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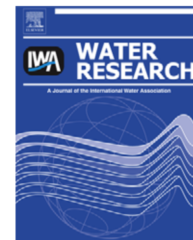

Effect of nitrate on the performance of single chamber air cathode microbial fuel cells

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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., 2005,b; 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 cm^2 projected surface area) and cathode (7 cm^2 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/cm^2 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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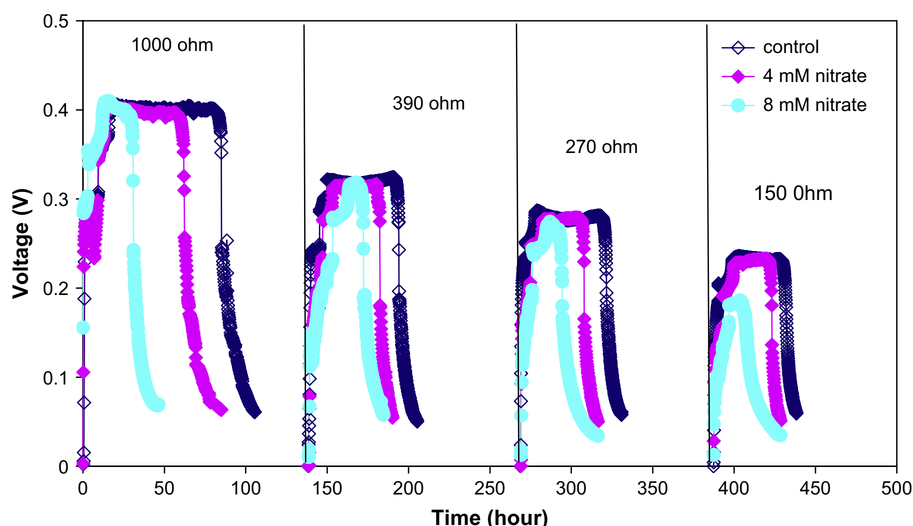

Fig. 1 – Effects of nitrate concentration and external resistance on power generation. No nitrate in the medium solution of the control experiment.

2.4.2. *Denaturing Gradient Gel Electrophoresis (DGGE)*

DGGE of the PCR products was carried out in a Dcode™ Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA). The 8% (w/v) polyacrylamide gels (16 cm × 16 cm gel, thickness of 1 mm) contains from 30% to 55% denaturing gradients (urea and formamide). Electrophoresis was conducted using a 1× TAE (Tris–Acetate–EDTA) buffer at 130 V and 60 °C for 5 h. After electrophoresis, the gel was stained with 1 μg/mL ethidium bromide (American Bioanalytical, Natick, MA, USA) in 1× TAE buffer for 15 min and destained in 1× TAE buffer for 10 min. The fragments were visualized under an UV transilluminator.

2.4.3. *16S rDNA gene sequencing and analysis*

Genomic DNA extracted from MFC was used for PCR-medicated amplification of 16S rDNA. The PCR product was cleaned using Qiagen PCR Purification kits (Qiagen, CA, USA) and cloned into *Escherichia coli* DH5α using the pGEM-T® easy vector system I (Promega, Madison, WI, USA) following the manufacturer's instructions. The sequencing of cloned 16S rDNA was performed by the Center for Genome Research and Biocomputing Core lab (CGRB) at Oregon State University.

3. Results and discussions

3.1. *Effect of nitrate concentration on voltage output*

Nitrate concentrations (0, 4.0, and 8.0 mM) did not affect the maximum voltage output at external resistances of 1000, 390, and 270 Ω (Fig. 1). Similar maximum voltage output was also obtained at 150 Ω for the MFCs fed with 4.0 mM nitrate and those without nitrate. However, when 8.0 mM nitrate solution was used, the maximum voltage at 150 Ω decreased about 20%, possibly due to the depletion of the available carbon source for exoelectrogens at a higher current as a result of the fast consumption of acetate by denitrifying bacteria at a higher nitrate concentration.

3.2. *Effect of nitrate concentration on Coulombic efficiency*

The presence of nitrate in the medium solution significantly affected the CE of MFCs (Fig. 2). The CE decreased with the increase of nitrate concentration for MFCs operated at all four

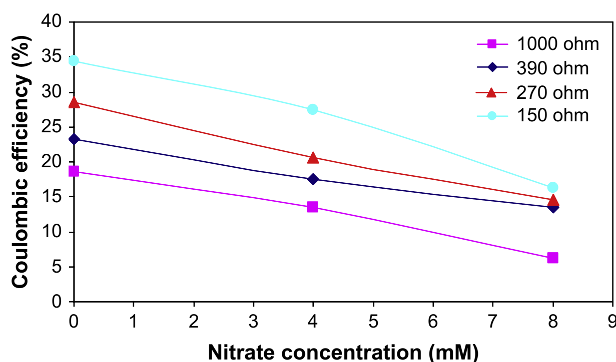

Fig. 2 – Effect of nitrate concentrations on Coulombic efficiency at various resistances.

