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Pyridine degradation in the microbial fuel cells

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ABSTRACT

The objective of this study was to investigate the feasibility of pyridine biodegradation in the microbial fuel cell (MFC), from which electricity was generated. Experiments were initially conducted in a graphite-packed MFC (G-MFC) using a pyridine concentration of 500 mg/L combined with different glucose concentrations. Pyridine of 500 mg/L only used as the G-MFC fuel resulted in a maximal voltage of 116 mV and a maximal power density of 1.7 W/m^3. The maximal voltage reached within 12 h when pyridine was totally depleted. The glucose supplement with concentrations of 500, 250, and 100 mg/L resulted in the maximum voltages of 623, 538, and 349 mV, respectively, correspondingly the maximal volumetric power densities were 48.5, 36.2, and 15.2 W/m^3. Pyridine biodegradation rates reached 95% within 24 h using the G-MFC. Interestingly, after 90 d of acclimation, the biodegradation rates of pyridine in the G-MFC using pyridine only as the fuel were higher than those using the glucose–pyridine mixtures. Further experiments were conducted using a graphite fiber brush MFC (B-MFC). Compared to the G-MFC, the B-MFC enhanced the electrical charges by 89, 186, and 586% for the mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, and 1:5, respectively. GC/MS analyses of the anode solution indicated that the metabolism of pyridine in the MFC was initiated by ring reduction and NH_3-N production. The results suggest that pyridine may be used as the MFC fuel in practical applications of wastewater treatment.

1. Introduction

Pyridine is classified as a hazardous substance in the USEPA (United States Environmental Protection Agency) list of priority pollutants [1,2]. As a typical representative of heterocyclic compounds, pyridine is often detected in the wastewaters from coking plants, pharmaceutical factories, and other relative industries. Because of pyridine's toxic and teratogenic nature, discharging of pyridine-containing wastes has an adverse impact on human health and the environmental quality [3–5]. It has been reported that pyridine and its derivatives are difficult to be degraded by bacteria under aerobic and anaerobic conditions for their toxicity to microbial communities unless enduring a long period of acclimation [3,6,7].

It has been reported that the microbial fuel cell (MFC) can be used for wastewater treatment [8]. Various types of biodegradable organic matter have been used as fuels to generate electricity in the MFC, including acetate, glucose, cellulose, and organic mixtures in the municipal wastewater [9–12]. However, different organics in the MFCs have resulted in different power outputs. The maximum power density of the MFC reported in the literature is generated using acetate as the fuel [13]. Catal et al. [14] demonstrated the possibilities of electricity production from furan derivatives and phenolic compounds using the MFC. Their results also showed that trans-cinnamic acid and 3,5-dimethoxy-4-hydroxy-cinnamic acid could not be used as the fuel and reduced the electricity generation from glucose at a concentration up to 10 mM/L. Syringaldeyhde, vanillin, trans-4-hydroxy-3-methoxy, and 4-hydroxy cinnamic acids can be used for electricity generation even at a concentration of 5 mM/L. Recently, it is reported that phenol can be used as fuel in the MFC and the maximum power density reaches to 9.1 W/m^3 [12]. From the point view of applications, the MFC may be a powerful tool for the wastewater treatment. However, various recalcitrant compounds are found in wastewaters. Therefore, it is essential to investigate the feasibility of biodegradation of recalcitrant compounds, such as pyridine, among the N-heterocyclic compounds, and their possible electricity generation using the MFC.

Pyridine has not previously been examined as substrate for electricity generation in the MFC and its degradation pathway in the MFC has not been investigated. In this study, we conducted various experiments to examine electricity production from pyridine and its biodegradation in the MFC. The metabolic pathway of pyridine or the mechanism of electricity production from pyridine in the MFC was also explored.

2. Materials and methods

2.1. MFC configuration

A graphite-packed MFC (G-MFC) was constructed as described by Luo et al. [12]. The MFC consisted of two chambers, which

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were separated by a proton exchange membrane (PEM, Nafion 212, Dupont Co., USA). Each chamber was with a volume of 27 cm^3 (9.0 cm × 2.0 cm × 1.5 cm) and stocked with graphite granule (16–20#), leaving a non-packed working volume of 8 cm^3. A piece of carbon cloth (9 cm × 2 cm; UT70-20, Toray Co., Japan) was inserted into each of the two chambers to collect current. In the cathode, potassium ferricyanide (50 mM) was used as the terminal electron acceptor because potassium ferricyanide was more stable than oxygen based on our experiment. Another type of the MFC was constructed using graphite fiber brush MFC (B-MFC). The B-MFC was almost the same as the G-MFC, but the electrodes were replaced by graphite fiber brush and the working volume of anode chamber was much larger (20 cm^3). To collect solution samples easily from the anode chamber during the experiments, an anode groove was set up, which was a brown bottle (500 mL capacity) connected with the anode chamber. Similarly, a cathode groove was set up to balance the flow rates in the two chambers. The anode bottle was filled with solutions of 200 mL throughout the experiments. A peristaltic pump was used to cycle the flow at a constant flow rate of 20 mL/min in the grooves.

The MFC was kept in a water bath with 30 ± 0.1 °C. Samples of the anode solution were taken at a time interval of 3 h from the anode groove. Concentrations of glucose, pyridine, and COD were measured using the samples. Cell voltages across a fixed external resistance of 1000 Ω were measured using a multimeter with a data acquisition system (DTCL-50, Australia). The acquisition system recorded the data at a time interval of 30 s.

