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Effect of electro-stimulation on activity of heterotrophic denitrifying bacteria and denitrification performance

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HIGHLIGHTS

- Nitrate could be effectively removed in developed bio-electrochemical reactor.
- Heterotrophic denitrifying bacterial activity could be enhanced by electro-stimulation.
- High ATP aggregate level was obtained at the current density of 200 mA/m^2.

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ABSTRACT

The effects of electro-stimulation on heterotrophic denitrifying bacterial activity and nitrate removal were investigated using a bench-scale bio-electrochemical reactor in this study. Results showed that the maximum nitrate removal efficiency was 100% at the optimum current density of 200 mA/m^2, at which low nitrite production and high ATP aggregate level were obtained. The activity of denitrifying bacteria was highest at the range densities of 200–250 mA/m^2, although the terminative pH increased to 8.62 at 200 mA/m^2 and 9.63 at 250 mA/m^2. This demonstrates that suitable current densities could improve the activity of denitrifying bacteria. Therefore, this study provides a number of useful information to improve the bio-electrochemical reactor designs and promote the removal efficiency of pollutants.

1. Introduction

Nitrate contamination in groundwater, a main source of drinking water, has recently become an increasingly serious problem, resulting from inputs of over fertilization in farms, industrial effluents, animal and human wastes (Ghafari et al., 2008). High concentration of nitrate in drinking water not only causes serious illnesses such as methemoglobinemia or blue baby syndrome (Mousavi et al., 2012), but could also lead to gastric cancer (Ghafari et al., 2009). Therefore, the maximum contaminant level (MCL) for nitrate stipulated by the World Health Organization (WHO, 2008) is at 11.29 mg-N/L in drinking water, and the value proposed by China is 10 mg-N/L (Standards for Drinking Water Quality, China: GB5749-2006).

Biofilm-electrode reactor (BER) has attracted considerable interests in treating polluted water by nitrate removal. Islam and Suidan (1998) used a continuous flow BER to evaluate biological denitrification at different currents (0–100 mA), and obtained 98% nitrate removal efficiency at 20 mA current. Ghafari et al. (2009) also treated contaminated water containing 20 mg/L NO_3^-–N in an upflow bioelectrochemical reactor (UBER) at currents 0–20 mA, and reported that the nitrate removal efficiency was 100% at optimum electric currents 10–16 mA. Zhou et al. (2009) found that concentrations of nitrate and nitrite decreased greatly to around 2 mg/L at 15 mA in a three-dimensional bio-electrochemical reactor (3D-BER). However, traditional BERs consume a lot of electrical energy to produce CO_2 and H_2 (Mousavi et al., 2012). In order to reduce the electric energy consumption and addition of organic carbon source, and to improve denitrification efficiency, Zhao et al. (2011) developed a HAD system using fiber threads as independent carrier with carbon rods as the anode and stainless steel wire as the cathode to provide H_2 for autotrophic bacteria, and they reported that the nitrate

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removal efficiency was over 97% at current of 40 mA and C/N of 0.75. Tong et al. (2013) used a similar HAD system but with a stainless steel rod as anode, which could avoid the generation of carbon dioxide during electrolysis process, and further observed 99.9% nitrate removal efficiency at current density of 200 mA/m^2. Hao et al. (2013) developed a three-dimensional biofilm-electrode reactor (3D-BER) with cooperative heterotrophic and autotrophic denitrification of wastewater treatment plant (WWTP) effluent, and they investigated the effects of C/N ratio and HRT on the denitrification performance and bacterial community under constant 40 mA supply. However, in previous study, the authors only reported the optimum current or set a constant amount of current as experimental conditions, and showed that the high current could inhibit microbial activity, or even kill microorganisms.

