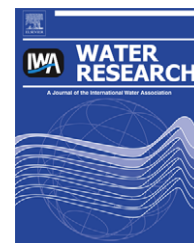


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Effect of nitrate on the performance of single chamber air cathode microbial fuel cells

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ABSTRACT

The effect of nitrate on the performance of a single chamber air cathode MFC system and the denitrification activity in the system were investigated. The maximum voltage output was not affected by 8.0 mM nitrate in the medium solution at higher external resistance (270–1000 Ω), but affected at lower resistance (150 Ω) possibly due to the low organic carbon availability. The Coulombic efficiency was greatly affected by the nitrate concentration possibly due to the competition between the electricity generation and denitrification processes. Over 84–90% of nitrate (0.8–8.0 mM) was removed from the single chamber MFCs in less than 8 h in the first batch. After 4-month operation, over 85% of nitrate (8.0 mM) was removed in 1 h after the MFC was continuously fed with a medium solution containing nitrate. Only a small amount of nitrite (<0.01 mM) was detected during the denitrification process. The similar denitrification activity observed at different external resistances (1000 and 270 Ω) and open circuit mode indicates that the denitrification was not significantly affected by the electricity generation process. No electricity was generated when the MFC fed with 8.0 mM nitrate was moved to a glove box (no oxygen), indicating that the bacteria on the cathode did not involve in accepting electrons from the circuit to reduce the nitrate. Denaturing Gradient Gel Electrophoresis (DGGE) profiles demonstrate a similar bacterial community composition on the electrodes and in the solution but with different dominant species.

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1. Introduction

Electricity generation using microbial fuel cells (MFCs) has drawn much attention recently as a new approach of wastewater treatment (Logan, 2005; Liu et al., 2004). Electricity can be generated from various biodegradable organic materials in MFCs, including carbohydrates (Liu and Logan, 2004), proteins (Logan et al., 2005), and various wastes and waste streams, such as dairy manure (Power et al., 2007), cow-waste slurry (Yokoyama et al., 2006), food processing wastewater (Logan, 2005), and domestic wastewater (Liu et al., 2004). While high

chemical oxygen demand (COD) removal has been achieved in many MFC systems (He et al., 2005; Jang et al., 2003; Kim et al., 2002; Moon et al., 2006; Rabaey and Verstraete, 2005; Zeikus, 2007; Cheng et al., 2006; Cheng and Logan, 2007; Lee et al., 2006; Liu et al., 2005b; Liu and Logan, 2004), further investigations are needed on nitrate removal in MFCs. The runoff of nitrate into lakes not only causes accelerated eutrophication, nitrate is of considerable public health significance.

Recently, it has been demonstrated that microorganisms can be used as catalysts on the cathode of electrochemically assisted cells to reduce nitrate to nitrite or nitrogen gas

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(Gregory et al., 2004; Goel and Flora, 2005; Park et al., 2005). In these studies electrical current was provided by external power supplies and electrodes served as direct electron donors for nitrate reduction by microorganisms in the family Geobacteraceae or adapted enrichment cultures. More recently, Clauwaert et al. (2007) reported biological cathodic denitrification without power input but coupled with energy recovery by using a biological anode of an MFC. It has been noticed that all these studies were conducted using medium solutions containing nitrate but without organic substances in cathode chambers. The fact is that many waste streams, such as those from food industries, wood product industries, farm and municipal waste streams, contain both high concentration of nitrogen and high level of COD. The effect of nitrate on power generation from organic materials and denitrification activity in single chamber MFCs have not been studied yet.

In this study, the effect of nitrate on the performance of single chamber air cathode MFCs was first investigated. Denitrification activities at various nitrate concentrations and external resistances were then examined. Microbial communities on the electrodes and in the solution were also analyzed and the roles of denitrifying bacteria in the electrode communities were discussed.

2. Materials and methods

2.1. MFC construction

Single chamber air cathode MFCs were constructed as described previously (Liu et al., 2005a). Briefly, the MFC consists of an anode (2 cm² projected surface area) and cathode (7 cm² projected surface area) placed in a plastic (Plexiglas) cylindrical chamber with an electrode spacing of 2 cm (Liu and Logan, 2004; Liu et al., 2004). The anode electrode was made of carbon cloth (Type A, without wet-proofing; E-TEK, USA). The cathode was prepared by coating 0.5 mg/cm² Pt catalyst (10% of Pt/C) on a carbon cloth (Type B, 30% wet-proofing; E-TEK, USA) following a published procedure (Cheng et al., 2006).

