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Bacterial communities in a bioelectrochemical denitrification system: The effects of supplemental electron acceptors

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ABSTRACT

Electrochemical treatment of nitrate (NO_3^-), nitrite (NO_2^-) and mixtures of nitrate and nitrite was evaluated with microbial catalysts on a cathode in three different bioelectrochemical denitrification systems (BEDS). The removal rates and removal percentage of nitrogen (N) compounds varied during biotic and abiotic operations. The biotic cathode using NO_3^--N as an electron acceptor showed enhanced removal percentages (88%) compared to the operation with NO_2^--N (85%). The simultaneous reduction of NO_3^--N and NO_2^--N occurred in the operation with a mixture of N compounds. The bacterial diversity from the initial inoculum (return sludge) changed at the end of bioelectrochemical denitrification operation after 55 days. The microbial community composition was different depending on the type of electron acceptor. BEDS operation with NO_3^--N and NO_2^--N was enriched with *Proteobacteria* and *Firmicutes* respectively. BEDS with a mixture of N electron acceptors showed enrichment with *Proteobacteria*. There was no clear, distinct microbial community between the cathode biofilm and suspended biomass.

1. Introduction

Nitrate (NO_3^-) and nitrite (NO_2^-) have been a worldwide groundwater contaminant mainly due to the use of fertilizers, industrial wastes, animal wastes and septic systems (Szpyrkowicz et al., 2006). High concentrations of nitrate and nitrite in drinking water can cause several health problems such as "blue baby syndrome" for infants, and also can lead to liver damage and cancer in adults (Tannenbaum et al., 1979). The maximum contaminant level of nitrate-nitrogen (NO_3^--N) in water is 10 mg/l in the United States and for nitrite-nitrogen (NO_2^--N) it is around 1 mg/l (Chebotareva and Nyokong, 1997; Park and Yoo, 2009). Various treatment methods such as reverse osmosis (RO), electrodialysis (ED), heterogeneous catalysis (HC), physicochemical, chemical and electrochemical processes have been employed in treating nitrates in water (Virdis et al., 2010). Even though all these methods are employed by choice, they have various disadvantages such as cost, the problem of disposing high saline content and production of toxic byproducts. Besides of all these process

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Bioelectrochemical denitrification system (BEDS) using biocathodes is an attractive way to convert nitrate into harmless nitrogen gas with easy operation and less capital costs (Park et al., 2005).

Biocathodic bioelectrochemical denitrification system is the modified form of a conventional microbial fuel cell (MFC), which can be operated either with an anode as a power source or by applying an external potential for carrying out the reduction of certain chemicals or metals like NO_3^- and perchlorate (Butler et al., 2010; Lovley, 2011; Park et al., 2005), and for generating value added products like hydrogen or methane (Cheng et al., 2009; Hu et al., 2009). In BEDS at high electrochemical potential, microorganisms are able to convey the electrons from the electrode to reduce or to oxidation of certain compounds. In these process microorganisms acts as a catalyst. The process of denitrification may differ in the presence of hydrogen or not. However, these aforementioned processes can be noted as autotrophic denitrification (Huang et al., 2013). Mostly the autotrophic bioelectrochemical denitrification process is considered to be more feasible technology for treating nitrate and nitrite contaminated waters than heterotrophic denitrification. Heterotrophic denitrification is only efficient in nitrate removal when adequate amounts of organic carbon are available (Watanabe et al., 2001). However, in potable water treatment, insufficient organic carbon may limit the application of heterotrophic denitrification unless external organic carbon sources are supplemented. The electrochemical biocathodic reduction process mainly depends upon applied potential and the type of microorganism involved in the denitrification process (Till et al., 1998).

