Int. J. Electrochem. Sci., 16 **(2021)** 150973, doi: 10.20964/2021.01.10

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Autotrophic Denitrification for Nitrate Removal from Groundwater with an Integrated Microbial Fuel Cells (MFCs)-microbial Electrolysis Cell (MEC) System

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Received: 2 September 2020 / *Accepted:* 25 October 2020 / *Published:* 30 November 2020

Bioelectrochemical system (BES) that is self-sufficient was developed to treat nitrate in groundwater, which consists of air cathode microbial fuel cell (MFC) and microbial electrolysis cell (MEC). Without external power, the highest nitrate removal rate and the highest removal efficiency of autotrophic denitrification MFC were 10.6 mg/(L·d) and 56.5%, respectively. However, the highest denitrification rate increased to 13.5 mg/(L·d) when three air cathode MFCs (0.8 V) were adopted in series as the power source for autotrophic denitrification MEC, which was 27.4% higher than that of autotrophic denitrification MFC. In addition, the nitrate removal efficiency was as high as 80.6%, which was the same to that of the conventional biological nitrogen removal (BNR). Thereby, autotrophic denitrification was significantly improved in MFCs-MEC and the hybrid system made BES more feasible in treating low ionic strength water (<1000 μS/cm) like groundwater. Furthermore, excessive organic input to groundwater was avoided by the autotrophic denitrification which might cause secondary pollution to it.

Keywords: nitrate removal; autotrophic denitrification; groundwater pollution; microbial electrolysis cell; microbial fuel cell

1. INTRODUCTION

Due to several factors, e.g., excessive use of chemical fertilizers, discharge of domestic wastewater, and industrial wastewater, nitrate pollution of groundwater has become a worldwide problem [1-3]. Pervasively, the nitrate concentrations in groundwater are in the range of 30-50 mg/L and even over hundreds in some areas [1-3]. There are commonly used physical and chemical techniques to remove nitrate, such as ion exchange, reverse osmosis, and electrodialysis [3]. Nevertheless, these

techniques are expensive and only separate nitrate from groundwater without complete treatment. By converting nitrate into nitrite and finally to nitrogen gas with denitrifying bacteria, biological denitrification can overcome these drawbacks [4]. The heterotrophic one which requires organic carbon as the carbon source for the growth of heterotrophic denitrifiers is the commonly used biological denitrification. However, groundwater is characterized by the absence of organics that heterotrophic denitrification in groundwater needs extra organic input [5]. As a result, due to the overdose of organics, this may cause the secondary pollution to groundwater.

Great attention has drawn by autotrophic denitrification as autotrophic denitrifiers use inorganic carbon (e.g., carbon dioxide and bicarbonate) as the carbon source and get electrons from inorganics (e.g., sulfur, hydrogen, and electrode) [6-10]. Furthermore, biocathode in bioelectrochemical system (BES) has been reported to provide electrons to autotrophic denitrifiers [8-10]. BES is an electrochemical system that converts chemical energy in organic waste to electricity or chemicals (e.g., hydrogen, metals) using microorganisms as catalysts [11]. It has been proved by Virdis et al. that nitrate was reduced into nitrogen gas on biocathode of microbial fuel cell (MFC), and it has been demonstrated by Puig et al. and Desloover et al. that nitrite and nitric oxide could function as electron acceptors in MFCs [12-14]. However, groundwater has low ionic strength (<1000 μS/cm), and the limited ion transfer would increase the overpotential of cathode, slow down the nitrate reduction rate, and accumulate the intermediate of denitrification [15,16]. Furthermore, the low ionic strength of water would severely affect the nitrate removal rate since autotrophic denitrifiers (the highest 849 mg/(L·d)) grow much lower than heterotrophic ones (~1700 mg/(L·d)) [1].

Microbial electrolysis cell (MEC) is also a kind of BES in which external power is applied between anode and cathode [11]. Not only could the applied voltage overcome the potential gap between electrodes to make nonspontaneous reactions take place; it could also accelerate in spontaneous reactions the electron transfer between electrodes and substrates. Thereby, MEC would be more feasible to treat low ionic strength water like groundwater; however, it requires external power supply which increases the cost of operation.

