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Performance of microbial fuel cells on removal of metronidazole

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ABSTRACT

The microbial fuel cells (MFCs) are the focus of extensive investigation as one of the promising technologies for renewable energy generation and wastewater treatment. Two-chambered MFCs were designed to investigate the removal of metronidazole and to quantify the effect of antibiotic on the efficacy of energy generation. Using 1,000 mg glucose L^{-1} containing different concentrations of metronidazole (0, 10, 30, 50 mg L^{-1}) as the fuels, the corresponding power densities were 141.94, 99.23, 25.44, 16.26 mW m^{-2}, respectively. The adverse effect on the performance of the MFCs was reversible. The removal of metronidazole achieved 85.4% within 24 hours in MFCs, while only 35.2% in open circuit. Current generation could account for the improved removal efficiency at these tested concentration levels. The findings of this paper indicated that antibiotics such as metronidazole could be removed in MFCs, which has implications for general wastewater treatment.

Key words | electricity generation, metronidazole, microbial fuel cells, removal

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INTRODUCTION

Microbial fuel cells (MFCs) are systems that combine biologically catalyzed reactions with electrochemical processes transforming the energy stored in the chemical bonds of organic matter to electricity. MFCs are the focus of extensive investigation as one of the promising technologies for renewable energy generation and wastewater treatment (Patil *et al.* 2010a, b, 2011). Literature survey indicated that a wide range of organic substrates could be used as fuels in MFCs, most of them were readily degradable compounds such as glucose, acetate and complex carbohydrates in food processing wastewater, domestic wastewater, brewery wastewater and starch processing wastewater (Oh & Logan 2005; Lu *et al.* 2009).

Various toxic or recalcitrant compounds could be detected frequently in wastewaters. Therefore, it is essential to investigate the feasibility of biodegradation of recalcitrant compounds using MFCs. Previous studies showed that there were only a few reports on biorefractory organics as fuels, such as phenols (Luo *et al.* 2009; Zhu & Ni 2009), nitrogenous heterocyclic compound (Zhang *et al.* 2009; Chen *et al.* 2010; Hu *et al.* 2011) and azo dye (Sun *et al.* 2012). These results indicated that some toxic and biorefractory organics might be degraded and produce electricity simultaneously after a period of acclimatization. Coupled with the benefit of power generation in offsetting the treatment cost, the MFCs may offer a new technique in enhancing biodegradation of recalcitrant contaminants.

Antibiotics, as an important group of pharmaceuticals, are widely used to treat humans. Substantial quantities of these compounds and metabolites are released into the environment through various sources such as pharmaceutical industry, hospital effluent and excretion from humans and animals. Therefore, it is necessary to treat the effluents containing antibiotics before they enter the environment and eventually reach drinking water. It was reported that wastewater containing antibiotics could be degraded efficiently in the biological system (Müller *et al.* 2013). MFC technologies are also involved in biological methods, and there are a few reports on MFCs with antibiotics added to the fuel in the available literature. Wen *et al.* adopted glucose–ceftriaxone sodium mixtures and glucose–penicillin mixtures as fuels, and found these antibiotics could be degraded and play an active role in electricity production (Wen *et al.* 2011a, b). Harnisch *et al.* demonstrated that sulfonamides could be removed from artificial wastewater using MFCs (Harnisch *et al.* 2013).

In this work, metronidazole (2-methyl-5-nitroimidazole-1-ethanol) was selected as a model antibiotic to

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study whether it could be removed in MFCs. Metronidazole is a common antibiotic drug which has been used for treating infections, and a recent study showed that its maximum concentration detected in hospital effluent was 9,400 ng/L (Fang *et al.* 2010). Various experiments were conducted to investigate the removal of metronidazole and the effect on the performance of MFCs. A comparison experiment, which was run in the open circuit, was also operated to examine removal of metronidazole in the absence of current generation. The major objectives of this work are: (1) to investigate the removal of metronidazole in MFCs; (2) to examine the influence of metronidazole on the power generation of MFCs.

MATERIALS AND METHODS

MFC configuration

A two-chambered MFC was made of perspex material, which consisted of an anode chamber and a cathode chamber, each with an operating volume of 140 mL (7 cm × 5 cm × 4 cm). Carbon papers (without waterproofing or catalyst) with a projected surface area of 16 cm^2 were used as electrodes. Prior to use, they were soaked in acetone for a period of 4 h to remove organic matter on the surface of the carbon papers and then soaked in 1 mol/L hydrochloric acid and 1 mol/L sodium hydroxide, respectively, for 24 h to wipe out impurities. A cation exchange membrane (CMI-7000) was sandwiched between the two chambers and they were held together with an external metal screw. Rubber gaskets were used to secure a seal between the glass walls and the membrane. Copper wires were used to connect the circuit with an external resistance of 1000 ohms and all leaks were sealed to maintain an anaerobic microenvironment in the anode chamber. 100 mmol/L $K_3[Fe(CN)_6]$ in 50 mmol/L phosphate buffer solution (PBS, pH = 7.0) was used as the electron acceptor.

