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Nitrate as an Oxidant in the Cathode Chamber of a Microbial Fuel Cell for Both Power Generation and Nutrient Removal Purposes

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Abstract Nitrate ions were used as the oxidant in the cathode chamber of a microbial fuel cell (MFC) to generate electricity from organic compounds with simultaneous nitrate removal. The MFC using nitrate as oxidant could generate a voltage of 111 mV (1,000 Ω) with a plain carbon cathode. The maximum power density achieved was 7.2 mW m^{-2} with a 470 Ω resistor. Nitrate was reduced from an initial concentration of 49 to 25 mg (NO_3^-−N) L^{-1} during 42-day operation. The daily removal rate was 0.57 mg (NO_3^-–N) L^{-1} day^{-1} with a voltage generation of 96 mV. In the presence of Pt catalyst dispersed on cathode, the cell voltage was significantly increased up to 450 mV and the power density was 117.7 mW m^{-2}, which was 16 times higher than the value without Pt catalyst. Significant nitrate removal was also observed with a daily removal rate of 2 mg (NO_3^-–N) L^{-1} day^{-1}, which was 3.5 times higher compared with the operation without catalyst. Nitrate was reduced to nitrite and ammonia in the liquid phase at a ratio of 0.6% and 51.8% of the total nitrate amount. These results suggest that nitrate can be successfully used as an oxidant for power generation without aeration and also nitrate removal from water in MFC. However, control of the process would be needed to reduce nitrate to only nitrogen gas, and avoid further reduction to ammonia.

Keywords Nitrate removal · Microbial fuel cell · Power generation · Cathode chamber · Electron acceptor

Introduction

Nitrate is a widespread pollutant to global water system, most commonly groundwater and surface water [1, 2]. The accumulation of nitrate has a negative impact on both the

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environment and human health. In most common case, domestic and agricultural wastewater contain high amount of nutrients such as nitrogen in the form of ammonia. However, in the rivers, nitrate and ammonium are the most common forms of nitrogen, nitrate alone accounts for over 80% of total nitrogen. For the period 1992–1996, over 65% of the rivers in the European Union had average annual nitrate concentration exceeding 1 mgN l^{-1}. During the same period, concentration of nitrate over 7.5 mgN l^{-1} was also found in approximately 15% of the cases. The highest concentration appears in Northwest Europe, where agriculture is intensive. It is reported that agriculture remains the largest source of nitrogen in water in Europe [3]. Meanwhile industrial wastewater also contains nitrogen, particularly water discharged by manufacturers of fertilizer or explosives, metal processing industries, and food processing industries [3].

The removal of nitrogen is essential and necessary. The conventional process for nitrogen removal involves two steps, nitrification and denitrification. In nitrification step, nitrogen in ammonia, the most common form of nitrogen in wastewater, is biologically oxidized to nitrate. Then, in a denitrification step, nitrate is reduced to nitrogen gas. The denitrification process is a heterotrophic process and needs electron donor, such as methanol, in order to complete reduction from nitrate to nitrogen gas. Since denitrification is a multiple reaction facilitated by microorganisms, a series of intermediate gaseous nitrogen oxide compounds are produced in the process, through which molecular nitrogen (N_2) is ultimately produced [4]. This respiratory process uses organic matter as the electric donor and electron acceptors in order of most to least thermodynamically favorable include nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O). The denitrification process completes the N cycle by returning N_2 to the atmosphere. The multistep reaction of nitrate to N_2 is given as follows (Eq. 1).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{1}$$

Other techniques such as reverse osmosis, ion exchange, and electrodialysis have been suggested to remove nitrate from drinking water [5, 6], however, these are relatively expensive procedures and lead to a discharge problem of the high saline content in waste streams [7].