Thus, in this study, MFCs-MEC hybrid system was constructed to treat nitrate in low ionic strength water. Specifically, treating domestic wastewater, the single-chamber air cathode MFCs were adopted as power supply for autotrophic denitrification MEC. Air cathode MFC has been studied for decades, and during the treatment of wastewater, it could provide steady power [17-21]. In this study, there were four tasks: First, autotrophic denitrification was studied in MFC without power supply. With different initial concentrations of nitrate, the nitrate removal effects and power generation were discussed. Second, autotrophic denitrification MEC was constructed with air cathode MFCs as power supply. To explore the effects of nitrate removal and determine the optimal power output of MFCs, different numbers of air cathode MFCs were connected in series with a MEC. Third, after the confirmation of the number of air cathode MFCs, nitrate of different initial concentrations was treated in MEC, and the effects were compared with those of autotrophic denitrification MFC. And finally, the importance of self-sustained MFCs-MEC was discussed treating nitrate-polluted groundwater.

2. MATERIALS AND METHODS

2.1 Setup of autotrophic denitrification BES

In order to establish the effect of power support in nitrate removal of BES, both autotrophic denitrification MFC and MEC were studied. Autotrophic denitrification MFC is comprised of anode and cathode chambers made of plexiglass. Separated by Nafion membrane (N117, DuPont Fuel Cells, DE), the volumes of both the chambers were 8 × 4 × 8 cm^3. Plain carbon cloth (8 × 4 cm^2) was used as electrodes, and an external resistance of 512 Ω was connected between anode and cathode. For MEC, except for applying power supply between electrodes instead of external resistance, the configuration was similar as autotrophic denitrification MFC. The system was completely sealed, and in the cathode chamber, nitrate worked as the electron acceptor. In this study, single-chamber air cathode MFC (4 × 4 × 4 cm^3) made of plexiglass was adopted as the power supply for MEC. In addition, anode was made of plain carbon cloth (4 × 4 cm^2) and immersed in the solution of MFC, and cathode was also made of carbon cloth with the solution-facing side loaded with Pt (0.5 mg/cm^2) and the air-facing side coated with PTFE [21]. Oxygen reduction reaction took place on cathode. For the sake of finding out the optimal voltage given by air cathode MFCs for the removal of nitrate in MEC, one to three air cathode MFCs were connected with a MEC in series to explore nitrate removal effects in MEC (Fig. 1). However, in maximum, only three were used as more MFCs in series might lead to electrode reversal and water electrolysis [22,23].

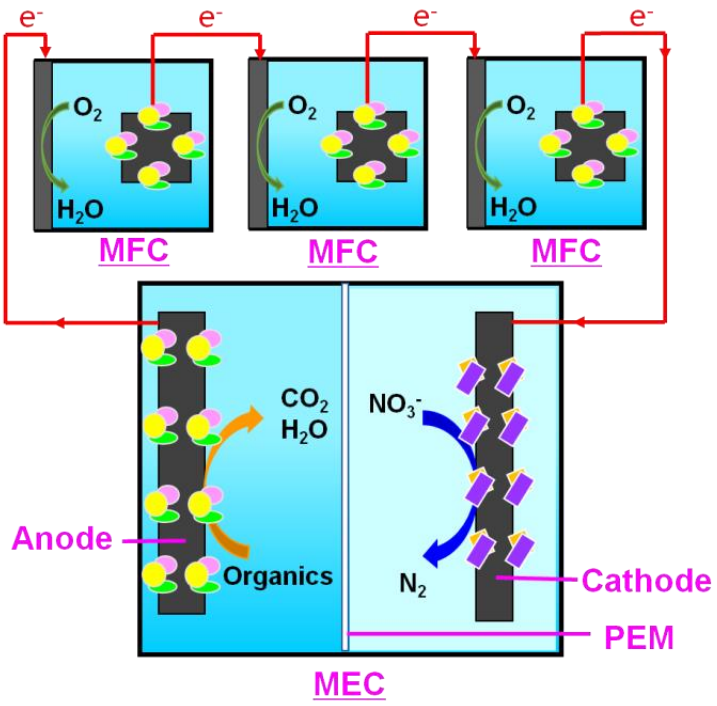

Figure 1. The schematic diagram of the microbial fuel cells (MFCs)-microbial electrolysis cell (MEC) system (PEM: proton exchange membrane)