MFC operation

Anaerobic sludge collected from a local wastewater treatment plant (Xinxiang, Henan, China) was used as the anodic inocula of MFCs. A cultivation solution added to the anode chamber for bacterial growth contained 1,000 mg glucose L^{-1} as electron donors; 50 mmol/L PBS (pH 7.0) containing (per litre deionized water): 3.32 g $NaH_2PO_4 \cdot 2H_2O$, 10.32 g $Na_2HPO_4 \cdot 12H_2O$, 0.13 g KCl, 0.31 g NH_4Cl, 12.5 mL vitamins and 12.5 mL mineral solution. The inoculated anaerobic sludge was controlled as 25% (volume) of the whole anode solution. N_2 gas was flushed continuously for 15 min to remove dissolved oxygen in the anodic chamber before each batch test. During the start-up and acclimation stage, the cultivation solution contained 1,000 mg glucose L^{-1} as the sole fuel. When the performance of the MFCs got stable, the cultivation solution was replaced with glucose–metronidazole mixture, which contained 1,000 mg glucose L^{-1} and different concentrations of metronidazole (10, 20, 30, 40 and 50 mg L^{-1}, respectively). The chemical oxygen demand (COD) loading rate and superficial loading rate of the reactor were 0.39 kg COD m^{-3} d^{-1} and 0.034 kg COD m^{-2} d^{-1}. The anode solution was replaced by fresh cultivation solution when the voltage decreased below 50 mV. Replacing of new cultivation solution, was also in order to prevent the accumulation of metronidazole and its poisoning effect on the anode. A comparison experiment was also operated, which was run in the open circuit mode to examine the degradation of metronidazole in the absence of current generation. All MFC experiments were conducted at 30 ± 1 °C in a constant temperature room.

Analysis and calculations

Absorbance and UV–vis absorption spectra were recorded by a UV–vis spectrometer (Beijing Purkinje General Instrument Co., Ltd, China).

The surface morphology of the bare carbon paper and the biofilm on the carbon paper were observed by environmental scanning electron microscope (SEM) Quanta 200, (Frequency Electronic, Inc.) and KYKY-EM3200, respectively.

The voltage of the MFCs was measured every 30 min with a digital multimeter, and all the data were automatically recorded by a computer. Polarization curves were obtained by changing external circuit resistance from 30,000 to 50 Ω.

The current density I_A (A m^{-2}) and the power density P_A (W m^{-2}) of the system were calculated using the formulae:

$$I_A = \frac{V}{R \cdot A} \quad (1)$$

$$P_A = \frac{V^2}{R \cdot A} \quad (2)$$

where V (V) is the cell voltage, R (Ω) is the external resistance and A (m^2) is the projected area of the anode.

Degradation of metronidazole was determined by monitoring the decrease in the absorbance at a wavelength of 319 nm. In order to determine the degradation efficiency of the metronidazole, samples (4 mL) were taken from MFCs at a time interval of 2 hour during every cycle with different concentrations of metronidazole, then the samples were centrifuged at 4,000 rpm for 15 min to remove suspended biomass from the anode solution and filtered through a 0.22 μm-pore-size syringe filter unit (2 mL), after that the samples were diluted to 4 mL prior to measurements. The blank solution was the anode solution taken from the MFC without metronidazole added to the fuel, and the following procedures for pretreatment were the same as the samples.

Degradation efficiency was calculated as the following:

$$R_d = \frac{A - B}{A} \times 100\% \tag{3}$$

where R_d is the degradation efficiency, A is the initial absorbance and B is the observed absorbance.

RESULTS AND DISCUSSION

Electricity generation from synthetic metronidazole wastewater in MFCs

Under external resistance of 1,000 Ω, the MFCs generated constant electrical power from anaerobic sludge after a successful acclimation period, yielding a maximum power density of 206.6 mW m^{-2} with 1,000 mg glucose L^{-1} as the sole fuel. As shown by Figure 1, when cultivation solution containing 1,000 mg glucose L^{-1} was added into the MFCs, an initial power density of 0.78 mW m^{-2} was immediately generated. It might be due to the difference of the potential between the two electrodes based on chemical and biological factors (Min et al. 2005). In the first two circles, the power density increased with the increase of time, and reached a maximum of 62.1 mW m^{-2} after 62 hours. From the third circle, the power density started to stabilize at 206.6 mW m^{-2}, indicating the biofilm formation and the bacterial adhesion reached a steady state on the anode.

