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Autotrophic denitrification performance and bacterial community at biocathodes of bioelectrochemical systems with either abiotic or biotic anodes

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Two-chamber bioelectrochemical systems (BESs) have recently been developed for nitrate removal from nitrate-contaminated water. In this study, we compared the nitrate removal performance of biocathodes of BESs when using abiotic and biotic anodes. Acetate was used as electron donor in BESs with biotic anode, whereas a direct current power supply was used as energy source in BESs with abiotic anode. The nitrogen removal efficiency increased from 18.1% to 43.0% when the voltage supplied to the BES with abiotic anode increased from 0.7 V to 0.9 V, whereas no higher removal efficiency was obtained at a higher supplied voltage (1.1 V). The highest efficiency (78.0%) of autotrophic nitrogen removal was achieved when electron transfer from the biotic anode chamber of BESs was used. Unexpectedly, control of the cathode potential did not enhance nitrate removal in BESs with biotic anode. Special attention was paid to elucidate the differences of bacterial communities catalysing autotrophic denitrification in the biocathodes of BESs with abiotic and biotic anodes. Data from denaturing gradient gel electrophoresis and phylogenetic analysis suggested that denitrification in BESs with abiotic anode could be attributed to *Nitratireductor* sp., *Shinella* sp., and *Dyella* sp., whereas the dominant bacterial denitrifiers in BESs with biotic anode were found to be *Pseudomonas* sp., *Curtobacterium* sp., and *Aeromonas* sp. These results implied that biocathodes of BESs with biotic anode are more efficient than those of BESs with abiotic anode for nitrate removal from nitrate-contaminated water in practical applications.

[**Key words:** Bioelectrochemical systems; Nitrate removal; Biocathode; Abiotic anode; Biotic anode; Autotrophic denitrification]

Nitrate (NO_3^-) is one of the common forms of nitrogen compounds found in water environments. Nitrate can enter into both surface water and groundwater as a consequence of excess application of inorganic nitrogenous fertilizers and manures, or improper treatment of wastewater (1). At moderate concentrations, nitrate is a harmless constituent of food and water. However, nitrate levels above 10 mg L^{-1}, which is the maximum contaminant level suggested by the Environmental Protection Agency, may cause methemoglobinemia in infants (2). To date, many technologies have been developed to remove nitrate from nitrate-contaminated groundwater and wastewater (3). Among them, biological technologies are commonly used for the treatment of nitrate-rich water treatment because of the lower impact on the surrounding environment and lower cost compared to other physical and chemical technologies (4).

The combination of biology and electrochemistry in a bioelectrochemical system (BES) is a novel approach for nitrate removal from nitrate-contaminated water, which has attracted the interest of numerous researchers (5–10). Autotrophic denitrification in biocathodes of BESs is now being evaluated by many researchers (6,11–14) when explaining the mechanisms of bioelectrochemical denitrification. A microbial fuel cell (MFC) is a BES with which chemical energy of organic matters can be converted into electrical energy. Recently, the abiotic cathode of a MFC has been replaced by a biocathode in which microorganisms were found to enhance denitrification (5). The autotrophic denitrification of nitrate to nitrogen gas in the biocathode of a MFC utilizing a cathode as electron donor can be described by 4 reactions, which are summarized in Table 1 (5,9,10). The denitrification performances obtained in biocathodes of MFCs were quite different when using various types and materials of reactors. The biocathode of a tubular MFC was able to remove up to 0.146 kg NO_3^-–N per m^3 net cathodic compartment per day (5). In another study, nitrate removal rates of up to 0.41 kg NO_3^-–N per m^3 net cathodic compartment per day were continuously achieved in the cathodic compartment of a rectangular MFC (15). Clauwaert et al. (16) reported that the amount of removed nitrogen could be increased from 0.22 to 0.50 kg NO_3^-–N per m^3 net cathodic compartment per day by maintaining the pH in the biocathode at 7.2. Autotrophic denitrification in a biocathode of a BES has been observed by coupling with heterotrophic denitrification for enhancing total nitrate removal efficiency (13,14). Coupling of autotrophic and heterotrophic denitrification could yield a nitrate removal rate of 1.09 ± 0.17 g m^{-3} h^{-1} in a BES supplemented with starch as a carbon source for microorganisms (13).

Autotrophic denitrification in biocathodes of BESs coupled with heterotrophic denitrification was previously investigated in various types of bioreactors with different materials. In addition, microbial communities related to autotrophic nitrate removal in different types of reactors were not fully investigated. This study, therefore,

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TABLE 1. Summary of reactions occurring during autotrophic nitrate reduction in the biocathode of bioelectrochemical system.

