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Mineralization of 4-chlorophenol and analysis of bacterial community in microbial fuel cells

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Abstract

4-Chlorophenol (4-CP) was co-metabolically degraded and mineralized with the presence of glucose in microbial fuel cells (MFCs), achieving a degradation rate of 0.58 ± 0.036 mg/L-h (7.2 ± 0.5 mg/g VSS-h) with an electricity generation of 5.4 ± 0.4 W/m^3 at an initial 4-CP concentration of 25 mg/L. Compared to the open circuit controls, current generation accelerated the removal of 4-CP. Coulombic efficiency decreased from 30.3 ± 2.9% at an initial 4-CP concentration of 5 mg/L to 6.3 ± 0.9% at 40 mg/L. 4-CP was degraded via the formation of phenol, which was further mineralized. Dominant bacteria most similar to both the exoelectrogenic and electrotrophic uncultured *Desulfovibrio*, the exoelectrogenic and recalcitrant degrader of uncultured *Desulfobulbus*, and the exoelectrogenic uncultured *Microbacterium* were identified in the biofilms. These results demonstrate that 4-CP mineralization using MFCs may be a promising process for remediation of water contaminated with 4-CP as well as for power generation.

Keywords: 4-chlorophenol; mineralization; bacterial community; microbial fuel cell

1. Introduction

4-chlorophenol (4-CP) is derived from the breakdown of pesticides and other chlorinated compounds, as well as the chlorination processes of phenol containing wastewater. It can be released into the environment through various human activities such as pulping industries, chemical industrious and are also found in industrial wastewaters, soils, surface waters and ground waters [1]. Co-metabolic degradation is regarded as an effective strategy for its removal [1-2]. One challenge to this degradation approach in practice, however, is low degradation rates, excess sludge generation, as well as high

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operating costs. New processes are needed to achieve more rapid 4-CP degradation and improve the existing process limitations.

One possibility for improved 4-CP degradation is to use a microbial fuel cell (MFC). An MFC is a device that uses microbes to convert the chemical energy stored in organic and inorganic compounds into electricity, providing a low-cost and low-maintenance reactor as well as a process that produces very little sludge [3]. While chemical cathode can de-chlorinate 4-CP, low 4-CP removal rate and accumulations of phenol (incomplete degradation) have limited its practical application [4]. Considering recalcitrant compounds including aromatic hydrocarbons, alkanes, chloroethane, pyridine, phenol and indole can be co-metabolically degraded and mineralized in MFCs [5], co-metabolism in MFCs might be useful for 4-CP mineralization. However, 4-CP mineralization has not yet been examined in MFCs.

In this study, we focused on co-metabolic degradation of 4-CP in one-chamber and air-cathode MFCs. Performance was evaluated in terms of 4-CP degradation, power production, coulomic efficiencies (CEs), biocatalytic activities, and bacterial community analysis. Identification and quantification of 4-CP metabolites were also investigated. Deeper insight into these aspects of 4-CP degradation in MFCs could enable future applications of MFCs for 4-CP bioremediation.

2. Materials and methods

2.1. MFC reactor construction and operation

The MFCs were constructed based on an efficient air cathode design, with a chamber 4 cm long and 3 cm in diameter [3]. The air cathode contained 0.5 mg/cm^2 Pt (water side) and four diffusion layers (air-side) on a 30 wt% wet-proofed carbon cloth (type B-1B, ETEK) [6]. A piece of stainless steel (mesh size 10×10 μm) (Xinxiang Zhengyuan Co. China) was placed in parallel with the cathode at a distance of 1.0 cm to avoid the contact of the cathode and the anode. Graphite felt (Sanye Co., Beijing) was packed in the anode to produce a net working volume of 20 mL.

The reactor was inoculated using effluent from the primary sedimentation tank of Lingshui Wastewater Treatment Plant in Dalian, China. Prior to use, wastewater was sparged with N_2 gas for 15 min. Wastewater was initially combined with an equivalent volume of nutrient solution which contained (per liter) glucose 1.0 g, NH_4HCO_3 0.386 g, $KHCO_3$ 0.149 g, $NaH_2PO_4 \cdot 2H_2O$ 3.31 g, $Na_2HPO_4 \cdot 12H_2O$ 10.31 g, $MgSO_4 \cdot 7H_2O$ 0.036 g, vitamins 12.5 mL/L and minerals 12.5 mL/L (pH 7.0, conductivity 6.5 mS/cm). After the formation of stable and repeatable power peaks, analytical grade 4-CP (Sigma, 99.8%) dissolved in 0.2 M NaOH, together with glucose in a nutrient solution, was refilled as indicated. The replacement of solution was done at the end of each fed-batch cycle (defined as a voltage of < 20 mV). All reactors were operated at a room temperature of 22 ± 3ºC.