A microbial fuel cell (MFC) has been regarded as a novel and elegant technology not only to remove organic wastes but also to recover energy from waste/wastewater. The electron acceptors in the cathode chamber of a bench-scale MFC were mainly oxygen or chemical compound such as ferricyanide. Ferricyanide used as an oxidant typically produces 1.5–1.8 times more power output than using dissolved oxygen in the same MFC [8]. However, there are several obvious disadvantages in using ferricyanide as a catholyte. Firstly, the ferricyanide itself is a toxic compound in the list of the EPA's Toxic Substances Control Act inventory [9]. Another disadvantage of ferricyanide is that when it is reduced to ferrocyanide, it has to be refilled with more solution to prevent its depletion in MFC operation. Oxygen can be constantly replenished by aeration in the cathode chamber, while the continuous aeration is an energy-consuming process [10]. Finally, ferricyanide is an expensive chemical compound, which does not permit industrial application. Therefore, new alternative oxidants for the MFC operation are needed to reduce the operational costs and recover sustainable energy.

There was research on simultaneous organics removal and bio-electrochemical denitrification in microbial fuel cells [11]. It has been shown that the redox potential of $NO_3^-/0.5N_2$ (+0.74 V at pH 7) has similarity to that of O_2/H_2O (+0.82 V at pH 7), and have demonstrated that nitrate is competitive to oxygen as an electron acceptor [11]. It was also mentioned in the literature that nitrate is of major interest due to the relatively high redox potential of the NO_3^-/N_2 (E'=+0.74 V; Eq. 2) [12]. In addition, the redox potential for ferri/ferrocyanide +0.55 V was pointed out by [13].

$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O(+0.74V) \qquad (2)$$

In our study, we investigated the feasibility of using nitrate as oxidant, without aeration and denitrifying bacteria in the cathode chamber for sustaining power in a microbial fuel cell. Additionally, nitrate removal in the cathode chamber was investigated by determining nitrate removal efficiency and also the forms of nitrogen such as nitrite and ammonium after an electrochemical nitrate reduction were checked. Pt-dispersed carbon electrode was used for improvement of electricity production and nitrate removal.

Materials and Methods

Domestic Wastewater and Chemicals

Domestic wastewater was collected from Lundtofte Wastewater Treatment Plant in Lyngby, Denmark. The non-diluted raw wastewater was used as the medium and inoculum for the anode chamber of the MFC without any modifications such as pH adjustment or the addition of nutrients or trace metals. Wastewater containing a diverse microbial community was added to the anode chamber. The microorganisms were firstly free suspended in the MFC, while with time they could attach to the electrode to form a biofilm layer. In the anode chamber, organic matter in the wastewater was oxidized by microorganisms, generating electrons and protons. Electrons were transferred to the cathode chamber through an external electric circuit, and the protons passed to the cathode chamber through a PEM membrane. Electrons and protons were consumed in the cathode chamber, combining with oxygen to form water. The microorganisms played a role in catalytic reaction that converted chemical energy to electrical energy. Potassium hexacyanoferrate (III) (100 mM) was initially used as the oxidant (electron acceptor) in the cathode chamber, and then nitrate as an oxidant at a concentration of 49 mg (NO_3^-–N) L^{-1} was used for nitrate removal tests. Phosphate buffer solution (100 mM), which consists of disodium hydrogen phosphate (8.19 g L^{-1}) and sodium dihydrogen phosphate (6.6 g L^{-1}), was added into cathode chamber as the start solutions. The purpose of adding phosphate buffer solution was to increase ionic strength and maintain the pH at the desired level. Sodium acetate solution, concentration of 20 mM was added to the anaerobic chamber together with wastewater medium as the main substrate. All the chemical solutions were prepared with distilled water. The pH in cathode chamber was initially adjusted to around 7.

