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Enhancement of bacterial denitrification for nitrate removal in groundwater with electrical stimulation from microbial fuel cells

Baogang Zhang [a, b, *], Ye Liu [a, b], Shuang Tong [a, b], Maosheng Zheng [c], Yinxin Zhao [d], Caixing Tian [a, b], Hengyuan Liu [a, b], Chuanping Feng [a, b]

[a] School of Water Resources and Environment, China University of Geosciences Beijing, Beijing 100083, China
[b] Key Laboratory of Groundwater Circulation and Evolution, China University of Geosciences Beijing, Ministry of Education, Beijing 100083, China
[c] Department of Environmental Engineering, Peking University, The Key Laboratory of Water and Sediment Sciences, Ministry of Education, Beijing 100871, China
[d] School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, China

HIGHLIGHTS

- Electricity from the MFC is applied to the BER directly as electrical stimulation.
- Nitrate removal from groundwater is accelerated by this means.
- Less intermediates accumulation is observed during that process.
- Denitrification bacteria proliferations and activities are promoted.

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ABSTRACT

Electricity generated from the microbial fuel cell (MFC) is applied to the bioelectrical reactor (BER) directly as electrical stimulation means for enhancement of bacterial denitrification to remove nitrate effectively from groundwater. With maximum power density of 502.5 mW m^{-2} and voltage outputs ranging from 500 mV to 700 mV, the nitrate removal is accelerated, with less intermediates accumulation, compared with control sets without electrical stimulation. Denitrification bacteria proliferations and activities are promoted as its number and Adenosine-5'-triphosphate (ATP) concentration increased one order of magnitude (3.5×10^7 in per milliliter biofilm solution) and about 1.5 folds, respectively. Effects of electricity from MFCs on enhancement of bacterial behaviors are demonstrated for the first time. These results indicate that MFCs can be applied in the in-situ bioremediation of nitrate polluted groundwater for efficiency improvement.

1. Introduction

Nitrate pollution in groundwater has become a serious issue in most parts of the world during past decades due to discharge of domestic, industrial wastewater and increased usage of nitrogenous fertilizers [1]. Excessive nitrate can do harm to humans and animals and it can be reverted to nitrite with more toxicity by microorganisms in human body, causing methemoglobinemia or blue baby syndrome in infants and gastrointestinal cancer in adults [2]. Thus the maximum contaminant levels (MCL) are stipulated to be 10 mg L^{-1} nitrate nitrogen (NO_3^-–N) and 1 mg L^{-1} nitrite nitrogen (NO_2^-–N) respectively by both USEPA and China.

Various physical–chemical and biological methods have been developed for nitrate removal, such as ion exchange [3], reverse osmosis [4], catalysis reduction [5] and bacterial denitrification [6]. The former can only separate nitrate from polluted groundwater, and cumbersome treatments are often inevitable, while the latter is able to remove nitrate efficiently and cost-effectively, drawing more and more attentions nowadays [7]. Bacterial denitrifications, both heterotrophic and autotrophic modes, are considered to be employed for nitrate removal [6,8].

To improve efficiencies of bacterial denitrification for nitrate removal, several meanings are employed, including nutrient elements and electron donors adjustment by extra addition (organics

* Corresponding author. School of Water Resources and Environment, China University of Geosciences Beijing, Beijing 100083, China. Tel.: +86 10 8232 2281; fax: +86 10 8232 1081.
E-mail address: zbgcugb@gmail.com (B. Zhang).

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and phosphorus) or electrochemically in-situ generation (hydrogen) [9]. These may increase operating costs or consume extra electric energy. Moreover, it is believed that proper electrical stimulation can promote microbial metabolism, thereby leading to higher biochemical performance [10]. Direct-current power supplies are used in almost all these previous studies and operated in relatively higher voltage outputs, ie above the decomposition voltage of water (1.2 V vs NHE) to obtain hydrogen [10]. Few studies are carried out to investigate the behaviors of denitrification under lower voltage without water decomposition, as some research reveals that low voltage stimulation (less than 1.5 V) without electrolytic O_2 generation can enhance the microbial degradation of PCB, by providing electron-donors/-acceptors to PCB dechlorination and microorganisms degradation [11]. Nowadays, microbial fuel cells (MFCs), devices that use bacteria as catalysts to oxidize organic or inorganic matters and generate current, are attracting increasing attention as they can convert chemical energy to electricity in mere one step [12–15]. Most reported voltage outputs from MFCs are less than 1 V and have been used to power sensors and generate hydrogen [16], while few studies concern on the enhancement of denitrification bacteria activities by directly electrical stimulation from MFCs for nitrate removal.

In the present research, the in-situ utilization of low voltage generated by MFC was considered. Electricity generated from MFCs was applied to bacterial denitrification directly as electrical stimulation means for effective nitrate removal from groundwater. The ability of voltage outputs of the MFC was evaluated. Nitrate removal and its reduced products in both aqueous solution and gaseous phase were measured. The enhancement of denitrification bacteria proliferations and activities under the stimulation of this low voltage was also monitored.