2.2. Microbial inoculum and MFC operation

Two hundred milliliters of mixed aerobic and anaerobic activated sludge collected from Liede Municipal Wastewater Treatment Plant of Guangzhou City, were used for inoculating the MFC. At the beginning, 1000 mg/L glucose was used as the MFC fuel. When the maximum voltage output reached 680 mV and repeatable cycles of power generation were achieved, indicating the biofilm was successfully inoculated onto the anode, pyridine–glucose mixtures were replaced as the substrate. The pyridine–glucose mixtures included two groups: the first group consisted of pyridine of 500 mg/L mixing with glucose of 500, 250 and 100 mg/L, respectively; and the second group consisted of glucose of 500 mg/L mixing with pyridine of 0, 500, 750, 1000 mg/L, respectively. A pyridine concentration of 340 mg/L has been reported to be toxic for several bacterial strains [15,16]. To explore the possibility of using pyridine as fuel in the MFC, the pyridine concentration of 500 mg/L was selected in this study. Meanwhile, a control experiment with graphite but without bacteria was conducted to examine the absorption effect of graphite on pyridine. The anodic solution contained (in 1 L deionized water): 4.0896 g Na_2HPO_4, 2.544 g NaH_2PO_4, 0.31 g NH_4Cl, 0.13 g KCl, 12.5 mL trace element solution, and 12.5 mL vitamin solution [17], and pH of the solution was adjusted to 7.0. Before operating, the anode groove was flushed with N_2 for 15 min to eliminate dissolved oxygen in the anode chamber.

Biodegradation processes of pyridine were measured in three different operation systems: the G-MFC, an anaerobic biodegradation system, and an aerobic biodegradation system. The anaerobic (or aerobic) biodegradation system was set up using the anaerobic (or aerobic) activated sludge (moisture of 70%) obtained from Liede Municipal Wastewater Treatment Plant. The sludge was put into a 300-mL flask containing 200 mL of 500 mg/L pyridine. The flasks were incubated at 30 °C on a rotary shaker at 150 rpm. Sampling methods and frequencies in the anaerobic and aerobic biodegradation systems were the same as that in the MFC.

After the starting period (about 20 d) using 1000 mg/L glucose as the fuel, the G-MFC was operated using the pyridine–glucose mixtures and pyridine in a period of more than 90 d. As shown in Table 1, the pyridine concentration of 500 mg/L was combined with glucose concentrations of 500, 250, and 100 mg/L sequentially as the pyridine–glucose mixtures used as the MFC fuel. Using the mixture with a glucose-to-pyridine ratio of 1:1, the MFC was operated from days 1 to 6. During the period, repeatable power cycles were obtained. Then the fuel was replaced with the mixture with a glucose-to-pyridine ratio of 1:2, operating from days 7 to 10. After repeatable power cycles were obtained, the fuel was replaced with the mixture with a glucose-to-pyridine ratio of 1:5, operating from days 11 to 15. Finally the fuel was replaced with pyridine only and the system was operated from days 16 to 90, when repeatable power cycles were generated.

Similarly the B-MFC was operated using the pyridine–glucose mixtures as the fuel after the starting period. The mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, and 1:5 were sequentially used as the MFC fuel. For each mixture, the B-MFC was operated for one electrical cycle. Then the mixtures were changed to 500 mg/L glucose combining with 0, 500, 750, and 1000 mg/L pyridine to repeat the operation.

2.3. Analyses and calculations

The area power density (P_A, W/m^2) and volumetric power density (P_V, W/m^3) are calculated using the following equations:

$$P_A = \frac{U^2}{RA} \quad (1)$$

$$P_V = \frac{U^2}{RV} \quad (2)$$

where U is the voltage (V) and R is the external resistance (Ω), A is the projected surface area of the electrode (cm^2), and V is the net volume of the anodic chamber (m^3). The Coulombic efficiency was calculated as the total coulombs (C) measured divided by the moles of electrons available from the added substrates [18]:

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

here U_i is the output voltage of MFC at time t_i, F is Faraday's constant (96485 C/mol electrons), b is the number of moles of electrons produced per mol of the COD (4 mol e^-/mol 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), and E is the coulombic number.

Pyridine concentrations were analyzed using a high performance liquid chromatography (HPLC) system (Agilient 1100 with UV detector; TC-C18 reverse-phase column, 250 mm × 4.6 mm, 5 μm). Samples were prepared by filtration with 0.2 μm pore diameter membrane and stored at 4 °C. The elution solvent consisted of a mixture of methanol and water (80:20, v:v) and the 254 nm wavelength was used to detect pyridine with a flow rate of 1 mL/min. Anode solutions were analyzed using GC/MS (QP2010) with a Finnigan Trace GC ultra coupled to a Finnigan Trace DSQ mass spectrometer to identify the possible metabolites of pyridine. The temperature of the GC/MS column (TR-5MS, 30 mm × 0.25 mm × 0.25 μm) was started at 40 °C and kept the temperature for 2 min, then increased at 5 °C/min to 250 °C and kept the temperature for 5 min, and finally increased to the temperature of 280 °C and kept for 2 min. The COD and NH_3-N were determined according to the standard methods [19]. Glucose was analyzed by the anthrone method [20].

Bacterial morphologies on the graphite (anode) were observed using a scanning electron microscope (SEM) (JSM-6330F, Japan). Before observations, the anodic graphite were fixed overnight with 2.5% glutaraldehyde in a buffer solution (0.1 M cacodylate, pH = 7.0, and 4 °C), washed six times in the buffer, dehydrated stepwise

Table 1
Concentrations and biodegradation rates of COD and pyridine in the G-MFC using different fuels within different operation periods.