2.2 Inoculation and operation of autotrophic denitrification BES and air cathode MFC

The anode and cathode of autotrophic denitrification MFC were inoculated with the influent taken from the Northern Suburb Sewage Treatment Plant in Changchun City, China. For anodes, to give adequate organic carbon for bacterial growth during inoculation, sodium acetate (1 g/L, chemical oxygen demand COD~1000 mg/L) was added. After 1 month of inoculation, the synthetic wastewater (0.4 g/L CH_3COONa, 10.3 g/L $Na_2HPO_4·12H2O$, 3.32 g/L $NaH_2PO_4·2H_2O$, 0.13 g/L KCl, 0.31 g/L NH_4Cl, 0.2 g/L $MgSO_4·12H_2O$, 0.015 g/L $CaCl_2$, 5 mL/L trace element solution) was used as anode solution for the experiments. Whereas for cathodes, along with sodium nitrate (total nitrogen concentration was 50 mg/L), the sewage was also adopted for the inoculation of autotrophic denitrifiers; it was gradually changed into the synthetic groundwater (0.5 g/L $NaHCO_3$, 0.92 g/L $NaH_2PO_4·2H_2O$, 0.004 g/L $MgSO_4·7H_2O$, 0.006g/L $CaCl_2·2H_2O$, 1 mL/L trace element solution) with no COD and low conductivity (974 μS/cm). When experiments started after 1 month of inoculation, the synthetic groundwater with different concentrations of nitrate (NO_3^-–N 20~50 mg/L) was tested in BES. By connecting with air cathode MFCs, some of the autotrophic denitrification MFCs were changed into MECs. Furthermore, the experiments were operated in batch mode, and when the voltage of autotrophic denitrification MFC dropped to 50 mV or the nitrogen concentration in nitrate was below 10 mg/L, the value for drinking water quality standard according to the World Health Organization (WHO) [22], the experiment was finished, and fresh wastewater was added for the next circle.

Used as power supply for autotrophic denitrification MEC, the air cathode MFCs were also inoculated with the sewage and sodium acetate (1 g/L) for the bacterial growth. After the experiments started, the solution was changed into the synthetic wastewater (0.4 g/L CH_3COONa, 10.3 g/L $Na_2HPO_4·12H_2O$, 3.32 g/L $NaH_2PO_4·2H_2O$, 0.13 g/L KCl, 0.31 g/L NH_4Cl, 0.2 g/L $MgSO_4·12H_2O$, 0.015 g/L $CaCl_2$, 5 mL/L trace element solution). To keep stable input voltage to MEC, the solution was changed oftentimes into fresh wastewater.

2.3 Analysis and calculations

Using a Keithley 2700 data logging system (USA), the output voltages of autotrophic denitrification MFCs over external resistance of 512 Ω were recorded every 30 min. With a series of external resistance (R, 12–3200 Ω), the polarization curve of air cathode MFC was measured, the current density was calculated according to $I = U/(R \times A)$, and the power density was calculated according to $P = U^2/(R \times A)$, where U is the measured voltage over R and A is the projected area of anode. Moreover, using a COD analyzer (DR1010, Water Quality Analyzer, Hach Instruments, USA), the COD value was measured. In addition, the nitrate was measured with ion chromatography (ICS-2100, Dionex, Thermo Scientific, USA) and nitrate concentration was represented by the concentration of nitrogen in nitrate in this study. At 30°C in duplicate, all the experiments were carried out.