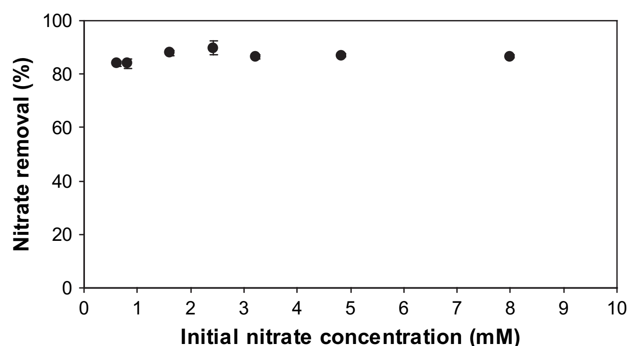

Fig. 3 – Nitrate removal as a function of initial nitrate concentration at 1000 Ω.

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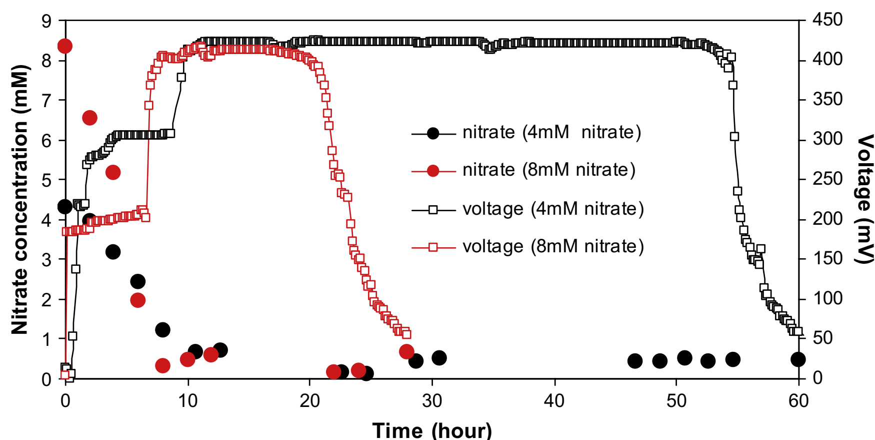

Fig. 4 – Denitrification kinetics for the MFCs containing 4 mM and 8 mM nitrate operated at 1000 Ω.

external resistances (1000, 390, 270, and 150 Ω). Increasing nitrate concentration from 0 to 8.0 mM resulted in 6.3–21.3% decrease in CE depending on the external resistance used. Decreasing external resistance at the same nitrate concentration, however, increased the CE. Similar trend was also found in a previous study in the absence of nitrate (Liu et al., 2004). Compared to a previous study with a similar cell configuration (Fan et al., 2007), the CE of 18–35% (150–1000 Ω; without nitrate) obtained in this study was comparable to the 14–42% (25–1000 Ω) using an MFC with 1.7 electrode spacing, but lower than the 24–71% (25–1000 Ω) when J-cloth was applied to the cathode surface.

Many factors could attribute to the electron loss in the air cathode MFCs, including substrate utilization for methanogenesis and the electron transfer from substrate to other electron acceptors in solution, such as nitrate and oxygen, resulting in a portion of the bacterial community being sustained by non-electricity-generating-processes and lowering the CE (Liu and Logan, 2004; Logan and Regan, 2006). Although the consumption of electrons for denitrification with 8 mM nitrate can only attribute to a maximum of 7% of the electron loss (70 mM acetate), the MFCs fed with 8 mM nitrate developed a much thicker biofilm (15.9 mg/cm^2 based on dry weight) in comparison with that without nitrate (2.9 mg/cm^2). The presence of nitrates in the solution also resulted in the growth of nitrate-utilizing bacteria in the solution, which can be seen based on the higher optical density at 600 nm. The consumption of electrons for denitrification and the growth of denitrifying bacteria may account for the lower CE of the MFCs fed with nitrate.