Ratio of concentrations (mg/L) of glucose-to-pyridine	Time (h)	COD (mg/L)	COD biodegradation rate (%)	Pyridine (mg/L)	Pyridine biodegradation rate (%)
500:500 (1–6 d)[a]	0	1371.7 ± 24.7[b]	0.0 ± 0.0	500.0 ± 0.0	0.0 ± 0.0
	3	565.3 ± 30.1	58.8 ± 1.4	351.7 ± 3.5	30.5 ± 1.4
	6	372.6 ± 23.7	72.8 ± 1.2	171.0 ± 10.0	69.4 ± 1.3
	9	334.8 ± 15.0	75.6 ± 0.8	124.0 ± 6.9	76.0 ± 1.0
	12	304.2 ± 31.2	77.8 ± 1.9	0.7 ± 1.2	81.7 ± 1.2
	24	277.9 ± 19.4	79.7 ± 1.0	ND[c]	99.9 ± 0.1
	36	221.8 ± 22.5	83.8 ± 1.9	ND	100.0 ± 0.0
	48	179.6 ± 11.1	86.9 ± 0.6	ND	100.0 ± 0.0
250:500 (7–10 d)	0	1169.0 ± 70.2	0.0 ± 0.0	500.0 ± 0.0	0.0 ± 0.0
	3	564.8 ± 13.7	51.6 ± 1.7	376.8 ± 6.8	23.9 ± 0.3
	6	421.6 ± 7.9	63.9 ± 1.5	231.5 ± 3.4	53.7 ± 0.6
	9	336.4 ± 5.6	71.1 ± 2.1	145.1 ± 4.4	71.2 ± 0.7
	12	305.3 ± 5.4	73.8 ± 1.9	132.9 ± 1.9	74.6 ± 1.1
	24	188.5 ± 1.4	83.8 ± 0.8	5.4 ± 3.0	98.8 ± 0.2
100:500 (11–15 d)	0	1138.8 ± 139.2	0.0 ± 0.0	500.0 ± 0.0	0.0 ± 0.0
	3	545.0 ± 22.0	51.8 ± 4.7	384.8 ± 8.9	23.2 ± 1.1
	6	439.0 ± 44.3	61.3 ± 2.7	237.6 ± 6.7	50.1 ± 1.8
	9	333.8 ± 15.0	70.5 ± 2.4	160.7 ± 9.5	66.4 ± 2.2
	12	280.7 ± 10.3	75.1 ± 3.6	121.6 ± 1.8	73.9 ± 3.4
	24	178.9 ± 16.5	84.0 ± 3.1	25.8 ± 4.1	94.9 ± 0.2
0:500 (16–20 d)	0	1128.3 ± 30.1	0.0 ± 0.0	500.0 ± 0.0	0.0 ± 0.0
	3	750.3 ± 49.5	33.5 ± 2.6	373.4 ± 10.1	27. 6 ± 2.0
	6	699.7 ± 50.0	38.0 ± 2.8	341.6 ± 16.1	34.0 ± 3.2
	9	613.3 ± 12.6	45.6 ± 1.3	261.3 ± 10.3	50.0 ± 2.1
	12	523.0 ± 25.6	53.6 ± 2.2	242.4 ± 9.2	53.6 ± 1.8
	24	110.0 ± 13.1	90.3 ± 0.9	27.9 ± 3.7	94.7 ± 0.7
0:500 (90 d later)	0	1135.7 ± 32.8	0.0 ± 0.0	500.0 ± 0.0	0.0 ± 0.0
	3	538.7 ± 16.3	56.2 ± 3.0	246.6 ± 5.7	48.0 ± 1.7
	6	365.6 ± 12.5	70.2 ± 2.9	222.9 ± 6.3	79.3 ± 0.5
	9	335.5 ± 6.1	72.7 ± 2.3	156.7 ± 7.6	88.7 ± 0.3
	12	285.9 ± 3.8	76.7 ± 2.1	ND	100.0 ± 0.0
	24	254.2 ± 8.2	79.4 ± 1.1	ND	100.0 ± 0.0
	36	223.3 ± 22.5	81.9 ± 1.4	ND	100.0 ± 0.0
	48	179.7 ± 19.2	85.4 ± 1.7	ND	100.0 ± 0.0

[a] The numbers in the parentheses are the operation periods in the MFC.
[b] The values are mean ± standard deviation ($n = 3$).
[c] Not detected.

in a graded series of ethanol/water solutions (30, 50, 70, 90, and 100%), and then dried (CO_2-critical point) for 3 h. Samples were then coated with Pt before the SEM observations.

Cyclic voltammetric (CV) measurements were performed with an electrochemical system (CHI 660c; Shanghai). A gold working electrode, a platinum counter electrode, and an Ag/AgCl reference electrode were used in test tubes filled with the anode solution. The CV was conducted at a scan rate of 25 mV/s [21] and with voltages ranging from −0.45 to 0.90 V.

3. Results

3.1. Power generation from pyridine–glucose mixtures

Results of the control experiment with graphite but without bacteria showed pyridine absorption by graphite was 2.5–5.0%, which was negligible. In the G-MFC, repeatable power cycles were observed with the pyridine–glucose mixtures as fuels. Typical six cycles (two cycles from each mixture) of electricity generation are shown in Fig. 1A. Voltages were rapidly generated with the pyridine–glucose mixtures, which was similar to the power generation with glucose alone as the fuel. The maximum voltage outputs were 623, 538, and 349 mV for the fuels with ratios of glucose-to-pyridine of 1:1, 1:2, and 1:5, respectively. Operation periods of the MFC were 49.5, 25.7, and 25.1 h for the mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, and 1:5, respectively. The maximum power densities (P_V) obtained were 48.5, 36.2, and 15.2 W/m^3 (the external resistance = 1000 Ω) at the concentrations of supplement glucose of 500, 250, and 100 mg/L, respectively.