3. RESULTS AND DISCUSSION

3.1 Performance of autotrophic denitrification MFC

Fig. 2 illustrated the concentration change of nitrate along the operation time. When the initial concentration was 20.6 mg/L, it went down to 10 mg/L immediately in 24 h with the average removal rate of 10.6 mg/(L·d). For nitrate with higher initial concentration, the reaction time became longer that the nitrate concentration turned down to 14.7 mg/L in 48 h with the start of 33.8 mg/L, and the average removal rate was 9.55 mg/(L·d). For nitrate which started at 50.5 mg/L, with slight change in the following time, the concentration decreased to 23.5 mg/L in 120 h. The average removal rate was 5.4 mg/(L·d), and the nitrate removal efficiencies were 51.4%, 56.5%, and 53.5% with the initial concentration of 20.6 mg/L, 33.8 mg/L, and 50.5 mg/L, respectively. Furthermore, the nitrate removal efficiencies were much lower in autotrophic denitrification MFCs than those of conventional biological nitrate removal (BNR) (60%–85%) [4]. This was presumably due to the low conductivity of the cathode solution that hindered mass transport and electron transfer between denitrifying bacteria and cathode [15,16].

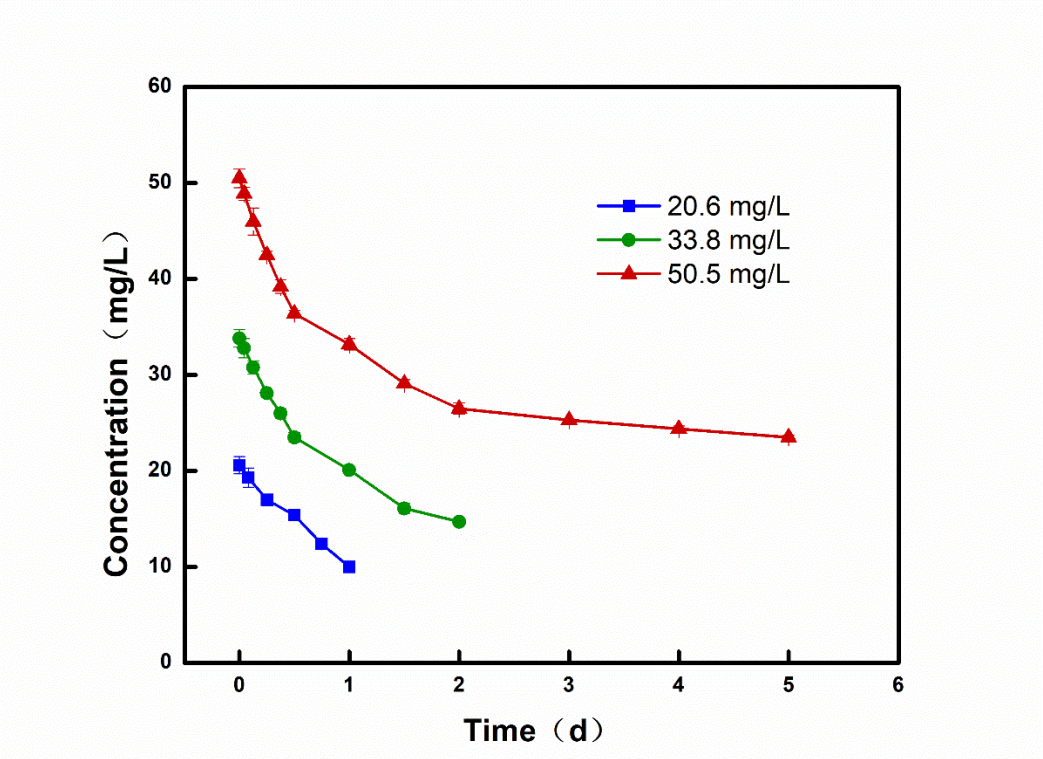

Figure 2. The concentration change of nitrate in autotrophic denitrification MFC with time at different initial concentrations of 20.6 mg/L, 33.8 mg/L and 50.5 mg/L