3.3. *Effect of nitrate concentration on denitrification activity*

Although the inoculum was taken from an MFC fed with acetate without nitrate for about 1 year and the MFCs started using the same medium solution, nitrate was reduced in the first batch using the medium solution containing nitrate. Over 84–90% of nitrate was removed from the MFCs operated with initial nitrate concentrations ranged from 0.8 to 8 mM at 1000 Ω by the end of the batches (Fig. 3). The high removal nitrate rate indicates the presence of denitrifying bacteria in the anode biofilms.

To investigate the kinetics of denitrification, nitrate and nitrite concentrations were monitored during the batch cycle with initial nitrate concentrations of 4.0 and 8.0 mM at 1000 Ω. The reduction of nitrate started right after the addition of medium solution containing nitrate (Fig. 4). Over 85% of nitrate was removed at both concentrations in less than 8 h. The nitrite concentration was always lower than 0.01 mM. A bulk of bubbles were observed in the nitrate-fed MFCs but not found in the control ones, indicating that the nitrate was possibly removed in form of nitrogen gas:

$$NO_3^- + 6H^+ + 5e \rightarrow 1/2\,N_2 + 3H_2O \quad (1)$$

Fig. 4 also demonstrated the relationship between the denitrification and electricity generation processes. For the MFC fed with medium solution containing 4 mM nitrate, the electricity generation process appeared to occur at two stages. At the first stage, a voltage of 0.27 V was generated in 2 h after the MFC was refilled with the new medium solution and the voltage slowly increased to 0.31 V in the following 7 h. The nitrate concentration continuously decreased with an average degradation rate of 0.37 mM h^{-1} in the first 10 h. At the second stage, when the nitrate concentration decreased to less than 0.68 mM, the voltage was jumped to 0.41 V and kept stabilized until the end of the batch. Similar trend was also found for the MFCs fed with 8 mM

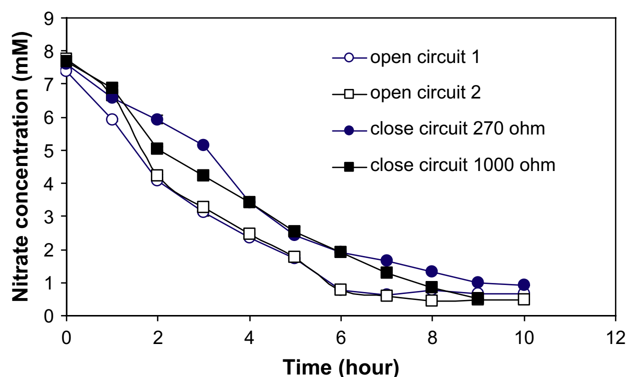

Fig. 5 – Denitrification activity at open and close circuit systems. Open circuit 1: MFC originally operated at 270 Ω; open circuit 2: MFC originally operated at 1000 Ω.

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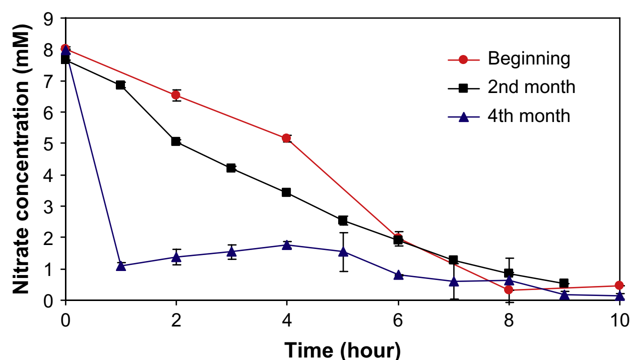

Fig. 6 – Effect of long-term operation on denitrification activity.

nitrate except that the voltage was even lower (0.19 V) in the first stage with a higher nitrate degradation rate of 1.1 mM h^{-1}.

This relationship between the electricity generation and nitrate reduction indicates that there might be two groups of bacteria on the anode of the MFC tested. One group can utilize the anode as electron acceptor and would not change the electron transfer pathways in the presence of nitrate. The other group of bacteria can use anode as electron acceptor in the absence of nitrate, but use nitrate as electron acceptor in the presence of nitrate. When high concentration of nitrate was present in the solution, only the first group of bacteria transferred electrons to the circuit, resulting in low current generation. When the nitrate was consumed, the second group of bacteria also started to transfer electrons to the circuit, achieving high current output. This result indicates that the effect of nitrate on the performance of the single chamber MFCs was mainly through the consumption of the electron donors.

3.4. *Effect of external resistance on denitrification activity*

To investigate if electricity generation affects the denitrification process, nitrate concentrations were monitored in two MFCs fed with same nitrate concentration (8 mM) but operated at two different external resistances: 1000 and 270 Ω, and at an open circuit mode as well. Fig. 5 demonstrates that the denitrification was not affected by the external resistances tested. The denitrification rate at close circuit mode was slightly slower than that at open circuit mode. This was possibly due to the competition between the anode and the nitrate for the electrons released from some bacteria on the anode when the MFCs were operated in the close circuit mode.