MFC Configuration and Operation

The MFC consisted of an anode and a cathode chamber (total volume 300 mL each) with the working volume of 250 mL. The two chambers were separated by a proton exchange membrane (Nafion® 117, Dupont Co., Wilmington, USA). The membrane was pre-treated by sequentially boiling in H_2O_2 (30%), deionised water, 0.5 M, H_2SO_4, and then deionised water, each step for 1 h [14]. Plain carbon paper (non-wet proofed) with the surface area of 36.6 cm^2 served as both anode and cathode electrodes. In some experiments, the cathode electrode was replaced with a Pt-dispersed carbon electrode (Pt loading rate=0.5 g cm^{-2};

wet proofed). In MFC studies, wet-proofed cathodes are typically used for oxygen reduction [15–17], due to its ability to reduce oxygen molecule dissolved in water.

In the first setup of the MFC experiments, potassium ferricyanide was used as the electron acceptor (oxidant) in the cathode chamber, and external resistance of 1,000 Ω was applied. The MFC was operated at 30 °C with a coil surrounding connected to a water bath. The anode chamber was filled with domestic wastewater mixed with 20 mM sodium acetate and 100 mM phosphate buffer at pH 7.0. The cathode chamber was filled with 100 mM potassium ferricyanide containing 100 mM phosphate buffer set at pH 7.0. When the voltage generation was stabilized in the preliminary experiment, nitrate solution 49 mg $(NO_3^-–N)$ L^{-1} with 100 mM phosphate buffer was then introduced to the cathode chamber to replace the ferricyanide solution. Nitrate solution $(NO_3^-–N)$ varied from 8.24 mg L^{-1} till 1,650 mg L^{-1} in order to investigate the power generation at different concentrations of the nitrate oxidant. We also tested the carbon paper electrode dispersed with Pt catalyst. We analyzed both the potential and nitrate removal rate with exactly the same procedure except the replacement of cathode electrode from normal carbon paper to Pt-dispersed carbon paper.

Analysis and Calculations

The cell voltage across a 1,000 Ω resistor was automatically registered every 10 min using a data acquisition program. In polarization tests, the load between two electrodes varied from 10 Ω to 33 kΩ (typically 5–10 min interval between the resistors), and the voltage and maximum power were obtained as a function of current density. The potential of each electrode was measured by immersing an Ag/AgCl reference electrode (Bioanalytical Systems, Inc.) to the anode and cathode solutions. After the power generation was stabilized, samples from both anode and cathode chambers were taken every day for acetate and nitrate measurements. All liquid samples were filtered through a 0.2 μm syringe membrane (Sartorius Minisart) before analysis. The chemical oxygen demand (COD) was measured using a standard method (method 5220; Spectroquant COD-Test, Merck) [18].

Acetate was analyzed by GC with a flame ionization detector and a 30 m×0.53 mm× 1 μm ZB-FFAP, nitroterephthalic acid-modified polyethylene glycol column. Ammonium $(NH_4^+–N)$, nitrite $(NO_2^-–N)$ and nitrogenous oxides $(NO_2^-–N+NO_3^-–N)$ concentrations were analyzed by a nitrogen auto analyzer, with cadmium coil. This automated procedure for the determination of nitrate and nitrite, uses the procedure whereby nitrate is reduced to nitrite by a copper–cadmium column reductor coil at a pH of 8.0. The nitrite ion then reacts with sulphanilamide under acidic conditions to form a diazole compound. This compound then couples with N-1-naphthylenediamine dihydrochloride to form a reddish-purple azo dye, which is colorimetrically measured at 550 nm [19].

Power density, P (W m^{-2}), was obtained according to $P=IV/A$, where I (A) is the current, V (V) the voltage, and A (m^2) the surface area of the anode. The half reaction in each chamber is shown in Eqs. 3 and 4.

$$\text{Anode reaction}:\ CH_3COO^- + 4H_2O \rightarrow 2HCO_3 + 9H^+ + 8e^- \tag{3}$$

$$\text{Cathode reaction}:\ NO_3^- + 3H_2O + 5e^- \rightarrow {}^1\!/\!_2N_2 + 6OH^- \tag{4}$$