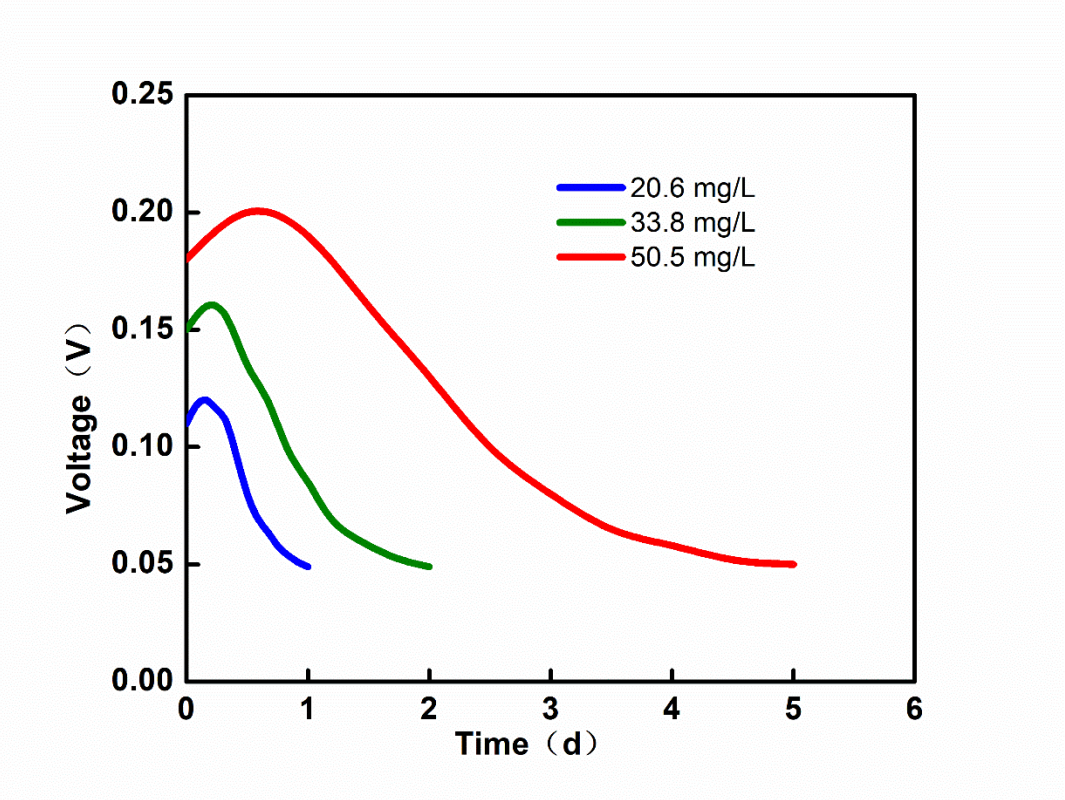

Figure 3. Voltage changes of autotrophic denitrification MFC with time at different initial nitrate concentrations of 20.6 mg/L, 33.8 mg/L, and 50.5 mg/L

Furthermore, during the operation, the voltage production was measured in MFCs (Fig. 3). In addition, the voltage decreased with the change of concentration correspondingly. For nitrate with initial concentration of 50.5 mg/L, the highest voltage of 0.2 V at the beginning of the experiment was observed, and after 120 h, it went down slowly to 0.05 V. The highest voltage of 0.16 V was seen in MFC with the start of 33.8 mg/L and 0.12 V for 20.6 mg/L. And as the voltage varied with the change of time, the power density was not measured. Compared with those of traditional MFCs treating domestic wastewater, the overall voltage production of autotrophic denitrification MFCs was low and not steady [17-21]. This also showed that nitrate reduction in cathode chamber was the limiting reaction of the system.