3.5. *Effect of long-term operation on denitrification*

One of the MFCs was also operated in batch fed mode for over 4 months (refill new solution with 8 mM nitrate every 3 days) to investigate the effect of operation time on the nitrate removal. Fig. 6 shows that less than 20% nitrate can be removed in 2 h for the beginning period, but more than 85% of nitrate can be removed in 1 h after 4-month operation with an estimated denitrification rate of 7 mM h^{-1}. A decrease in voltage generation from 0.41 to 0.35 V was observed after 2-month operation. One possible reason for such a decrease is that the presence of nitrate in the medium solution resulted in the development of nitrate-utilizing but non-electricity-generating bacteria in the anode biofilm, which limited the mass transfer of substrate. The other possible reason is that the thick biofilm developed on the cathode after 2-month operation increased the cathode polarization and internal resistance of the MFC system.

3.6. *MFC performance in the presence of nitrate but absence of oxygen*

To examine if the bacteria on the cathode involved in the electricity generation process by accepting electrons from circuit, experiments were also conducted by examining the MFC performance in a glove box (without oxygen). The MFC had been operated with nitrate-containing medium for over 2 months before being moved to the glove box. A voltage less than 0.05 V was produced after adding the medium solution and quickly decreased to less than 0.01 V (Fig. 7). This result indicates that in the tested MFC system, the bacteria on the

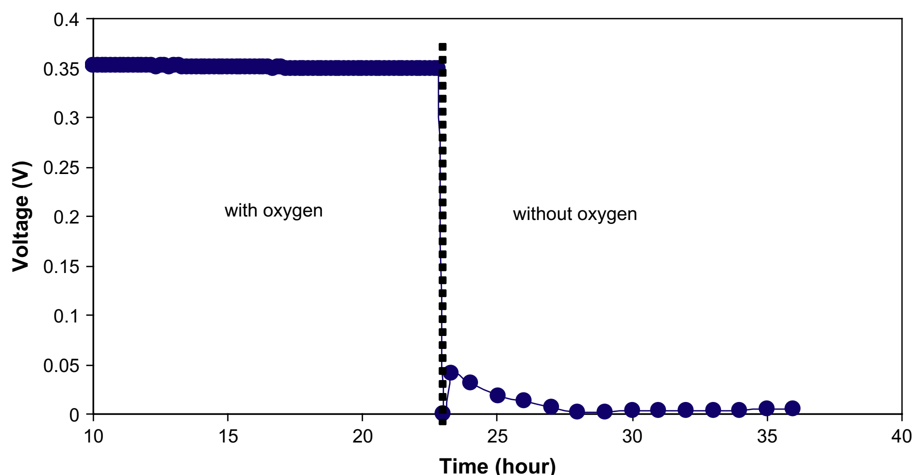

Fig. 7 – Power generation in the presence and absence of oxygen at 400 Ω with 8 mM nitrate in the medium.

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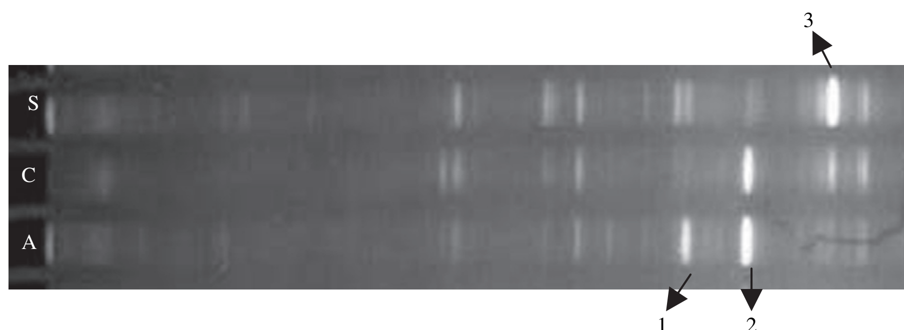

Fig. 8 – PCR-DGGE analysis of 16S rDNA extracted from the anode (A), cathode (C), and solution (S).

cathode did not involve in accepting electrons from the circuit to reduce nitrate. It differs from the previous studies conducted in the two chamber systems (Gregory et al., 2004; Goel and Flora, 2005; Park et al., 2005) that cathode served as the electron donor for denitrifying bacteria in the absence of carbon sources and resulted in denitrification.