Results from the B-MFC showed that the maximum voltages increased to 652, 605, and 453 mV for the fuels with pyridine concentration of 500 mg/L, mixing with glucose concentrations of 500, 250, and 100 mg/L, respectively (Fig. 1B). The maximum power densities (P_V) obtained were 21.3, 18.3, and 10.3 W/m^3 (the external resistance = 1000 Ω) with ratios of pyridine to glucose of 1:1, 2:1, and 5:1, respectively. As shown in Fig. 1C, the maximum voltages from the B-MFC were 663, 652, 645 and 127 mV for fuels of 500 mg/L glucose combining with 0, 500, 750 and 1000 mg/L pyridine, respectively. The corresponding maximum power densities were 22.0, 21.3, 20.8, and 0.8 W/m^3, respectively.

3.2. Power generation from pyridine as the fuel

After 90 d of acclimation using the pyridine–glucose mixed substrates in the G-MFC, stable and reproducible voltages were successfully obtained using 500 mg/L pyridine as the pure substrate (Fig. 2). The maximal voltage obtained was 116 mV and the power density was estimated from the stabilized voltage with the 1000 Ω external resistance. The maximal volumetric power density was 1.7 W/m^3 at a current density of 14.5 mA/m^3, and the corresponding maximal area power density was 7.5 mW/m^2 at a current density of 64.0 mA/m^2 during 290 h of power generation.

A biofilm of rod-shaped bacteria attached to the anode surface when 500 mg/L pyridine was used as the fuel in the MFC (Fig. 3A).

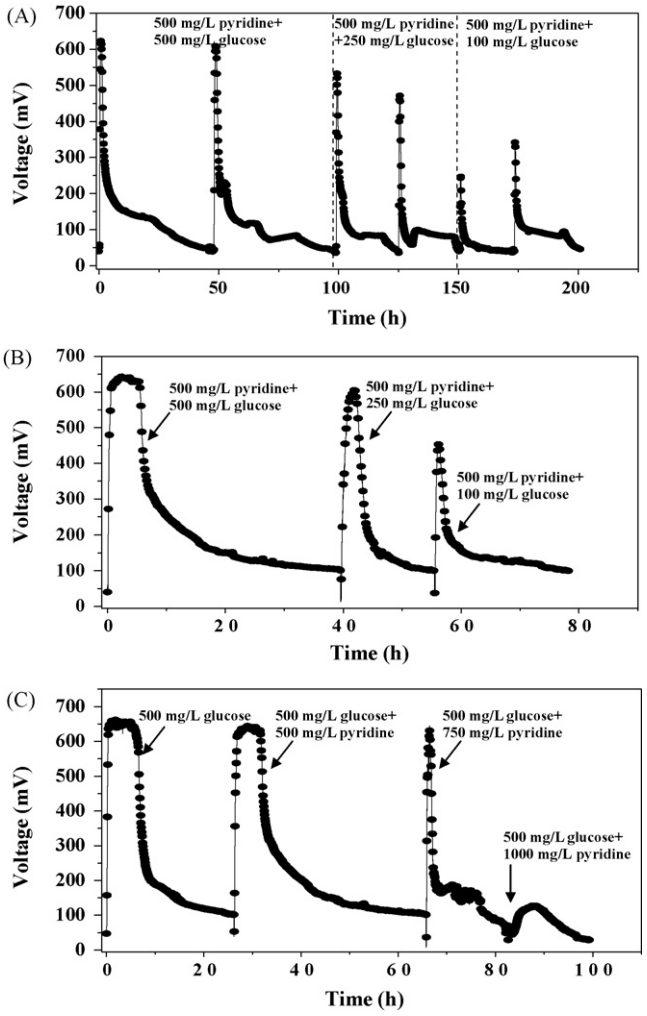

Fig. 1. Voltages generated using (A) an initial pyridine concentration of 500 mg/L with different glucose concentrations (500, 250, and 100 mg/L) in the G-MFC, (B) an initial pyridine concentration of 500 mg/L with different glucose concentrations (500, 250, and 100 mg/L) in the B-MFC, and (C) an initial glucose concentration of 500 mg/L with different pyridine concentrations (0, 500, 750 and 1000 mg/L) in the B-MFC.

However, a thicker biofilm of rod-shaped bacteria was observed on the anode surface with 500 mg/L glucose as the fuel (Fig. 3B), which might be responsible for the higher power generation.

The initial NH_3 concentration was zero in the MFC. The highest intermediate NH_3-N concentration reached 11.8 mg/L at 12 h when

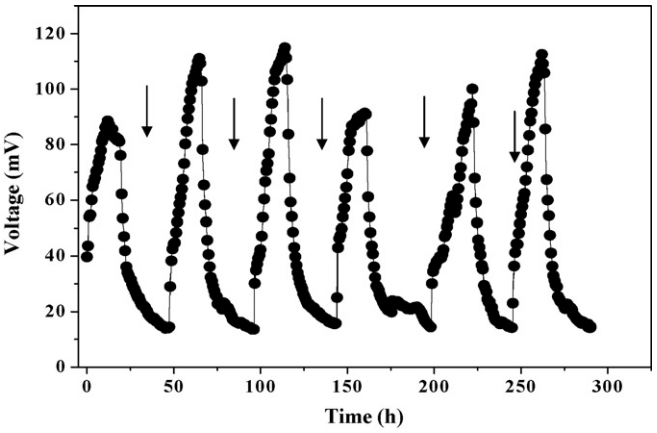

Fig. 2. Voltage output using pyridine of 500 mg/L as the fuel in the G-MFC. The arrows show the substrate refreshment.

pyridine became undetectable (Fig. 4). About 13% of the nitrogen from pyridine converted into NH_3 that was directly detected, while a large portion of NH_3-N converted from pyridine was used in the biomass synthesis.