2.2. Analyses

Power density, polarization curve and Coulombic efficiency (CE) were obtained as described [3]. Chemical oxygen demand (COD) and biomass were measured using standard methods [7]. Samples (0.5 mL) were periodically withdrawn from the reactors and filtered through 0.22 μm pore diameter membrane filters. A high performance liquid chromatograph (HPLC Agilent 1100), equipped with a C_{18} capillary column (4.6 mm in diameter and 250 mm in length, ODS-2 Hypersil, Thermo) was performed to analyze 4-CP and phenol. The mobile phase was prepared by dissolving trifluoroacetic acid with ultrapure water (pH = 2.8) and the ratio of this solution and methanol was 20:80 (V/V).

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. Genomic DNA extraction, PCR amplification, and DGGE analyses were performed as previously described [8].

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 between –0.6 and +0.6 V (vs SHE) was performed at a scan rate of 1.0 mV/s under quiescent conditions.

3. Results and discussions

3.1. Reactor acclimation

After three cycles and using glucose as a sole fuel, MFC exhibited a stable and peak power (9.1 ± 0.5 W/m^3), illustrating the accomplishment of acclimation for exoelectrogenic biofilms. When 5 – 10 mg/L 4-CP was added, power was not apparently adversely affected and reached the same values (Fig. 1). However, adding 15 mg/L of 4-CP decreased power to 7.0 W/m^3, showing an inhibition of the activity of exoelectrogenic biofilms at this concentration. Power production of around 5.0 W/m^3 was in accordance with a high 4-CP of 25 mg/L, demonstrating an apparently negative effect of 4-CP (25 mg/L) on power production.

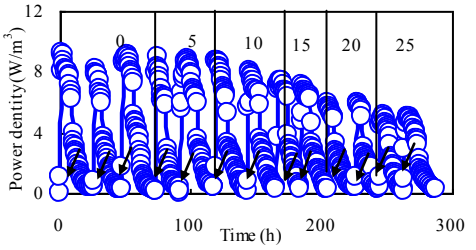

Fig. 1. Power production from MFCs. (Arrows indicate when reactors were fed with fresh medium; numbers indicate concentration of 4-CP (mg/L); 4-6 repeated cycles were performed at each 4-CP concentration, although only two cycles were shown here).

3.2. Polarization curve

Polarization curves and electrode potentials under various 4-CP concentrations are shown in Fig. 2A, 2B and 2C). The addition of 4-CP did not apparently decrease open circuit potential, achieving 0.62 V at 4-CP 25 mg/L, in comparison with 0.71 V in the absence of 4-CP (Fig. 2A). There was no appreciable effect on the maximum power density with the addition of 4-CP from 5 mg/L to 10 mg/L, exhibiting a maximum power production of 9.8 – 10.1 W/m^3 at 49.5 – 50.8 A/m^3 (Fig. 2B). However, in the presence of 15 mg/L 4-CP, the maximum power was only 7.4 ± 0.2 W/m^3, compared to 10.3 ± 0.2 W/m^3 when only glucose was added. A 4-CP concentration of 25 mg/L further decreased power production to 5.4 ± 0.4 W/m^3, demonstrating an apparent inhibition of 4-CP on the activity of exoelectrogenic biofilms at this concentration (Fig. 2B).

Both anode and cathode showed less potential change with change of current density at 4-CP concentrations from 5 mg/L to 10 mg/L (Fig. 2C). 4-CP concentrations of 15 – 25 mg/L led to an apparent decrease of both anode and cathode potentials with change of current density. The rapid decrease of both anode and cathode potentials at high 4-CP concentrations of 15 – 25 mg/L within the

same range of current density (0 – 37 A/m^3) indicates the happening of more severe polarization at this current density.