The coulombic efficiency (CE) was calculated according to Eq. 5. The coulombic efficiency was deduced as follows: CE=CP/CT · 100%, where CP is the total coulombs calculated by integrating the current over time. CT is the theoretical amount of coulombs that can be produced from the substrate, which should be calculated as follows: CT=*FbSv*/*M*, where *F* is the Faraday's constant (96,485 °C per mole of electrons), *b* is the number of moles of electrons produced per mole of substrate, *S* is the substrate concentration, *v* is the liquid volume, and *M* is the molecular weight of the substrate [11]. The coulombic efficiency can be an indicator for the efficiency of the process to utilize the organic matter to power generation.

$$\text{Coulombic efficiency} = C_1/C_2 \tag{5}$$

$$C_1 = (\text{moles}_{\text{initial}} - \text{moles}_{\text{final}}) \times 96,500\text{coulomb/mol}$$

$$C_2 = \text{current(coulomb/s)} \times \text{time}$$

Results and Discussion

Power Generation with Nitrate as Electron Acceptor

In a preliminary experiment, the MFC was started with nitrate solution 49 mg (NO_3^-–N) L^{-1} in the cathode chamber and wastewater modified with 20 mM acetate addition in the anode chamber. However, a very small voltage (less than 5 mV) was obtained in 5 days operation (data not shown). In order to fast develop of an active biofilm on the anode electrode, potassium ferricyanide was initially used as the electron acceptor in the cathode chamber of the MFC. A circuit voltage of 250 mV was generated with a fixed 1,000 Ω resistor within the first 2 h, and then it was rapidly increased up to 666 mV within 16 h (Fig. 1). The average power density was 121.2 mW m^{-2} during 14 h (between 16 and 30 h). When the power output was stabilized, the ferricyanide solution in the cathode chamber was replaced with nitrate solution of 49 mg (NO_3^-–N) L^{-1} at hour 31. With the nitrate oxidant, voltage was immediately developed, but the cell voltage decreased down to 111 mV (1,000 Ω; power density was 3.4 mW m^{-2}), which was nearly six times lower than in the previous set up with ferricyanide solution. With ferricyanide vs. an Ag/AgCl reference electrode, a voltage of +666 mV was obtained between anode and cathode. While with nitrate, only +111 mV was gained between the two electrodes. The anode potential with nitrate was 0 mV, and with a cathode potential of +111 mV, i.e., the overall cell voltage with nitrate was 111 mV. The initial cathode potential OCP ($\text{OCP}_{\text{cathode}}$) of 49 mg ($NO_3^-$–N) L^{-1} nitrate solution was +0.409 V which was a little lower than the theoretical redox value (0.74 V NO_3^-–N/N_2). In addition, the obtained $\text{OCP}_{\text{cathode}}$ from this experiment was very similar to the range of the cathode potential of 0.399 to 0.425 V with oxygen as oxidant in two or single chamber MFCs [20]. The typical $\text{OCP}_{\text{cathode}}$ with ferricyanide was +0.332 V which is lower than the values with a nitrate oxidant obtained here [10]. The OCP of the anode in this study was −0.278 V which was similar to the theoretical value (−0.29 V) [21]. If we consider a positive potential difference between anode acetate and cathode nitrate, when acetate is used as electron donor ($E^{0\prime}_{\text{HCO3/CH3COO}}$=−0.29 V vs. SHE) and nitrate is used as electron acceptor, oxidant ($E^{0\prime}_{\text{NO3/NO2}}$=+0.433 V vs. SHE) or nitrite ($E^{0\prime}_{\text{NO2/NO}}$=+0.350 V vs. SHE),