Concentrations and biodegradation rates of COD and pyridine in one typical electrical cycle are shown in Table 1 for different pyridine and glucose mixtures. The raw COD values were 1372, 1169, 1139, and 1136 mg/L for the substrates with ratios of glucose-to-pyridine of 1:1, 1:2, 1:5, 0:5, respectively. The COD biodegradation rates were in the range of 81.4–86.7% and pyridine biodegradation rates exceeded 95% at the end of each electrical cycle. Electrical charges obtained were 90.3, 49.6, and 36.5 C/g COD degradation, respectively, from using mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, and 1:5, while electrical charges were only 7.1 C from using pyridine.

3.3. Cyclic voltammetry of the bacterial culture

Solution samples were obtained from the anode chamber at the end of electrical cycle when using 500 mg/L pyridine as fuel at the initial state of acclimation and the stage of successful power generation after the bacteria completely degraded the substrate. The solutions included the anode cell suspension with the medium. Analyses of voltammetry (CV) were conducted using the solution samples [21]. The CV at each stage was obtained by repeatedly scanning the sample for six times. The CVs obtained at the different stages showed different shapes of oxidation and reduction peaks (Fig. 5). From the solution collected at the stage of successfully power generation, oxidation and reduction peaks were observed

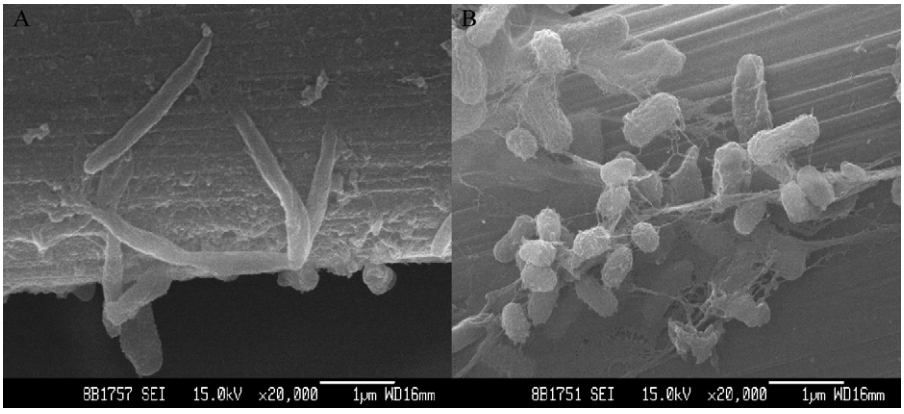

Fig. 3. SEM images of (A) bacteria on the anode surface using 500 mg/L pyridine and (B) bacteria on the anode surface using 500 mg/L glucose.

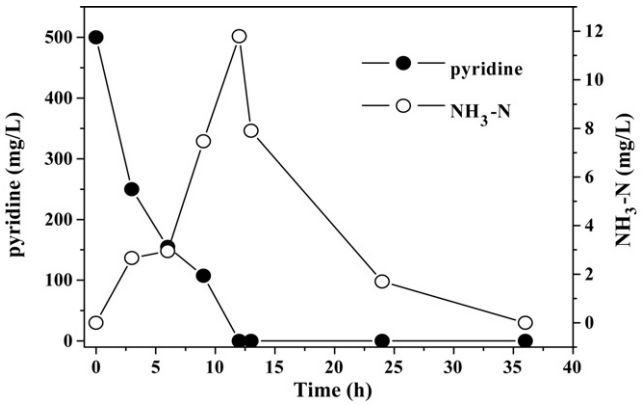

Fig. 4. Concentrations of pyridine and NH_3-N for the experiment using 500 mg/L pyridine as the fuel of the G-MFC.

with an apparent oxidation potential of 0.307 V (1.380 μA) and a redox potential of 0.690 V (−2.60 μA), respectively. The peaks indicated the presence of a mediator that was cell-membrane associated [21]. No obvious peak was detected from the oxidation and reduction curves at the initial stage of acclimation.

3.4. Degradation kinetics of pyridine

With the pyridine concentration of 500 mg/L and different glucose concentrations from 100 to 500 mg/L, the pyridine biodegradation in the G-MFC was well described with the following first-order kinetic model:

$$\ln C = 6.215 - kt \quad (4)$$

where C is the concentration of pyridine, k is the biodegradation rate (1/h), and t is time (h). Since the initial concentration of pyridine was 500 mg/L, the first constant at the right hand side was fixed as 6.215 (ln(500) = 6.215) during the fitting process. Coefficients of determination (r^2) of the fitting results were from 0.932 to 0.995. The pyridine biodegradation rates were 0.127, 0.162, and 0.204 l/h for the pyridine–glucose mixtures with the glucose concentrations of 100, 250, and 500 mg/L, respectively.

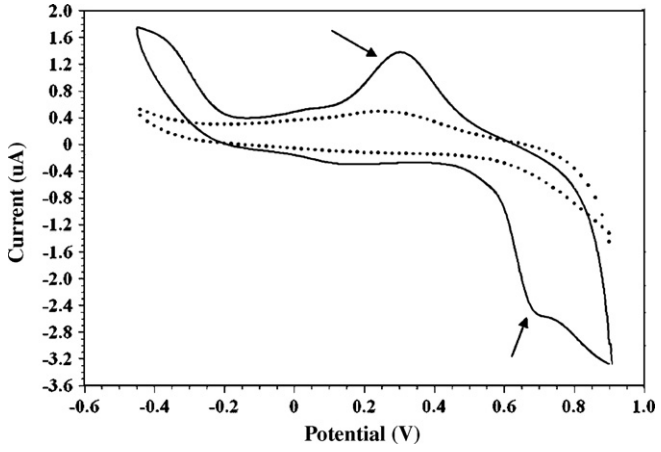

Fig. 5. Comparison of cyclic voltammograms fueled with 500 mg/L pyridine at two stages: the initial stage of acclimation and the stage of successful power generation. The solid curve represents the CV of the anode solution obtained from the G-MFC during successful power generation and the dashed curve represents the CV of anode solution obtained at the initial stage of acclimation. Arrows indicate peaks.