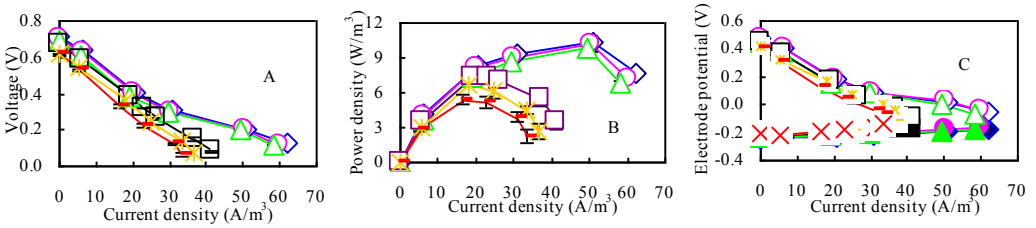

Fig. 2. Comparison of (A) voltage output, (B) power density, and (C) anode and cathode potentials as a function of current density under various 4-CP concentrations (◊, no 4-CP control; ○, 5 mg/L; △, 10 mg/L; □, 15 mg/L; *, 20 mg/L; -, 25 mg/L; ♦ in (C), no 4-CP control; ●, 5 mg/L cathode potential; ▲, 10 mg/L cathode potential; ■, 15 mg/L cathode potential; +, 20 mg/L cathode potential; ×, 25 mg/L cathode potential).

3.3. Time course of power generation and 4-CP degradation, and effect of initial 4-CP concentration on degradation rate and CE

A peak power of 5.0 W/m^3 was achieved at an operation time of 4.5 h and an initial 4-CP concentration of 25 mg/L (Fig. 3A). After a slow decrease period of 7 h, power production experienced a more apparent abatement and was thereafter decreased gradually. Concomitant with power generation, 4-CP concentration was decreased from the initial 25 mg/L to the final 7.7 ± 1.7 mg/L at an operation time of 24 h. The intermediate of phenol reached the peak concentration of 1.1 ± 0.2 mg/L at 12 h and decreased thereafter. The decrease of phenol demonstrates the further degradation and cleavage of phenol in the present MFCs (Fig. 3A).

4-CP degradation rate increased with initial 4-CP concentrations, reaching 0.58 ± 0.036 mg/L-h (7.2 ± 0.5 mg/g VSS-h) at 4-CP of 25 mg/L whereas CE decreased from 30.3 ± 2.9% at 5 mg/L to 6.3 ± 0.9% at 40 mg/L (Fig. 3B). This degradation rate was 2.4 times of the conventional biological processes [2]. In the open circuit controls (OCCs), 4-CP degradation rates were much lower than the closed circuit condition, illustrating current generation accelerated 4-CP removal (Fig. 3B). Our observation was in line with previous reports with other chemicals, where the degradation rates of diesel and chloroethane were improved by current generation [9-10].

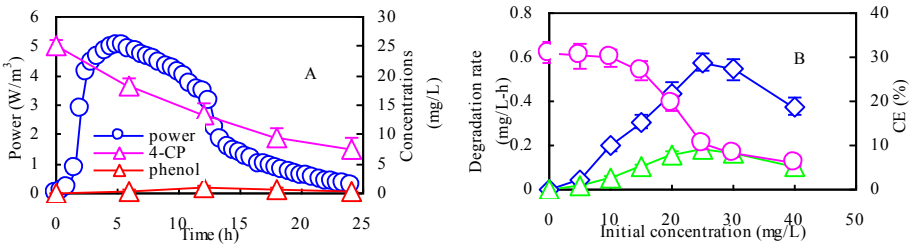

Fig. 3. (A) Time course of power production, 4-CP degradation and phenol production, and (B) effects of initial 4-CP concentrations on degradation rate and CE (◊: degradation rate in closed circuit condition; △: degradation rate in open circuit condition; ○: CE).

3.4. Bacterial morphologies and community, and interactions of bacteria with electrodes

SEM revealed that the anode was covered with sparse bacteria (Fig. 4). It is agreed that the numerical

abundance of microorganisms in biofilms cannot be assumed a priori to correlate to capacities of the predominant species for high power production [11]. Thus, this microbial consortia developed at the presence of 4-CP and sparsely distributed on the anode may have experienced a hard microbial selection and competition, and preserved responsible for electricity generation. The competitors killed off under these selective and competitive conditions may contribute to the apparent sparse bacteria on the electrode surface.