2. Materials and methods

2.1. Experimental apparatuses and electrolyte

The configuration of the experimental apparatus was shown in Fig. 1. It consisted of an MFC and a bioelectrical reactor (BER), with another two electrochemical cells as controls. The single-chamber MFC was in cubic shape and had been reported in our previous study [17], with an effective volume of 125 mL (5 cm × 5 cm × 5 cm). The anode was carbon fiber felt (1 cm thickness, 4 cm length and width, Beijing Ever grow Resources Co. Ltd, Beijing, China). The cathode made of plain carbon paper (with

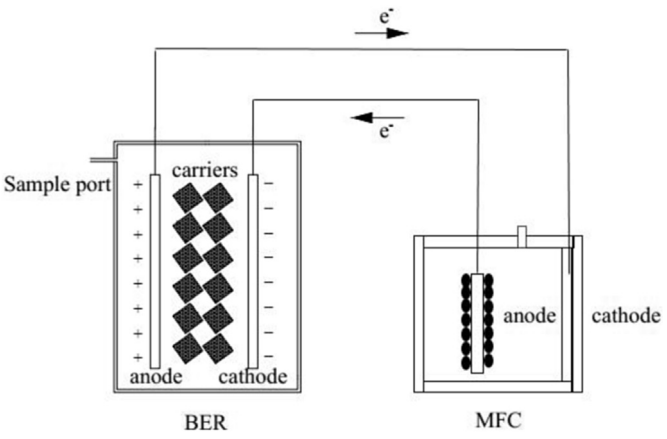

Fig. 1. Experimental apparatus. The left: MFC unit, 1 cathode (Pt coated carbon paper); 2 anode (carbon fiber felt). The right: BER unit, 3 anode (stainless steel plate); 4 cathode (stainless steel plate); 5 carriers (functional polyurethane foams).

0.5 mg cm^{-2} of Pt on one side) with a projected surface area of 16 cm^2 was placed on the opposite site of the anode. The voltage outputs of the MFC were recorded by a data acquisition system (PMD1208LS, Measurement Computing Corp., Norton, MA, USA) at an interval of 5 min [18]. The MFC had been well developed before present experiment. The electrolyte contained the following components (per liter): $C_6H_{12}O_6$ (0.75 g); NH_4Cl (0.31 g); KCl (0.13 g); $NaH_2PO_4 \cdot H_2O$ (4.97 g); $Na_2HPO_4 \cdot H_2O$ (2.75 g); vitamin solution (1.25 mL) and 12.5 mL trace mineral element solution.

The BER was designed in sealed cuboid shape and the total volume was 480 mL. Both anode and cathode were made of stainless steel (15 cm × 4.3 cm), with the electrode spacing of 2 cm. Functional Polyurethane Foams (FPF) with the specific surface area of 35,000 m^2 m^{-3} were added as carriers to immobilize the microorganisms in the BER [17]. The electrodes of MFC were connected to those of BER using copper wires. Another two electrochemical cells were built in the same specification as the BER. One was not inoculated (Control 1) and another was inoculated (Control 2). The BER and the Control 2 were respectively inoculated with 50 mL anaerobic sludge, which was collected from the Qinghe Sewage Treatment Plant (Beijing, China) and had been well acclimated before the formal trial. Synthetic groundwater (per liter of tap water) contained 0.364 g $NaNO_3$, 0.044 g KH_2PO_4, and 0.21 mL CH_3OH. The concentration of NO_3^-–N was prepared as around 60 mg L^{-1}.

2.2. Experimental procedure

The MFC and BER as well as control sets were filled with fresh electrolyte and synthetic groundwater, respectively. The BER and Control 2 were domesticated separately for 4 weeks to achieve the same performance before the formal experiments. Then BER was connected with MFC through copper wires to domesticate denitrification bacteria in the BER to accommodate the electrical stimulation for about 30 days, with refreshing electrolyte and synthetic groundwater every 3 d and 5 h, respectively. After that, Nitrate removal in a typical cycle was evaluated compared with the control sets. Intermediates of nitrate reduction, both in aqueous solution and gaseous phase, were also measured at the same frequency. Differences of denitrification bacteria in the aspects of amount and activity between BER and Control 2 were investigated to reveal the mechanisms of electrical stimulation, compared with electrolysis effect simulated in Control 1. The abilities of electricity generation in MFC were evaluated during these procedures as well. All the experiments were carried out at room temperature (25 ± 2 °C).

2.3. Analytical methods

NO_3^-–N, NO_2^-–N and NH_4^+–N in the aqueous solution of BER were determined by ultraviolet spectrophotometer (DR 5000, HACH, the USA) according to standard methods, with three duplications for each data point. Chemical oxygen demand (COD) was measured by the standard method of potassium dichromate. N_2O and N_2 in the gaseous phase of BER was detected by gas chromatography (GC, 7890A, Agilent, the USA) with electrical conductivity detector (ECD) and mass spectrometry (MS), repectively [19]. N_2O in the liquid phase was estimated using Henry's function. NO was monitored by nitrogen oxides gas analyzer (42i series, Thermo Fisher, the USA) [20].

Adenosine-5'-triphosphate (ATP) reflecting the activity of denitrification bacteria was quantitatively evaluated by enzymes labeling instrument (Zenyth 340rt, Biochrom Anthos, Austria) with CellTiter-Glo luminescent cell viability assay, which was performed according to previous research and luminescences in the form of RLU (relative light unit) were recorded, as they have a linear