Process	Cathodic reduction reaction	E'_0 (V vs. Ag/AgCl)
Nitrate reduction	$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O$	+0.233
Nitrite reduction	$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O$	+0.150
Nitric oxide reduction	$NO + e^- + H^+ \rightarrow 0.5N_2O + 0.5H_2O$	+0.975
Nitrous oxide reduction	$0.5N_2O + e^- + H^+ \rightarrow 0.5N_2 + 0.5H_2O$	+1.155
Overall denitrification	$NO_3^- + 6H^+ + 5e^- \rightarrow 0.5N_2 + 3H_2O$	+0.549

aimed to evaluate separately autotrophic nitrate removal in biocathodes of BESs by using either an abiotic or biotic anode to compare the denitrification performances. The bacterial communities related to the autotrophic denitrification of nitrate in these reactors were also elucidated.

MATERIALS AND METHODS

BES configuration and operation Experiments were conducted at room temperature (25°C) using a BES consisting of an anode chamber and a cathode chamber placed on opposite sides with a total volume of 350 mL per chamber. Every chamber was fixed with a rectangular graphite felt electrode (3 × 4 cm; GF-S6-06, Electrolytica, USA) connected to a platinum wire. A proton exchange membrane (Nafion 117, DuPont Co., Wilmington, DE, USA) was used as separator placed between anode and cathode chambers. An Ag/AgCl electrode (assumed +0.197 V vs. standard hydrogen electrode) was used as reference electrode. Fig. 1 shows the schematic diagram of BESs with abiotic and biotic anodes that were used in this study to treat nitrate-contaminated water. Synthetic nitrate-contaminated water applied to BESs contained 3.375 g L^{-1} of KH_2PO_4, 4.35 g L^{-1} of K_2HPO_4, 0.584 g L^{-1} of NaCl, and 0.1 g L^{-1} of $MgSO_4 \cdot 7H_2O$. The nitrate concentration was maintained at a final concentration of 50 mg L^{-1} NO_3^-–N by adding 0.361 g L^{-1} of KNO_3. $NaHCO_3$ (2.94 g L^{-1}) was only added to cathode chambers as an inorganic carbon source for microorganisms. The reactor was added with bacterial inoculum and run without enrichment. After completion of the set-up, 300 mL of medium was added to the reactor, which was then monitored for 14–18 d. At that point, the denitrification process had achieved a steady state and the treated medium was completely removed and replaced with an equal amount of new medium. The operating conditions of all bioreactors designed in this study are tabulated in Table 2.

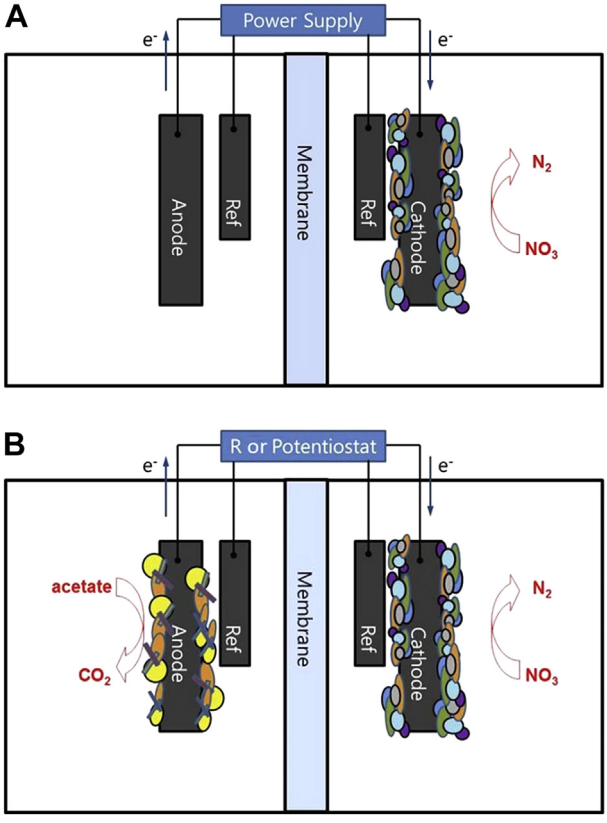

FIG. 1. Schematic diagram of bioelectrochemical systems with an abiotic anode (A) and a biotic anode (B) for autotrophic nitrate removal used in this study.