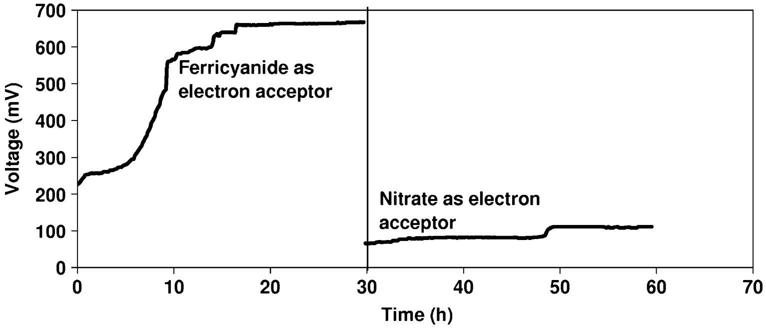

Fig. 1 Voltage with domestic wastewater in anode chamber (1,000 Ω). *Left curve* potassium ferricyanide was used as electron acceptor in cathode chamber. *Right curve* sodium nitrate was used as electron acceptor in the cathode chamber (no catalyst on cathode electrode)

this process is theoretically feasible to produce energy [22]. It was shown that nitrate could act as oxidant in MFC and result in a high potential due to a reasonable OCP. However, an effective catalyst such as Pt would be needed on the cathode for increasing power density in the MFC.

Power Generation as a Function of External Resistance and Nitrate Concentrations

The polarization curve was obtained by loading various circuit resistances (10 Ω to 30 kΩ) between the electrodes in order to determine the maximum power generation (optimal external resistor) in the system (Fig. 2). The maximum power density was 7.2 mW m^{-2} at a current density of 64.5 mA m^{-2}, and the optimal resistance was 470 Ω. The maximum power density we achieved in this study with nitrate as oxidant was almost 10 times lower compared to maximum power outputs (70 mW m^{-2}) with ferricyanide as oxidant from two chamber MFCs that our previous study operated [23]. In the previous study, we used the

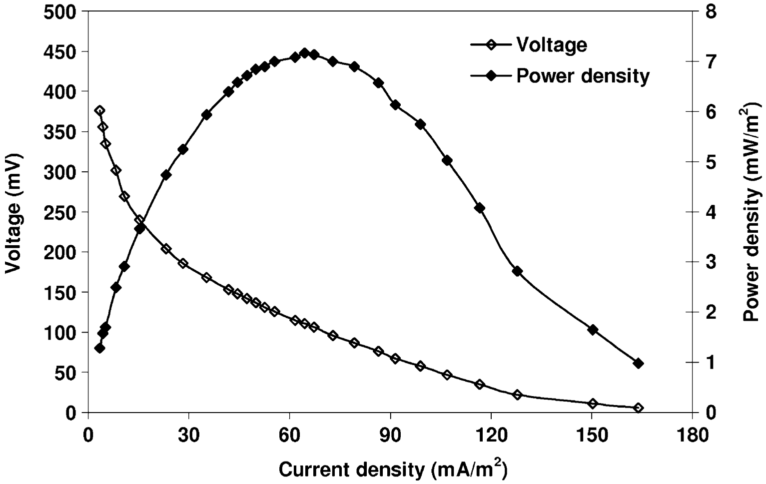

Fig. 2 Polarization curve, potential, and power density as a function of current density obtained using external resistors of 10 Ω–30 KΩ with sodium nitrate as the electron acceptor (no catalyst on cathode electrode)

same setup and operation conditions, with the only difference that ferricyanide was used as oxidant in the cathode chamber.

Power output was measured as a function of different concentrations of nitrate solution in cathode chamber (Fig. 3). The concentration ranged from 8.24 mg (NO_3^-–N) L^{-1} to 1,650 mg (NO_3^-–N) L^{-1} together with 100 mM phosphate buffer. The maximum voltage of 73 mV (470 Ω) was observed at 450 mg L^{-1} of nitrate, but there was no significant difference in voltage obtained over the whole ranges of concentrations. The indifference of voltage to nitrate concentrations could be due to lack of difference in the cathode potentials. The OCP cathode potential fluctuated around −300±15 mV and short circuit potentials ranged from +2 mV at nitrate concentration 49 mg L^{-1} to +6.9 mV at a nitrate concentration of 1,650 mg L^{-1}. We can see from open circuit potentials at different concentrations of nitrate solution that nitrate can be functioned as the electron acceptor in cathode chamber. The cell potential, anode potential, and cathode potential varied from +408 to +420 mV, −402 to −415 mV, and +2.0 to +6.9 mV, respectively.