The sizes of oxidation-reduction peaks of the biofilms in 4-CP fed MFCs became smaller than those in the absence of 4-CP (Fig. 4 left), illustrating negative effects of 4-CP on electrochemical activities. In the abiotic controls, no apparent oxidation-reduction peaks were observed, reflecting the importance of biofilms responsible for the electrochemically catalytic activities.

Bacterial communities analyzed by DGGE indicated several prominent bands (Fig. 4 right). Band of G-C-4 shared sequences belonging to uncultured *Desulfovibrio*, which were recently found to be both exoelectrogenic and electrotrophic [12]. Similarly, the sequences of G-C-2 were most similar to the exoelectrogenic uncultured *Microbacterium* and bacterium MFC-R7 [13], as well as the uncultured *Coriobacteriaceae*, found in wastewater treatment plant influent [14]. G-C-6 was closely related with the well-known exoelectrogen and pentachlorophenol degrader of *Desulfobulbus* [15-17]. The survival of these exoelectrogenic and electrotrophic bacteria as well as the pentachlorophenol degrader explained the good 4-CP degradation and electricity generation. Interesting, band G-C-1, G-C-3, G-C-5 and G-C-7 formed several distinct clusters distant from the cluster of the *Rhodococcus*, *Propionibacteriaceae*, and uncultured bacterium (AM712557, JF623715, FJ753410), which are known to efficiently degrade chlorophenol, fluorophenol and polyaromatic hydrocarbons [18-21].

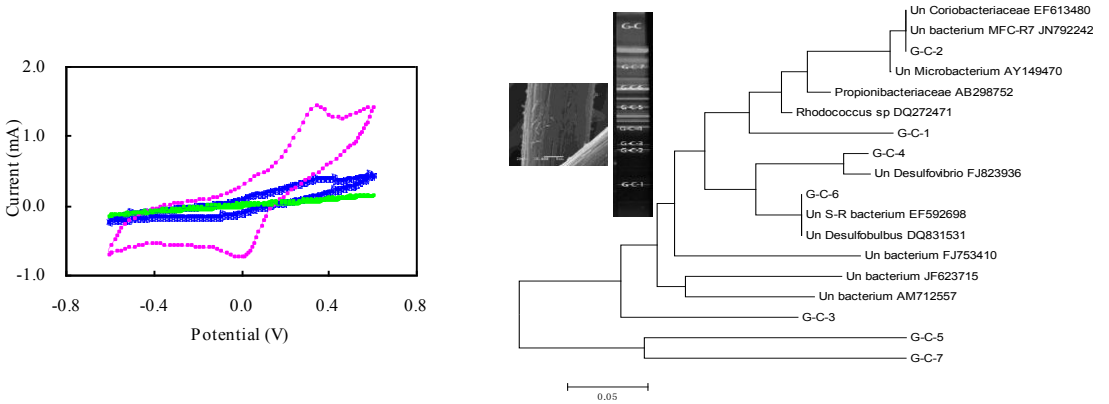

Fig. 4. (Left) cyclic voltammetry tests carried out on the anode ((▒) 4-CP and glucose, (—) glucose in the absence of 4-CP, and (▬) abiotic control). (Right) Neighbor-joining tree based on 16S rRNA gene sequences derived from the DGGE band using Clustal X 2.0 (Un indicates uncultured, and S-R indicates sulfate-reducing. Bands G-C-1~G-C-7 represented selected DGGE bands that were excised and sequenced. Insert figure: (left) bacterial morphologies and (right) bacterial community profiles revealed by DGGE.

4. Conclusions

4-CP was firstly demonstrated to be co-metabolically mineralized in MFCs. 4-CP degradation rate increased and CEs decreased with the increase of initial concentrations ranging from 5 mg/L to 25 mg/L, achieving a degradation rate of 0.58 ± 0.036 mg/L-h (7.2 ± 0.5 mg/g VSS-h) and an electricity generation of 5.4 ± 0.4 W/m^3 at an initial 4-CP of 25 mg/L. Current generation accelerated 4-CP degradation via the

formation of phenol, which was further degraded in the present MFCs. These results could potentially enable future applications of MFCs for 4-CP bioremediation.

Acknowledgement

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