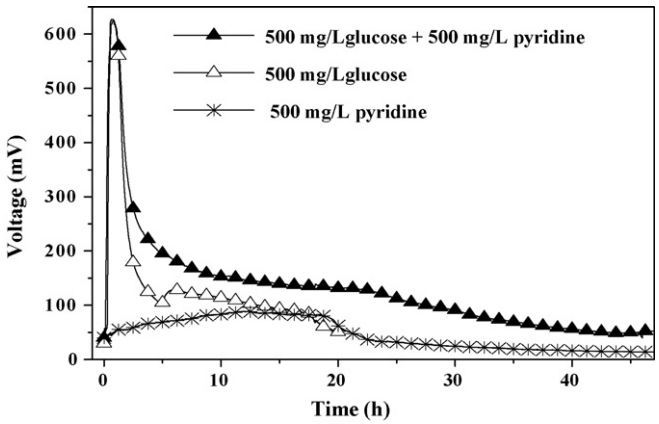

Fig. 6. Comparison of voltage output for the G-MFC using a pyridine–glucose mixture, pure glucose, and pure pyridine as the fuel.

4. Discussion

4.1. Power generation

Repeatable electricity cycles were obtained from pyridine in this study. The results showed that the G-MFC could utilize pyridine as the fuel after a long term of acclimation was conducted in the system with suitable conditions. As shown in Fig. 6, the bacteria in the anode chamber could be cultivated to be electrochemically active under the conditions employed. However, it was also observed that pyridine restrained the microbial activities to generate electricity, by comparing the maximal voltage output 663 mV using glucose (Fig. 1C) as the fuel with 116 mV using pyridine (Fig. 2). As shown in Fig. 1C, the maximum voltage was sharply decreased when pyridine concentrations were larger than 750 mg/L. It seemed that the pyridine concentration above a particular level produced an inhibition. Results from this study indicated that pyridine concentration exceeding 1000 mg/L produced an inhibition on electrochemical active bacteria in the anode chamber and consequently reduced the maximum voltage output. Nevertheless, the coulombic number from using the pyridine–glucose mixture as the fuel was 3.7 C higher than the sum of coulombic numbers using from individual pyridine and glucose as the fuel. The voltage outputs from the different fuels are shown in Fig. 6. The result suggested that the MFC using the mixtures might show different characteristics of power generation by using the mixture compared to using the pure

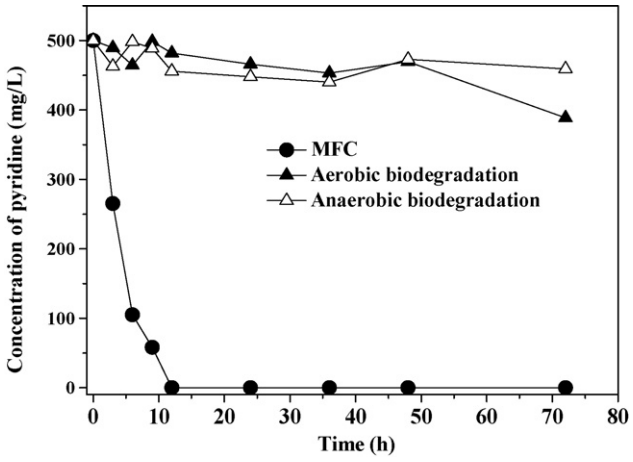

Fig. 7. Biodegradation of pyridine with an initial pyridine concentration of 500 mg/L in three systems: in the G-MFC, the anaerobic biodegradation system, and the aerobic biodegradation system.

organics. Similar to the phenol results of Luo et al. [12], co-substrates with glucose enhanced the pyridine degradation and electricity generation simultaneously. The result is very useful in practice because besides pyridine, easily biodegraded organics, such as glucose, acetate and others can be found in the wastewater, therefore, the MFC may be used to generate the electricity and degrade pyridine simultaneously. Our experimental results showed the possibility of the MFC application on the industrial wastewater treatment containing pyridine.

4.2. Substrate utilization and coulombic recovery

The degradation kinetics of pyridine indicated when using the pyridine–glucose mixtures as the fuel, the degradation rates of pyridine were enhanced with the increasing of glucose concentrations. The results were attributable to the microbial populations increasing with the glucose metabolism. It was interesting that after 90 d of acclimation, the biodegradation rates of pyridine in the G-MFC using pyridine only as the fuel were higher than those using the glucose–pyridine mixtures. For example, in one typically repeatable cycle shown in Table 1, within 6 h, the biodegradation rates of pyridine in the G-MFC were 69.4, 54.7, 50.1, and 79.3% for using the mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, 1:5, and pyridine, respectively. Within 12 h, the biodegradation rates of pyridine in the G-MFC were 81.7, 74.6, 73.9, and 100.0% for using the mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, 1:5, and pyridine, respectively. When using 500 mg/L pyridine as fuel at days of 16–20, no power was generated and the biodegradation rates were the lowest (Table 1). However, after a longer period of acclimation of 90 d, the microbial communities might have been changed and were prone to degrade pyridine quickly.