Nitrate Removal on the Plain Carbon Electrode

The cathode chamber of MFC was initially filled with 49 mg (NO_3^-–N) L^{-1} (0.8825 mmol). At this time, the average voltage with a 470 Ω resistor was 96 mV (current=0.2 mA) for 42 days of operation. Based on the current generation and Eq. 3 in Materials and Methods, the initial acetate (1.64 g L^{-1}) should be completely consumed theoretically within 25 days of MFC operation. However, 0.24 g L^{-1} acetate was left after 42 days of operation (33.3 mg acetate L^{-1} day^{-1}). Coulombic efficiency based on the substrate (acetate) removal and current generation was 26.2 % during this operation. At the same time, nitrate concentration in the cathode chamber decreased from 49 to 25 mg L^{-1} (Fig. 4). Nitrate was reduced to ammonium and at day 29, a concentration of 7.6 mg (NH_4^+–N) L^{-1} was measured in the cathode chamber. Nitrite was also detected, but it was only 0.8 mg (NO_2^-–N) L^{-1}. The major N-containing compound after nitrate reduction was nitrogen gas with an electrode as direct electron donor in a biofilm-electrode reactor [24]. It was also reported that microbial fuel cell coupled with aeration chamber and biocathode converted 72% of nitrate to nitrite and the remaining might have been converted to nitrous oxide or nitrogen gas [25]. In this

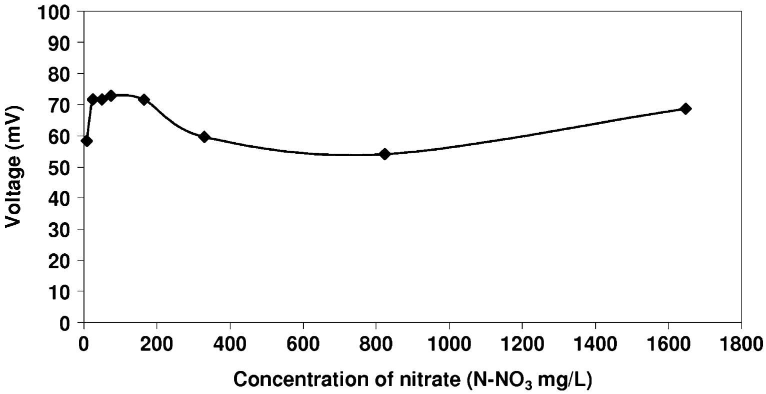

Fig. 3 Cell voltage as a function of different concentrations of nitrate solution in the cathode chamber. (470 Ω, no catalyst on the cathode electrode)

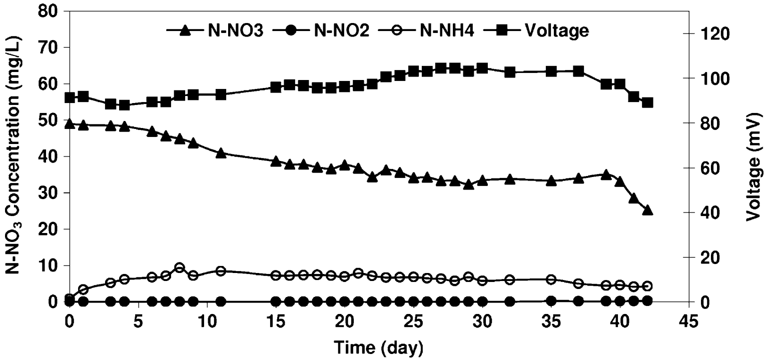

Fig. 4 Nitrogen concentration changes and power generation based on different products from nitrate reduction with time (no catalyst on cathode electrode)

study, we assumed the rest of nitrate went to nitrogen gas; it would be 15.6 mg (N_2–N) L^{-1}. Nitrogen balance was made and is shown in Eq. 6:

$$NO_3^- - N(24mg) \rightarrow NO_2^- - N\ (0.8mg) + NH_4^+ - N(7.6mg) + N_2 - N(15.6mg) \quad (6)$$