SEM images further proved successful bacterial growth on the carbon paper. As shown in Figure 2, the bare carbon paper consists of a number of carbon fibers with a relatively smooth surface (inset plot of Figure 2). In contrast, a clearly visible homogeneous biofilm has developed on the anode surface after the acclimation period, and most bacteria are globular and arranged in chains.

When the cultivation solutions containing metronidazole–glucose mixtures were added into the MFCs, as can be seen in Figure 1, the power density were obviously decreased. In comparison with MFCs using glucose as the sole fuel, the power density output were reduced by 48.6% for 10 mg L^{-1} metronidazole, 62.9% for 30 mg L^{-1} and 75.2% for 50 mg L^{-1}. These results might be attributed to the following factors. (1) The effect of metronidazole on microorganisms. Metronidazole works by reduction of the nitro group in its molecule to an amino group firstly, and then the activated reduced metronidazole molecule would bind nonspecifically to bacterial DNA, and finally inhibit the metabolism, growth and reproduction of the bacteria (Wang 2000). (2) It was speculated that the reduction of

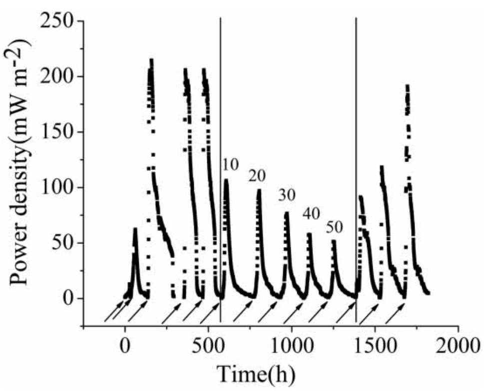

Figure 1 | Power production from MFCs in the presence of glucose, and glucose with metronidazole (arrows indicate when reactors were fed with fresh medium; numbers indicate the concentration of metronidazole (mg L^{-1})) under external resistance of 1,000 Ω.

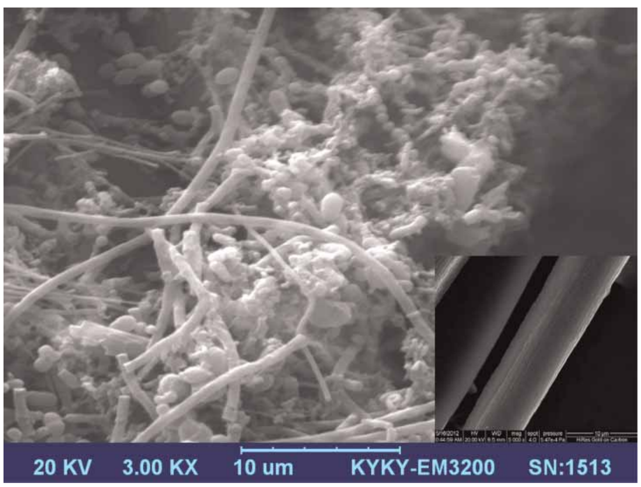

Figure 2 | SEM image of the biofilm on the carbon paper. Inset is the SEM image of the bare carbon paper.

metronidazole would consume some electrons produced from glucose degradation in MFCs, so there will be competition between metronidazole and electricity-producing bacteria (Cao *et al*. 2010). Both of the factors would impair the power output.

It was also noteworthy that the power density was able to recover to the original level after replacement of the anodic solution containing no metronidazole for three cycles (the last three cycles in Figure 1). This phenomenon was probably owing to the inhibited activity of electricity-generating bacteria on the anode being regained when metronidazole was removed from the anolyte in MFCs.

Polarization data were obtained to characterize the performance of MFCs at different concentrations of metronidazole. Figure 3 showed the presence of metronidazole significantly affected the MFC polarization behavior. With the external resistance varied from 30,000 to 50 Ω, the MFCs using glucose as the sole fuel generated a maximum power density of 141.94 mW m^{-2} at a current density of 941.8 mA m^{-2}. While using 1,000 mg L^{-1} glucose and 10 mg L^{-1} metronidazole as the fuel, the maximum power density decreased by 30%, to 99.23 mW m^{-2} at a current density of 787.5 mA m^{-2}. When 30 mg L^{-1} of metronidazole was added, the maximum power density decreased to 25.44 mW m^{-2} at a current density of 398.8 mA m^{-2}, which was 82% lower than that obtained without metronidazole. Increasing the metronidazole concentration to 50 mg L^{-1} further decreased the maximum power density by 88.6%, resulting in a value of 16.26 mW m^{-2} at a current density of 318.7 mA m^{-2}. These results also suggested that metronidazole was inhibitory to the bacteria and not actively involved in electricity production.