In our experiments, the COD biodegradation rates exceeded 81% in all the treatments (Table 1). However, the *CE* values were smaller than 5%, which suggested that various by-product reactions might happen in the MFC. Most of the COD removed was used to support the microbial growth and production of methane or hydrogen. The mass balance in the MFC includes the masses of electron, carbon, and proton. It was reported that in an MFC experiment using acetate as the substrate, the carbon balance had high errors (9–20%) [22]. If glucose is used in the MFC, it is more challenging to set up the mass balance because of the production of methane or hydrogen, which is difficult to be measured accurately. In addition, the mass balance changes with the operation conditions. The quick degradation of pyridine suggests that the recalcitrant organic can be degraded efficiently through the MFC, though the organic may not make a great contribution to the electricity generation.

Battersby and Wilson [23] indicated that susceptibility to biodegradation of pyridine increased after 10 weeks of acclimation. As shown in Fig. 7, pyridine degradation rates in the MFC with an acclimation period of 90 d were faster than those in the anaerobic and aerobic biodegradation methods without acclimation periods. Pyridine was degraded completely within 12 h in the MFC, whereas pyridine biodegradation rates were only 8.2 and 22.2% at 3 d under the aerobic and anaerobic biodegradation conditions, respectively (Fig. 7). The microbial communities in the MFC might be prone to degrade pyridine after a long period of acclimation using the pyridine–glucose mixtures as the fuel. Similarly Mathur et al. [24] isolated a bacteria strain *S. putrefaciens* from biofilters, which degraded 500 mg/L of pyridine completely within 140 h.

The CEs of the G-MFC were about 1% using the pyridine–glucose mixtures or pyridine as the fuel, indicating that most of electrons exhausted in the MFC and did not contribute to the power generation [25]. Furthermore, the degradability of substrates had a great influence on power generation, which agreed with other studies [12,25]. Compared to the G-MFC, the B-MFC showed that CEs were enhanced by 89, 186, and 586% for the mixtures with ratios of glucose-to-pyridine of 1:1, 1:2, and 1:5, respectively, and the corresponding electron charges increased by 90.2, 192.0, and 577.2% (calculated based on the cell voltages higher than 100 mV). The enhancement of CEs and electron charges attributed to the high specific surface area of the brush and the lower internal resistance [26].

The intermediate NH_3-N was consumed totally within 36 h in the G-MFC. This result disagreed with Bai et al. [27], who reported that NH_3-N was not utilized by bacteria for a limiting factor of C:N = 4.3:1. Compared with the pure bacteria used by Bai et al. [27], the interaction of mixed bacteria used in our study might result in the difference [28]. Furthermore, the anaerobic environment in the MFC enhanced NH_3-N consuming. Similarly, Mudliar et al. [29] report that 22% nitrogen from pyridine may have been used in biomass synthesis during pyridine biodegradation in a rotating rope bioreactor.

4.3. Metabolic pathway of pyridine degradation

Biodegradation of heterocyclic compounds under an anaerobic environment is largely dependent upon the presence of electron acceptors if organic carbon is readily available [30]. The metabolic pathway of pyridine under anaerobic conditions is initiated either by ring reduction or ring hydroxylation [2,31]. In our study, NH_3 was detected in the anode solution, indicating that electricity was generated from the pyridine biodegradation to NH_3. The transformation of the carbon and nitrogen of pyridine were detected within 48 h. The value of pH was slightly decreased from the initial stage of 7.0–6.7 at 12 h, suggesting that some acid intermediates were produced. No heterocyclic intermediates of pyridine were detected by GC–MS and only one peak for pyridine was detected by the HPLC analysis during the biodegradation. Therefore, it seemed not possible for pyridine to be degraded in the pathway proposed by Zefirov et al. [32]. Pyridine may be degraded in the metabolic pathways proposed by Rhee et al. [33], in which pyridine ring was cleaved between the C_2 and N, and deaminated to glutaric dialdehyde subsequently, followed by successive oxidation to glutarate semialdehyde.

5. Conclusions

Electricity was successfully generated using pyridine as the fuel in the G-MFC after acclimation for 90 d. Pyridine with concentrations up to 500 mg/L was biodegraded efficiently and was not detectable within 12 h. After the acclimation, the bacteria in the anode chamber of the MFC degraded pyridine efficiently with pyridine as fuel. The biodegradation rates were even faster than those with the supplementary of glucose in the earlier operation periods. The results suggested that the metabolism of pyridine in the MFC was cleaved between the C_2 and N and deaminated to glutaric dialdehyde subsequently, followed by successive oxidation to glutarate semialdehyde. This study shows the potential of biodegradation of recalcitrant compounds and their possible electricity generation using the MFC.