Power Generation and Nitrate Removal with Carbon Electrode Dispersed with Pt

The effect of Pt catalyst at the cathode electrode was investigated for power generation and nitrate removal. The cathode chamber was filled with nitrate solution at 49 mg (NO_3^-–N) L^{-1}. The voltage increased and was stabilized at 450 mV after 16-h operation, which was almost 4.7 times higher than the values obtained with the plain carbon cathode electrode (without Pt; Fig. 5). The power density was 117.7 mW m^{-2}, which was almost 16 times higher than with a Pt-free cathode (7.03 mW m^{-2}) measured with optimal resistance 470 ohm. Platinum is often used in cathode electrodes due its excellent capacity in reducing oxygen [26]. Pt-carbon cathode and dissolved oxygen (saturated) as oxidant were previously used in MFC for power generation from wastewater sludge amended with 20 mM [10]. A maximum power of 0.097 mW within 120 h after inoculation was reached [10]. In our study, we reached maximum power of 0.43 mW, which was more than four

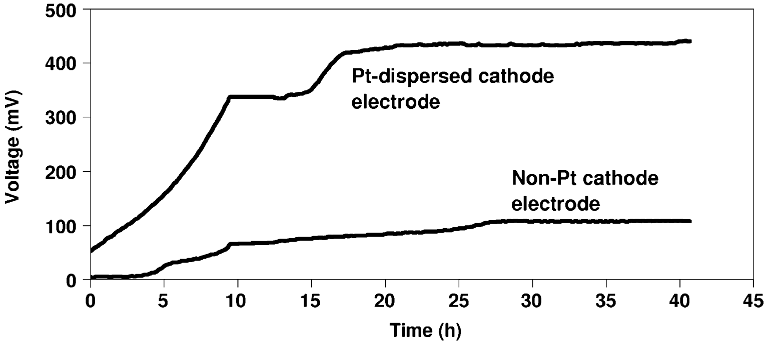

Fig. 5 Power generation with Pt-dispersed and non-Pt carbon paper as cathode electrode versus time

times compared to the previous study. Use of nitrate as oxidant, has the advantage that no aeration is needed, which would reduce the electricity consumption. Moreover, oxygen has the disadvantage of diffusing through the Nafion® membrane to the anodic chamber, which is hard to avoid in cathode-aerated MFCs [27]. An optimized MFC system with aeration needs to be developed to minimize the oxygen supply while sustaining high power output from organic matters.

The cathode potential was +341 mV, and anode potential was −349 mV for nitrate with Pt-dispersed cathode electrode. Coulombic efficiency based on substrate removal and current generation was 66% with Pt catalyst, which was 2.5 times higher than the value (26%) without Pt catalyst. Coulombic efficiency was increased with increased cathode potential. A significant nitrate removal in cathode chamber at a daily removal rate of 2 mg L^{-1} $day^{-1}$was observed in Fig. 6 with Pt catalyst, which was 3.5 times higher than without Pt catalysts (0.57 mg L^{-1} day^{-1}). Jia et al. [11] made an investigation on simultaneous organics removal and bio-electrochemical denitrification using MFC. Their highest power output obtained on the MFC was 1.7 mW m^{-2} and daily removal rate of 3–4 mg L^{-1} day^{-1} with 1,000 ohm resistance. However, in our study, although our daily nitrate removal rate was half of their value by using 470 ohm resistance, our coulombic efficiency was around 3.7 times higher without Pt and nine times higher than their results. The coulombic efficiency in their study was about 7%. However, we got maximum power density without Pt as catalyst 7.03 mW m^{-2}, more than four times higher their result and 117.7 mW m^{-2} around 70 times higher. From above studies, we can conclude that use of Pt as catalyst gives higher potentials, higher power density, and better nitrate removal. The MFC performance was positively enhanced by applying catalyst Pt.