3.2 Power generation of MFCs as the power supply for autotrophic denitrification MEC

In order to enhance the autotrophic denitrification rate, air cathode MFCs were used as power supply to MEC treating domestic wastewater (Fig. 1). The highest power density of the air cathode MFC reached 154.4 mW/m^2 with the highest current density of 736.8 mA/m^2 (Fig. 4). Furthermore, the constant voltage input to MEC was around 0.35 V (Table 1). In addition, for the sake of providing higher voltage to MEC, two and three of air cathode MFCs with the same power generation ability were connected in series to MEC, respectively. Two MFCs connected in series provided the input voltage of

0.58 V for MEC, and due to extra ohmic losses during connection, three MFCs connected in series reached 0.8 V (Table 1).

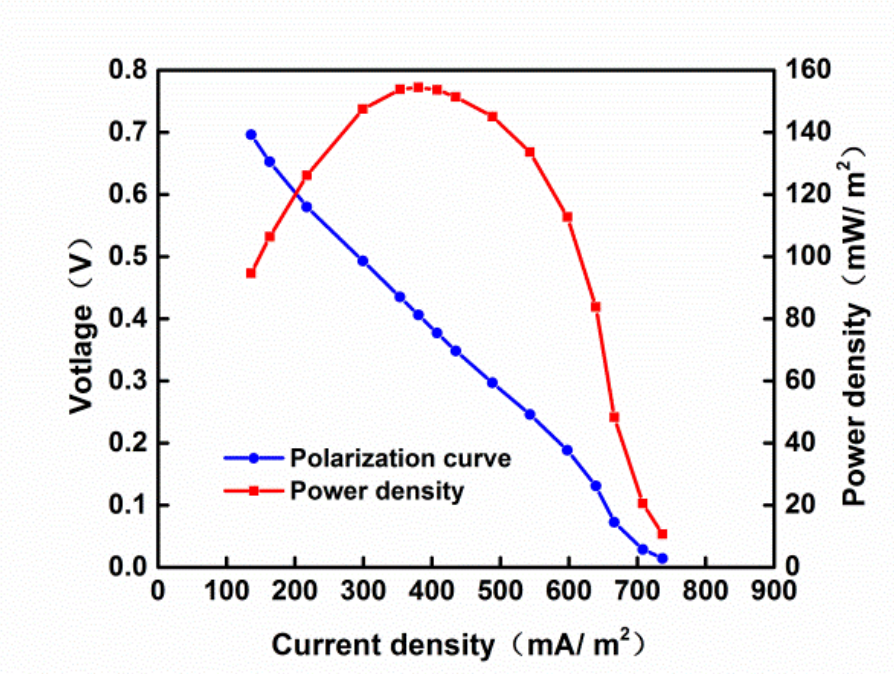

Figure 4. Polarization curve and power output of an air cathode MFC at the external resistances of 12-3200 Ω

With the initial concentration of 20.6 mg/L, nitrate was treated in MEC to explore the optimal output voltage from air cathode MFCs. It has been noted from Table 1 that with the increase of supporting voltage, the nitrate removal efficiencies in MEC increased. The removal efficiencies reached 57.3%, 61.2%, and 65.5% in 24 h with supporting voltage of 0.35 V, 0.58 V, and 0.8 V from MFCs, respectively. Besides, with voltage input of 0.8 V, the average removal rate was as high as 13.5 mg/(L·d), which was 27.4% higher than that of autotrophic denitrification MFC (Table 1). Nitrate removal rate enhanced with the increase of air cathode MFCs indicating that low applied voltage stimulated the bacteria activity and enhanced subsequently the nitrate removal rate.

Table 1. Nitrate removal efficacy of MEC with different number of air cathode MFCs as power supply during 24 h

Air cathode MFC number	MFC voltage output (V)	Effluent NO_3^- (mg/L)	NO_3^- removal rate (mg/(L·d))	NO_3^- removal efficiency (%)
0	0	10	10.6	51.4
1	0.35	8.8	11.8	57.3
2	0.58	8	12.6	61.2
3	0.8	7.1	13.5	65.5

Furthermore, it had been reported that the low direct current could stimulate the growth of bacteria and further improve the pollutants removal efficiency [25-27]. Ding et al. reported that with the increase of applying voltage to 0.8 V, the methane generation in MEC enhanced [25], while further increase affected adversely the bacteria metabolism and reduced methane generation. Moreover, the applied voltage of 0.8 V could give the most negative potential for cathode that facilitated electron transfer between cathode and autotrophic denitrifiers [3,28]. Therefore, to treat nitrate of different initial concentrations in MEC for the following research, three air cathode MFCs in series were used as power source.