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References

[1] D.H. Lataye, I.M. Mishra, I.D. Mall, Removal of pyridine from aqueous solution by adsorption on bagasse fly ash, Ind. Eng. Chem. Res. 45 (2006) 3934–3943.
[2] K.V. Padoley, A.S. Rajvaidya, T.V. Subbarao, R.A. Pandey, Biodegradation of pyridine in a completely mixed activated sludge process, Bioresour. Technol. 97 (2006) 1225–1236.
[3] J. Leenheer, T. Noyes, H. Stuber, Determination of polar organic solutesin oil-shale retort water, Environ. Sci. Technol. 16 (1982) 714–723.
[4] S. Fetzner, Bacterial degradation of pyridine, indole, quinoline and their derivatives under different redox conditions, Appl. Microbiol. Biotechnol. 49 (1998) 237–250.
[5] D. Mohan, K.P. Sing, S. Sinha, D. Gosh, Removal of pyridine from aqueous solution using low cost activated carbons derived from agricultural waste materials, Carbon 42 (2004) 2409–2421.
[6] Z. Ronen, J. Bollag, Biodegradation of pyridine and pyridine derivatives by soil and subsurface microorganisms, Int. J. Environ. Anal. Chem. 59 (1995) 133–143.
[7] S. Mudliar, K. Padoley, P. Bhatt, M. Sureshkumar, S. Lokhande, R. Pandey, A. Vaidya, Pyridine biodegradation in a novel rotating rope bioreactor, Bioresour. Technol. 99 (2008) 1044–1051.
[8] H. Liu, R. Ramnarayanan, B.E. Logan, Production of electricity during wastewater treatment using a single chamber microbial fuel cell, Environ. Sci. Technol. 38 (2003) 2281–2285.
[9] H. Liu, B. Logan, Electricity generation using an air cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, Environ. Sci. Technol. 38 (2004) 4040–4046.
[10] C. Sukkasem, S. Xu, S. Park, P. Boonsawang, H. Liu, Effect of nitrate on the performance of single chamber air cathode microbial fuel cells, Water Res. 42 (2008) 4743–4750.
[11] S. Oh, B. Logan, Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies, Water Res. 39 (2005) 4673–4682.
[12] H. Luo, G. Liu, R. Zhang, J. Song, Phenol degradation in microbial fuel cells, Chem. Eng. J. 147 (2008) 259–264.
[13] Y. Fan, H. Hu, H. Liu, Enhanced Coulombic efficiency and power density of air-cathode microbial fuel cells with an improved cell configuration, J. Power Sources 171 (2007) 348–354.
[14] T. Catal, Y. Fan, K. Li, H. Bermek, H. Liu, Effects of furan derivatives and pyridineic compounds on electricity generation in microbial fuel cells, J. Power Sources 180 (2008) 162–166.
[15] M. Doughlas, Von Nostrands Scientific Encyclopedia, Von Nostrand Rein Hold Company, New York, 1976.
[16] K. Verschueren, Handbook of Environmental Data on Organic Chemicals, Rein Hold Company, New York, 1977.
[17] D. Lovley, E.J.P. Phillips, Novel mode of microbial energy metabolism: organism carbon oxidation coupled to dissimilatory reduction of iron and manganese, Appl. Environ. Microbiol. 54 (1988) 1472–1480.
[18] H. Liu, S. Cheng, B. Logan, Production of electricity from acetate or butyrate in a single chamber microbial fuel cell, Environ. Sci. Technol. 39 (2005) 658–662.
[19] State Environmental Protection Administration of China, Monitoring and Analysis Methods of Water and Wastewater, third ed., China Environmental Science Press, Beijing, 1989, pp. 254–256, 354–356.
[20] K. Raunkjaer, T. Hvitved-Jaclbse, P.H. Neilsen, Measurement of pools of protein, carbohydrate and lipids in domestic wastewater, Water Sci. Technol. 28 (1994) 251–262.
[21] B.E. Logan, Microbial Fuel Cells, John Wiley & Sons, Inc., Hoboken, New Jersey, 2008, pp. 58–60.
[22] S. Freguia, K. Rabaey, Z. Yuan, J. Keller, Electron and carbon balances in microbial fuel cells reveal temporary bacterial storage behavior during electricity generation, Environ. Sci. Technol. 41 (2007) 2195–2921.
[23] N. Battersby, A. Wilson, Survey of the anaerobic biodegradation potential of organic chemicals in digesting sludge, Appl. Environ. Microbiol. 55 (1989) 433–449.
[24] A. Mathur, C. Majumder, S. Chatterjee, P. Roy, Biodegradation of pyridine by the new bacterial isolates *S. putrefaciens* and *B. sphaericus*, J. Hazard. Mater. 157 (2008) 335–343.
[25] B. Logan, B. Hamelers, U. SchrÖeder, Microbial fuel cells: methodology and technology, Environ. Sci. Technol. 40 (2006) 5181–5192.
[26] B.E. Logan, S. Cheng, V. Watson, G. Estadt, Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells, Environ. Sci. Technol. 41 (2007) 3341–3346.
[27] Y. Bai, Q. Sun, C. Zhao, D. Wen, X. Tang, Microbial degradation and metabolic pathway of pyridine by a *Paracoccus* sp. strain BW00, Biodegradation 19 (2008) 915–926.
[28] T. Wilkinson, H. Topiwala, G. Hamer, Interactions in a mixed bacterial population growing on methane in continuous culture, Bioresour. Technol. 16 (2005) 41–49.
[29] S.N. Mudliar, K.V. Padoley, P. Bhatt, M. Sureshkumar, S.K. Lokhande, R.A. Pandey, A.N. Vaidya, Pyridine biodegradation in a novel rotating rope bioreactor, Bioresour. Technol. 99 (2008) 1044–1051.
[30] D. Grbić-Galić, Microbial degradation of homocyclic and heterocyclic aromatic hydrocarbons under anaerobic conditions, Develop. Ind. Microb. 30 (1989) 237–253.
[31] G.K. Watson, R.B. Cain, Microbial metabolism of the pyridine ring metabolic pathways of pyridine biodegradation by soil bacteria, Biochem. J. 146 (1975) 157–172.
[32] N. Zefirov, S. Agapova, P. Terentiev, I. Bulakhova, N.I. Vasyukova, L. Modyanova, Degradation of pyridine by *Arthrobacter crystallopoeites* and *Rhodococcus opacus* strains, FEMS Microbiol. Lett. 118 (1994) 71–74.
[33] S.-K. Rhee, G. Lee, S.-T. Lee, Influence of a supplementary carbon source on biodegradation of pyridine by freely suspended and immobilized *Pimelobacter* sp., Appl. Microbiol. Biotechnol. 44 (1996) 816–822.