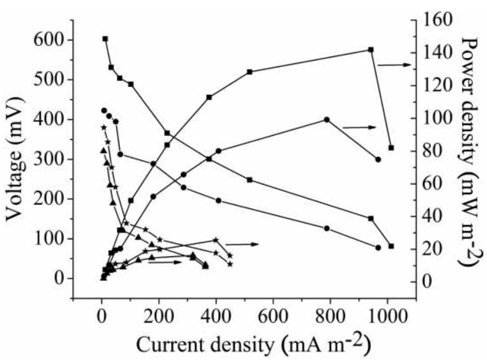

Figure 3 | Polarization and power density curves of MFCs operating on 1,000 mg L^{-1} glucose (■) and 1,000 mg L^{-1} glucose containing different concentrations of metronidazole (10 mg L^{-1}•, 30 mg L^{-1}★, 50 mg L^{-1}▲) at an external resistance of 1,000 Ω. The arrows indicate the corresponding curves were the power density curves.

Metronidazole removal in the MFCs

Removal performances of metronidazole at different concentrations (10, 30, 50 mg L^{-1}) in MFCs and in open circuit are shown in Figure 4. Although metronidazole could be removed in both reactors, there were obvious differences between them. Firstly, MFCs achieved better removal performance than in open circuit at any given time during 24 h. For example, when the concentration of metronidazole was 10 mg L^{-1}, the metronidazole was removed by almost 85.4% during 24 h in the MFCs, but only 35.2% for the open circuit. The accelerated removal of metronidazole in this study might be attributed to the current generation. The continuous electron transfer made the anode a sufficient anaerobic terminal electron acceptor, which would increase the metabolic rate of anaerobic bacteria and accelerate the oxidation of the glucose, thereby producing more electrons for metronidazole reduction (Morris *et al*. 2009; Zhang *et al*. 2010; Wen *et al*. 2011a).

Secondly, the removal efficiency decreased with the increase of metronidazole concentration in both conditions. Over 85% of the metronidazole was removed from the solution within 24 h at an initial metronidazole concentration of 10 mg L^{-1}. At 30 mg L^{-1} of metronidazole, the removal efficiency had a slight decrease, with 69% removed within 24 h. It is noteworthy that metronidazole removal was strongly inhibited at higher concentrations of 50 mg L^{-1}, with only 44% of the metronidazole removed after 24 h.

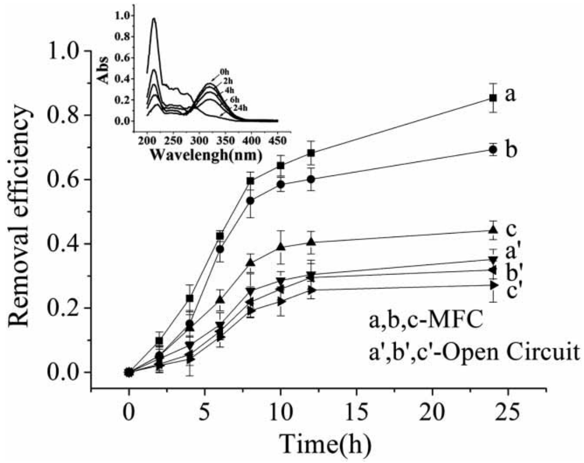

Figure 4 | Removal performance of metronidazole at different concentrations (10 mg L^{-1} for a and a'; 30 mg L^{-1} for b and b', 50 mg L^{-1} for c and c') in MFC and in open circuit conditions. Inset are the UV–vis spectra of anode solutions taken from the MFC with 1,000 mg L^{-1} glucose and 10 mg L^{-1} metronidazole as substrate at different operation times.

These results indicated that an increase of metronidazole concentration would reduce the removal efficiency, which may be due to the increasing toxic effect on the bacteria. But the microbial consortium still presents a certain degree of toxic tolerance to metronidazole at the tested concentration levels.

To further illustrate the metronidazole removal performance in MFCs, the UV–vis spectra of samples taken from MFCs were recorded (inset plot of Figure 4). The UV–vis absorption spectra revealed that the absorbance at 319 nm for metronidazole decreased, and at the same time the absorbance in the UV region at 200–250 nm increased during 24 h of operation. The noteworthy absorbance decrease at 319 nm and increase in other UV regions indicated that the metronidazole was indeed removed in MFCs and might have formed corresponding degradation products simultaneously. The removal pathways of metronidazole and the effects of metronidazole on microbial communities in MFCs will need further research.

CONCLUSION

Metronidazole removal could be achieved using glucose as cosubstrate in MFCs. More than 85% metronidazole (10 mg L^{-1}) was removed within 24 hours, and the removal efficiency in MFCs was almost 2.5 times as high as that in the open circuit. But the removal efficiency gradually decreased with the increase of the concentration of metronidazole. Metronidazole showed adverse effects on the performance of MFCs, the power output obviously decreased with the increase of concentration of metronidazole. Our results indicated that antibiotics such as metronidazole could be removed in MFCs, which may present a new way to remove antibiotics from wastewater.

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