3.7. *Microbial community*

Fig. 8 illustrates the DGGE profiles of the 16S rDNA gene fragments amplified from the extracted DNA of the biofilms on the electrodes and the bacteria in the solution at the end of the batch. Each band on the DGGE profile represents a specific species in the microbial community and the staining intensity of a band represents the relative abundance of the corresponding microbial species. Among the detectable bands in the DGGE profiles of the three samples, eight bands were common in all the samples. While the bands 1 and 2 demonstrated very high intensity in the anode sample but only band 2 showed high intensity in the cathode sample. On the other hand, another band, band 3, demonstrated high intensity in the solution (Fig. 8). The similarity of the banding patterns with different staining intensity indicates that although the dominant bacterial species on the electrodes and in the solution are different, the presence of nitrate in the medium solution may result in the growth of denitrifying bacteria on both electrodes and in solution. Our preliminary cloning results demonstrated the presence of some potential exoelectrogens, such as *Geobacter* sp. Ply1 and *Shewanella* algae on the anode. Philippot (2005) tracked nitrate reducers and denitrifiers in the environment and found many *Geobacter* and *Shewanella* species in the nitrate-reducing community. In addition, *Halomonas* sp. *Ap*-5, *Mesorhizobium* sp. 4FB11 and *Rhizobium* sp. UMR7372 were also found in the MFC samples. These species may be related to denitrification or nitrogen fixation (Kaneko et al., 2000; Peyton et al., 2001; Tubb, 1976).

3.8. *Implication for denitrification in wastewater treatment*

MFC technology provides a potential new approach for removing COD from wastewater and generating electricity at the same time. This study demonstrates that MFC can also be very effective in denitrification in wastewater treatment. Although electricity generation, mainly CE, in MFCs was affected by the nitrate due to the competition between the anode and the nitrate for electrons from carbon sources, the simultaneous removal of the COD and nitrate could significantly reduce the cost for wastewater treatment, especially those containing both high COD and nitrate.

High nitrate removal rate was achieved in the first batch when the fed solution was switched from nitrate-free to nitrate-containing medium solution. This indicates that many electricity-generating bacteria are denitrifying bacteria, which can transfer electrons to anode in the absence of nitrate, but directly donate the electrons to nitrate otherwise. Thus no additional enrichment process is needed when the influent of MFCs switches between nitrate-free and nitrate-rich waste streams.

By using the single chamber MFC system, it took less than 1 h to remove 85% of 8.0 mM (112 mg NO_3^-N/L) nitrate after 4-month operation of the MFCs. Such a removal rate is comparable to that using some highly efficient biological denitrification processes (Zayed and Winter, 1998). Although the exact reasons for causing such a high nitrate removal rate are still not clear, the enrichment of highly efficient denitrifying bacteria in MFCs and the higher C/N ratio (48 g COD/g NO_3^-N) in comparison with that in some traditional denitrification process (e.g. 3 g COD/g NO_3^-N; Bernet et al., 1996) may attribute to the high denitrifying activity.

Since only 2 cm^2 anode surface area (7 cm^2 cathode) was used in the study to reduce the limitations on anode, we expect an even higher nitrate removal rate by increasing the anode surface area and the electrode area to reactor volume ratio. Nitrite production is known to be one of the main problems in biological denitrification, because nitrite ions are inhibitors of bacterial growth (Almeida et al., 1995). However, in the MFC system, no nitrite accumulation (less than 0.01 mM) was observed during the fast denitrification process.

For waste stream containing other form of nitrogen, for example, ammonia, the use of MFC for electricity generation from ammonia has yet to be demonstrated. Although ammonia removal has been found in MFCs when treating animal wastewater (Min et al., 2005), later study by the same group shows that ammonia-oxidizing bacteria were not detected on the electrodes. They concluded that ammonia removal was possibly due to the release of ammonia gas from the liquid to the gas phase caused by the increase of local pH on cathode (Kim et al., 2008). Future study is needed to

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investigate the possibility of simultaneous removal of nitrate and ammonia in single chamber MFCs.

4. Conclusions

- Over 85% of nitrate (8 mM) can be removed in the single chamber MFCs in less than 8 h at the first batch, and in 1 h after 4-month operation.
- The maximum voltage output was not affected by the nitrate at higher external resistance, but affected at low resistance possibly due to the low organic carbon availability.
- The CE was greatly affected by the nitrate due to the competition between the electricity generation and the denitrification processes.
- The denitrification was not significantly affected by the electricity generation process and the bacteria on the cathode did not involve in accepting electrons from the circuit to reduce nitrate.

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