2.2. MFC inoculation and operation

The MFCs were inoculated with active microorganisms from the anode of an MFC, which was initially inoculated with wastewater from Corvallis wastewater treatment plant (Corvallis, OR) and has been operated in semi-continuous mode using acetate as carbon source for over a year. The medium solution contains 70 mM sodium acetate, 100 mM phosphate buffer (pH 7) and nutrients as described previously (Liu and Logan, 2004). All MFCs were operated at a fixed external resistance of 1000 Ω initially. Electricity was generated in the first batch and the solution was replaced with a new medium solution when the voltage started decreased (Liu and Logan, 2004). The system was considered to be operating under steady conditions when the voltage output was reproducible after refilling the reactor with medium at least two times. The start up normally takes about 3–4 days.

A series of tests were conducted to investigate the effect of nitrate concentration on MFC performance and denitrification

activities in MFCs. In the first set of tests, potassium nitrate was dissolved in the medium solution containing 70 mM acetate and nutrients at room temperature to obtain final nitrate concentrations of 0.8 mM, 1.6 mM, 2.4 mM, 3.2 mM, 4.0 mM, 4.8 mM and 8.0 mM. The nitrate-containing culture medium solutions were then added to individual MFCs operated under steady conditions at 1000 Ω . One control (no nitrate) MFC was operated under the same condition. Nitrate was measured at the beginning and the end of each batch test. In the second set of tests, the effect of nitrate concentration (0, 4.0, 8.0 mM) on the voltage output of MFCs was investigated at various external resistances, including 1000 Ω , 390 Ω , 270 Ω and 150 Ω . The nitrate and nitrite concentrations were monitored during one of the batch cycles with initial nitrate concentrations of 4.0 mM and 8.0 mM to investigate the kinetics of denitrification at 1000 Ω . In the third set of tests, the MFCs fed with 8.0 mM nitrate were first operated at 270 Ω and 1000 Ω external resistances, and then switched to an open circuit mode. Nitrate concentrations were monitored during the process to investigate the effect of electricity generation on denitrification activity. In the fourth set of tests, the MFCs fed with 8.0 mM nitrate medium solution and connected with 400 Ω were moved to a glove box to investigate if the bacteria on the cathode involved in accepting electrons from the circuit in the absence of oxygen. One of the MFCs was also operated for over 4 months to investigate if the nitrate removal rate was affected by operation time. All tests were conducted in 30 $\pm$ 2 $^{\circ}$ C temperature-controlled chamber.

2.3. Analysis and calculations

Cell voltage across external resistor was recorded using a multimeter with a data acquisition system (2700, Keithley). The Coulombic efficiency (CE) based on total acetate added was calculated as previously reported (Liu et al., 2004). Nitrate and nitrite concentrations were measured using Ultraviolet Spectrophotometric Screening Method (APHA, AWA, WPCF, 1995) and Colorimetric Nitrite Assay (Hageman and Hucklesby, 1971), respectively.

2.4. Microbial community analysis

2.4.1. DNA Extraction and Polymerase Chain Reaction (PCR) amplification

Biofilms were scratched from the anode and cathode of MFC fed with 8.0 mM of nitrate and 70 mM acetate for about 20 days (around 10 batches). Bacterial genomic DNA was extracted from the biofilms and the medium solution (end of the batch) using the DNeasy tissue Kits (Qiagen, CA, USA) according to the manufacturer's instructions. The universal primer set 357F-GC (5'-GC-clamp-CCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3') (Invitrogen, Carlsbad, CA, USA) was used to amplify the V3 region of bacteria 16S ribosomal DNA (rDNA) from the extracted genomic DNA (Muyzer et al., 1993). PCR amplification was performed in a thermocycler (Thermo hybaid, MBS 0.2G, Thermo, MA, USA) following a previous procedure (Muyzer et al., 1993).

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