Although bacterial communities associated with heterotrophic denitrification have been well established, information about the microorganisms responsible for cathodic bioelectrochemical denitrification of either nitrate or nitrite is limited. Therefore, in this study, we conducted a bioelectrochemical denitrification process using nitrates, nitrites and a mixture of nitrates and nitrites with the same operational conditions, and an analysis of the bacterial biofilm community for each operation were performed. A pyrosequencing technique was used for monitoring the 16S rRNA gene for possible recovering of uncovered species as in previous studies (Hamady et al., 2010). Based on the data generated from pyrosequencing in this study, we hope to present a detailed analysis of the community structure of cathodic denitrifying bacteria and an analysis of the phylogenetic affiliations corresponding to cathode denitrification in the presence of different nitrogen (N) compound. Electrochemical techniques like cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were also carried out to better understand the factors that limit biocathodic nitrate reductions of different nitrogen ions.

2. Materials and methods

2.1. Bioelectrochemical system set-up

BEDS were operated simultaneously in three different reactors with variation of N compounds such as NO_3^-, NO_2^- and mixture of NO_3^-, and NO_2^-. The bioelectrochemical system was an "H" type two-compartment cell with a total volume of 270 ml and a working volume of 200 ml for each chamber. Toray carbon paper with a surface area of 22.4 cm^2 was used as an anode and cathode electrode (Fuel Cell Earth, USA). The electrical connections on the electrode were established using a copper wire. The connections were sealed using a non-conductive epoxy resin (Hardex, USA). A pretreated proton exchange membrane (Nafion 117, Dupont Co., USA) was used to separate the anode and cathode compartments. Membrane pretreatment was carried out as described in previous studies (Kondaveeti and Min, 2013). To prevent membrane swelling when placed in the bioelectrochemical system, membranes were stored in deionized water prior to being used. An Ag/AgCl reference electrode (+0.197 V *vs* SHE, model: MF-2052, BASi Inc., Korea) was placed in the cathode chamber during the electrochemical analysis. The anode and cathode electrodes were connected to an external DC power supply (Array 3645A, Circuit Specialists Inc., USA) and an external cell voltage of 0.7 V was applied as described previously (Kondaveeti and Min, 2013). All the experiments were carried out in triplicate cycles to observe the consistency in results.

2.2. Reactor inoculum and operating conditions

Initially all the BEDS are operated in abiotic conditions in triplicate. Later the cathode chambers of the reactor were inoculated with return sludge, which was collected from the Giheung Respia Wastewater Treatment plant (Yongin-si, Korea). The biofilm formation on the cathode electrode was initialized as described previously (Kondaveeti and Min, 2013). After approximately 25 days of operation (Optimization period) the consistencies in denitrification were observed and further experimental studies have been carried out. During the entire operation (55 days in biotic conditions and 30 days in abiotic conditions), the cathode chambers were fed with a medium containing 100 mM phosphate buffer solution (PBS: $NaH_2PO_4 \cdot H_2O$, $Na_2HPO_4 \cdot H_2O$), 2 g/l $NaHCO_3$, 12.5 ml/l minerals and 5 ml/l trace metal solution (Anderson et al., 2003). 50 mg nitrate-N/l or 50 mg nitrite-N/l was added into the cathode chamber. In the case of nitrate–nitrite reduction test, the cathode chamber was supplemented with both 50 mg nitrate-N/l and 50 mg nitrite-N/l. The biotic and abiotic cathodes differed in the presence of $NaHCO_3$, which was supplemented a carbon source. In entire case the anode chambers for all of the reactors were fed with 100 mM PBS. The reactors were sealed using silicone gel (Wacker Inc., Germany) to ensure strict anaerobic conditions and operated in fed-batch mode. Prior to sealing the reactors, headspace and fluids of both chambers were purged with argon gas (purity >99.9%) for 10 min. Cathode and anode solutions were completely replaced in all the reactors when the concentration of N reached less than 5 mg/l. The solutions were completely mixed by continuously stirring the reactors on a magnetic stirring plate at 160 rpm (ATL-4200, Anytech Co., Korea). BEDS were operated in a temperature controlled incubator (VS-1203P1, Vision Inc., Korea) at 30 ± 1 °C. Biotic control experiments were carried out in an open circuit mode by disconnecting power supplier from the system to determine N reductions in the absence of external power source.