3.3 Nitrate removal in MEC supported by MFCs

After the confirmation of supporting voltage for autotrophic denitrification MEC, the treatment effects of nitrate were also explored individually in MEC with initial concentration of 33.8 mg/L and 50.5 mg/L. For nitrate removal with the initial concentration of 33.8 mg/L, within 48 h, the concentration reached 9.1 mg/L with the removal efficiency of 73.1% (Fig.5); therefore, the average nitrate removal rate was 12.4 mg/(L·d) (Fig. 6). In addition, for nitrate removal with initial concentration of 50.5 mg/L, within 120 h, the concentration went down to 9.8 mg/L with the removal efficiency of 80.6% (Fig. 5); thus, the average removal rate was 8.14 mg/(L·d). Hence, the nitrate removal rate and efficiency declined with the increase of the initial concentration.

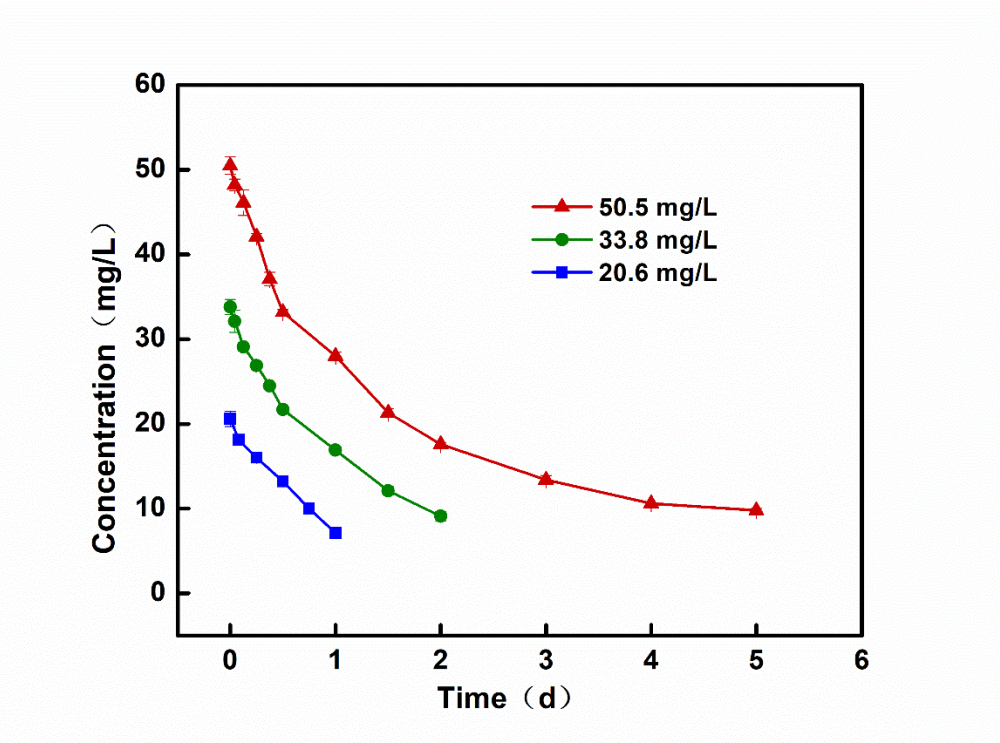

Figure 5. The concentration change of nitrate in autotrophic denitrification MEC with time at different initial concentrations of 20.6 mg/L, 33.8 mg/L, and 50.5 mg/L