Nitrite and ammonium were detected on the seventh day at 0.09 mg (NO_2^-–N) L^{-1} which corresponding to 0.6% of the total NO_3^-–N and 7.25 mg (NH_4^+–N) L^{-1} which accounts 51.8% of the total NO_3^-–N, respectively. If the rest of the nitrate was converted into nitrogen gas, the calculated nitrogen removal for N_2 gas was 6.66 mg (NO_3^-–N) L^{-1}. Virdis et al. [28] could reduce nitrate into nitrite (below detection limit), ammonium (below detection limit), nitrogen gas (90%), and even N_2O (10%) with a simultaneous electricity generation. In their study, the cathode chamber for nitrate reduction contained biocathode such as granular graphite.

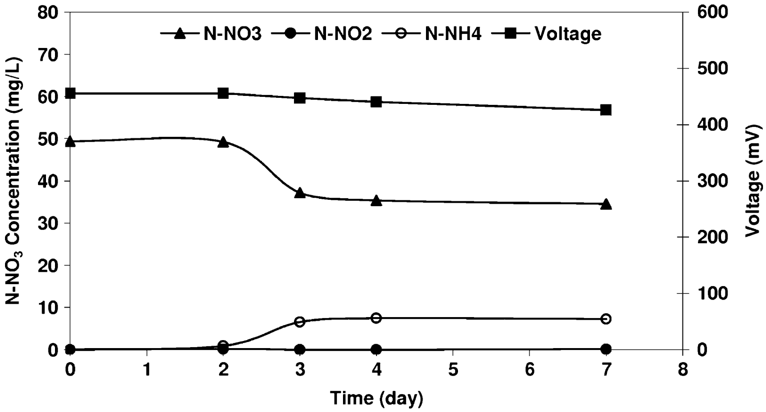

Fig. 6 Nitrogen concentration changes and power generation based on different products from nitrate reduction with time (Pt catalyst on cathode electrode)

Since ammonia is also a pollutant, various redox potentials or other catalysts can be applied to the cathode electrode for converting nitrate to mostly nitrogen gas without ammonia production in the cathode chamber. Additionally, control of nitrate reduction would be needed, for confining reduction to only nitrogen gas and not to further reduction to ammonia. In the future, an advanced MFC configuration can be practical with efficient electrode and catalyst, so that both power output and nitrate removal will be possibly improved.

Conclusions

When nitrate was used as oxidant in the anode chamber of an MFC, electricity was generated, and simultaneously nitrate was removed. The cell voltage of 111 mV was obtained with nitrate as the electron acceptor with a plain cathode (without Pt). The polarization curve showed that the maximum power density appeared at 7.2 mW m^{-2} (64.5 mA m^{-2}) at the optimal resistance 470 Ω. Nitrate was simultaneously removed from the cathode chamber at a removal rate of 0.57 mg (NO_3^-–N) L^{-1} day^{-1}. The cathode electrode dispersed by Pt catalyst could significantly increase the power generation up to 117.7 mW m^{-2} (450 mV), which was 16 times higher than without Pt catalyst. Nitrate removal was also much increased to 2 mg (NO_3^-–N) L^{-1} day^{-1}. The coulomb efficiency for Pt-dispersed electrode was 66% higher than with Pt-free cathode (26.2%). The results from this study demonstrate that nitrate oxidant can be successfully used for electricity generation and also for nitrate removal in MFC operation. However, control of the nitrate reduction process is needed.

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