When using an abiotic anode, BES-abe was connected to a direct current power supply at a voltage of 0.7 V, which was then increased to 0.9 and 1.1 V. Bacterial inocula added to the cathode chamber were activated sludge collected from a domestic wastewater treatment plant in Busan, South Korea. An open circuit control system (BES-abo), to which no electrical voltage was applied, and an abiotic control system (BES-aae), to which no activated sludge was added, were set up in an identical way.

In order to run a BES with a biotic anode similar to a MFC, acetate (as CH_3COONa) was added to the anode chamber as electron donor at a final concentration of 150 mg L^{-1} of chemical oxygen demand (COD). Activated sludge was added to both the cathode and anode chambers as bacterial inocula. The anode and cathode of the BESs were connected through a resistor of 1000 Ω. Two BESs with biotic anodes (BES-bbc and BES-bbe) were first set up and operated identically; then, one of them (BES-bbe) was connected to a potentiostat (WMPG1000, WonATech, Seoul, Korea) to control the cathode potential first at −0.1 V and +0.35 V, and increased it later to +1.0 V with the aim to enhance the denitrification process.

Analytical techniques and calculations During the operation of BESs, samples were periodically taken from the reactors and filtered through a 0.22-μm Whatman nylon syringe filter. The samples were then introduced to an ion chromatograph (IC DX-300, Dionex, Sunnyvale, CA, USA) for determining nitrate (NO_3^-–N) and nitrite (NO_2^-–N) concentrations. Total nitrogen and ammonium levels were determined using the Humas Kit (Humas Co. Ltd., Daejeon, Korea) according to the manufacturer's instructions.

Total nitrogen removal efficiency (EN) and total nitrogen removal rate (RN) were calculated according to equations Eqs. 1 and 2, respectively, as follows:

$$\mathrm{EN}(\%) = \frac{\mathrm{TN}_0 - \mathrm{TN}_t}{\mathrm{TN}_0} \quad (1)$$

$$\mathrm{RN}\left(\mathrm{mg} \cdot \mathrm{L}^{-1} \cdot \mathrm{d}^{-1}\right) = \frac{\mathrm{TN}_0 - \mathrm{TN}_t}{t} \quad (2)$$

TN_0 represents the total nitrogen concentration at the initial period, TN_t denotes the total nitrogen at the investigated period and t represents the duration of the denitrification process.

Bacterial community analyses The biofilms that formed on the electrode were collected at the end of the experiment and DNA was extracted using a PowerSoil DNA isolation kit (Mo Bio Lab., Carlsbad, CA, USA) according to the manufacturer's instructions. The bacterial 16S rRNA genes were amplified by polymerase chain reaction with the universal primers Eub 8F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and Eub 1392R (5′-ACG GGC GGT GTG TAC AAG-3′) according to procedures described by Yu et al. (17). Denaturing gradient gel electrophoresis (DGGE) was performed as described by Yu et al. (17,18) but at 100 V for 16 h. Position and intensity of bands in the DGGE profile were determined using the Fingerprinting II Informatix software (Bio-Rad, Hercules, CA, USA). Principal component analysis was performed to define the relationships in the band profile using the SPSS program (version 14.0, SPSS Inc., Chicago, IL, USA). DNA fragments extracted from the DGGE bands were amplified by polymerase chain reaction using the universal primers Eub 27F (5′-ACG GGC GGT GTG TAC AAG-3′) and Eub 518R (5′-ATT ACC GCG GCT GCT GG-3′). The partial 16S rRNA sequences were then defined using an ABI 3730XL capillary DNA sequencer (Applied Biosystems, Franklin Lakes, NJ, USA) by a professional company (Solgent Co., Korea).

The obtained sequences were compared to those found in the GenBank database by performing nucleotide BLAST on the website of the National Center for Biotechnology Information (19). The determined sequences were then aligned with additional sequences of closest related sequences, which were obtained from GenBank nucleotide collection and 16S ribosomal RNA sequence (Bacteria and Archaea) databases. Alignments were performed using Clustal W and phylogenetic trees were constructed using the Neighbour-Joining method of Saitou and Nei (20). The percentage of replicate trees in which the associated taxa clustered together were determined using a bootstrap test based on 100 replicates (21). Evolutionary distances were computed using the Jukes–Cantor method (22) and are presented by the units of the number of base substitutions per site. All positions containing

TABLE 2. Operating condition of the bioreactors designed in this study.

Bioreactor name	Anode condition	Cathode condition	Connection condition
BES-abe	Abiotic	Biotic	Electrical support by power supply
BES-abo	Abiotic	Biotic	Open circuit
BES-aae	Abiotic	Abiotic	Electrical support by power supply
BES-bbc	Biotic	Biotic	Circuit connection
BES-bbe	Biotic	Biotic	Electrical support by potentiostat