 7B) or glucose-fed (Fig. 7D) MFCs in the absence of PCP, where dense biofilms

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**Figure 5.** Cyclic voltammetry tests carried out on the anode fed with (A) PCP–acetate and (B) PCP–glucose (triangle in solid: abiotic control, diamond in solid: pH 5.0, circle in open: pH 6.0, diamond in open: pH 7.0, square in open: pH 8.0, triangle in open: pH 9.0, circle in solid: pH 10.0, temperature: 22°C, initial COD: 780 mg/L, initial PCP: 15 mg/L). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

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Figure 6. A, C: Voltage (solid) and power (open), and (B and D) cathode (open) and anode (solid) potentials as a function of current density in PCP–acetate (A and B) or PCP–glucose (C and D) fed MFCs under temperature of 50 °C (circle), 35 °C (triangle), 22 °C (diamond) and 4 °C (square) (pH: 7.0, initial COD: 780 mg/L, initial PCP: 15 mg/L). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

Figure 7. Morphological features of biofilms on the anode in (A) PCP–acetate or (C) PCP–glucose MFCs compared to the controls of (B) acetate or (D) glucose MFCs (pH: 7.0, initial COD: 780 mg/L, initial PCP: 15 mg/L).
covered the anodes. The different performance and bacterial abundances in these systems were presumably due to the changes induced by the presence of PCP.

Bacterial communities by DGGE indicated that PCP–acetate and PCP–glucose anodes had several common and prominent bands, but also some different bands from acetate and glucose controls lacking PCP (Fig. 8). Bands of A2 from acetate controls and G2 from glucose controls shared sequences belonging to the well-known exoelectrogenic Geobacter sulfurreducens (Kiely et al., 2011; Logan, 2009), which were absent in PCP–glucose or faint in PCP–acetate reactors (Fig. 8), implying the negative effect of PCP on this strain. In addition, predominant bands were identified to be most similar to exoelectrogenic Bacteroidetes sp. (A1) and Comamonadaceae sp. (A3) in acetate MFCs, and Desulfovibrio desulfuricans (G1), Clostridium sp. (G3) and Burkholderiales sp. (G4) in glucose reactors (Borole et al., 2011; Kan et al., 2011; Logan, 2009; Prasad et al., 2006). In the case of PCP–acetate and PCP–glucose MFCs, however, the prominent strains experienced apparent shifts (Fig. 8). The sequences of PA1 from PCP–acetate MFCs were most similar to Pseudomonas putida and Pseudomonas sp., both of which were frequently found to cometabolize PCP, 1,3-dichlorobenzene, alpha-halocarboxylic acid, or crude-oil (Field and Sierra-Alvarez, 2008; Marchesi and Weightman, 2003; Mulet et al., 2011). Pseudomonas putida has been found to be exoelectrogenic (Juang et al., 2011). The sequences of band PA2 from PCP–acetate anode were most similar to uncultured Alicyclobacillus sp., and Bacterium rA3 and rP5, which were found to be present in

Figure 8. Neighbor-joining tree based on 16S rRNA gene sequences derived from the DGGE band using Clustal X 2.0 (Un indicates uncultured, and C nitrativorans indicates Comamonas nitrativorans). Inset figure: anodic bacterial community profiles revealed by DGGE. (From left to right: acetate without PCP; glucose without PCP; PCP–glucose; PCP–acetate. Bands A1–A3, G1–G4, PG1–PG5 and PA1–PA2 represented selected DGGE bands that were excised and sequenced from acetate, glucose, PCP–glucose, and PCP–acetate fed MFCs, respectively).
chlorophenol degrading aerobic granular sludge, or responsible for the breakdown of a phenol-digesting activated-sludge process (Watanabe et al., 1998, 1999). The band PA2 was most similar to Comamonas nitrativorans, dominant in a microbial community for treating carbazole-containing wastewater or landfill leachate (Etchebehere et al., 2001; Tan and Ji, 2010), but its exoelectrogenic activity is unknown. The presence of these diverse bacteria that are capable of degrading multiple recalcitrant compounds, as well as the presence of bacteria with exoelectrogenic activities, can therefore explain the successful dechlorination of PCP and mineralization with simultaneous power generation in acetate fed MFCs.

In the case of PCP–glucose anode, band PG1 shared a high similarity to phytase-producing Shigella dysenteriae, whereas sequences of PG2 were similar to Klebsiella sp., Klebsiella variicola, and Klebsiella pneumoniae (Fig. 8). Klebsiella pneumoniae has been shown to be exoelectrogenic with glucose (Logan, 2009; Zhang et al., 2008) and it can degrade the recalcitrant compound citrinin (Chen et al., 2011), suggesting that this microorganism plays a role in both PCP degradation and power generation. The band PG4 present in the PCP–glucose MFC, but not in the control, belonged to an uncultured gamma proteobacterium, which was presumably developed due to the presence of PCP. These results for both the acetate and glucose fed MFCs demonstrate that there is a large diversity of bacteria in these reactors, and that the communities are altered by the presence of PCP.

Conclusions

PCP was shown for the first time here to be completely mineralized under a combined anaerobic–aerobic condition created in air-cathode, single-chamber MFCs. PCP degradation rates were improved by 35% (acetate) and 92% (glucose), and power production by 300% (acetate) and 200% (glucose) compared to previous results using two-chambered MFCs (Huang et al., 2011c). A more acidic pH of 5 improved PCP degradation rates, but power production was maximized at higher pHs of 9 for acetate, and 8 for glucose. For both acetate and glucose fed MFCs, a temperature of 35°C was optimal for not only PCP degradation but also power production. Dominant bacteria that are known exoelectrogens and that were identified in PCP–acetate and PCP–glucose fed biofilms include the Pseudomonas and Klebsiella species. These results show that MFCs can be used for successful bioremediation of PCP-contaminated water using co-substrates such as acetate or glucose. The use of single chamber MFCs in particular can result in complete mineralization of PCP under optimum operational conditions of PCP concentrations below 20 mg/L.

We gratefully acknowledge financial support from the National Basic Research Program of China (No. 2011CB936002) and the Natural Science Foundation of China (Nos. 21077017 and 51178077).

References