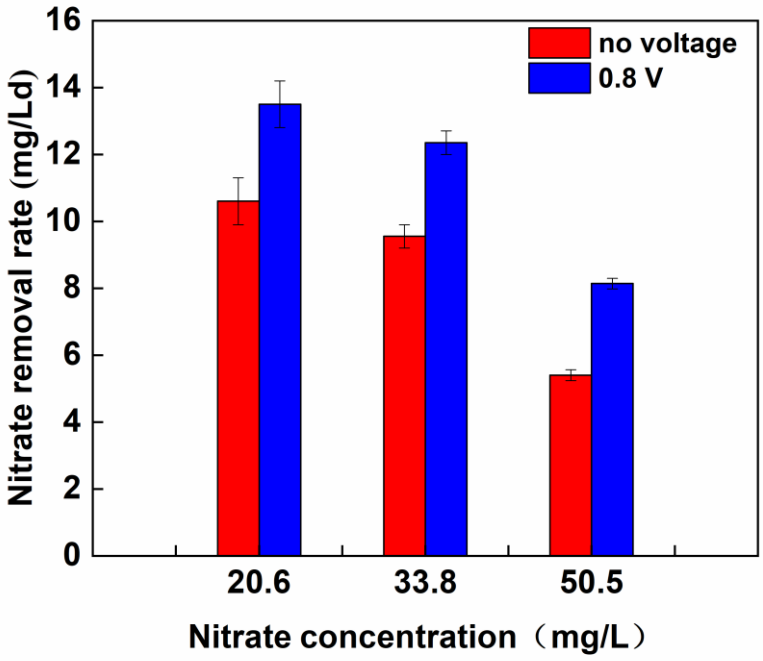

Figure 6. The comparison of nitrate removal rates between autotrophic denitrification MFC with no voltage support and MEC with the voltage input of 0.8 V

Nevertheless, the autotrophic denitrification rates still improved 29.3% (initial concentration of 33.8 mg/L) and 50.7% (initial concentration of 50.5 mg/L), respectively, in MFCs-MEC than those of MFC without power support (Fig. 6). This indicated that in the treatment of nitrate with higher initial concentration, applying voltage was more effective that the high voltage (0.8 V) and initial nitrate concentration (50.5 mg/L) enriched the autotrophic denitrifiers and accelerated the nitrate reduction subsequently [25]. It was reported in previous studies that autotrophic denitrification rates in BES were in the range of 3–13 mg/(L·d) using single nitrogen species (either nitrate or nitrite) as the electron acceptor [29-32]. In this study, the achieved nitrate removal rates were 8.14–13.5 mg/(L·d) in MFCs-MEC even with low ionic strength solution. In addition, 65.5%–80.6% were the autotrophic nitrate removal efficiencies of MFCs-MEC which were the same with those of the conventional BNR (60%–85%).

3.4 Significance of autotrophic denitrification in MEC supported by MFCs

The integrated MFCs-MEC was a self-sustained system and exhibited several advantages over conventional BNR. First, with the limited ionic strength of cathode solution in MEC, similar to conventional BNR, the system still reached the nitrogen removal efficacy without addition of extra organics. In conventional BNR, when initial nitrate concentration was 50 mg/L, 175–250 mg/L of COD (3.5–5.0 of COD/NO_3^-–N) was required theoretically [4]. Second, energy was harvested in air cathode MFCs that about 0.8 kW·h/m^3 was gained in three MFCs in series during the operation time of 120 h. Instead of external power source, this was applied to MEC for the aid of autotrophic denitrification.

Thereby, compared with that of autotrophic denitrification MFC, the autotrophic denitrification rate was accelerated. Finally, by converting energy between wastes and electricity, self-sustained autotrophic denitrification MFCs-MEC could simultaneously treat sewage wastewater and nitrate in low ionic strength water (e.g., groundwater).

4. CONCLUSION

To treat nitrate in groundwater, a self-sustained hybrid MFCs-MEC was designed. In autotrophic denitrification MFC, the nitrate removal effect was impeded by the low ionic strength of solution. However, with the aid of air cathode MFCs as power supply for autotrophic denitrification MEC, the nitrate removal effect substantially improved. Therefore, autotrophic denitrification in MFCs-MEC was feasible in treating nitrate with low conductivity and power generation from air cathode MFCs saved external power source for the system.

ACKNOWLEDGEMENTS
The work was financially supported by the National Natural Science Foundation of China (grant number 41907163) and Department of Education of Jilin Province (JJKH20201012KJ).

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