The potential effects of low current and suitable current to improve microbial activity has been rarely reported or tested. Also, knowledge on the mechanisms of how current affects activity of the denitrifying bacteria remains to be understood. In fact, the effect of electric current on bacterial activity and viability is a major concern in applying bio-electrochemical method. Some studies pointed out that the electro-stimulation could affect microbial growth and activity, and proper electro-stimulation could promote microbial metabolism (Thrash and Coates, 2008). Kojima et al. (1992) used 0.2–0.6 V potential to stimulate MKN45 cells (Carcinoma cells) in a culture dish with Pt as the anode and cathode, and showed that current stimulation could change the DNA, protein synthesis, membrane permeability and cell growth. Furthermore, heterotrophic bacterial viability was also observed to be not significantly affected when the applied electric current density was less than 6.2 A/m^2, but was partly inactivated at current densities more than 12.3 A/m^2 in a membrane reactor (Wei et al., 2011). The same observation was also true for the autotrophic species where the denitrification and viability drastically decreased at electrical stimulation with densities more than 16 mA/cm^2 but remained active and viable at 2 and 4 mA/cm^2 (Safari et al., 2013).

In this study, to further explore the effects of electro-stimulation on the activity of heterotrophic denitrifying bacteria and denitrification efficiency, a small bench-scale bio-electrochemical reactor was developed. Specifically, this study aims to: (1) determine the optimum current density by investigating nitrate removal efficiency under different current densities, (2) investigate the activity of heterotrophic denitrifying bacteria under different current densities, and (3) elucidate the mechanisms of electro-stimulation on the activity of denitrifying bacteria in a bio-electrochemical nitrate treatment.

2. Methods

2.1. Synthetic groundwater preparation

Synthetic groundwater was prepared by dissolving 0.304 g $NaNO_3$, 0.044 g KH_2PO_4, and addition of 0.21 mL methanol (CH_3OH) in a liter of tap water. The concentration of NO_3^-–N was prepared as 50.00 mg/L. The original pH was around the range of 7.25 ± 0.02 and needed no further adjustment.

All chemical reagents used in the experiments were analytical grade.

2.2. Experimental apparatus

The experimental apparatus consisted of a bench-scale bio-electrochemical reactor, a DC regulated power supply and a constant temperature magnetic stirrer. The bio-electrochemical reactor composed of a conical flask (1 L), an anode and a cathode. The anode was a stainless steel rod (diameter 3 mm, length 250 mm), while the cathode was made of a spiral iron wire (iron wire diameter 1 mm, spiral diameter 35 mm and spiral height 30 mm). The cathode was concentrically installed in the conical flask, and the anode was fixed in the center of the cathode. The conical flask was then sealed with a rubber plug through which a sampling pipe was inserted. A constant temperature magnetic stirrer (Hijiu, H01-1C, China) was used to keep the synthetic groundwater at constant temperature of 30 ± 2 °C and ensure homogeneous distribution of the bacterial suspension in the reactor during the experiments. A DC regulated power supply (PS-302D, Shenzhen, China) was used to provide a constant DC for the different runs.

2.3. Acclimation of sludge

Activated sludge for inoculation of denitrifying bacteria was collected from the Qinghe wastewater treatment plant (Beijing, China), and was washed three times with tap water prior to cultivation in 1 L liquid nutrient medium and incubation at 30 °C. The media was also supplanted with nutrients such as $NaNO_3$ (7 mM), KH_2PO_4 (0.3 mM) and glucose (10 mM), and was replaced every 4 days. Acclimation was considered successful when the nitrate removal efficiency became stable (maintained at >95%) and also when the sludge turned dark grey.

2.4. Experimental start-up

The acclimated sludge (4 mL) and synthetic groundwater (1 L) were added to the bio-electrochemical reactors at the beginning of each experiment. Each group of experiment was independent and the acclimated sludge in each experiment was re-added to inhibit growth of autotrophic denitrifying bacteria.

To investigate the effects of different current densities and initial pH on nitrate removal efficiency and bacterial activity, the applied current densities were set as 0, 50, 100, 150, 200, 250, 300, 350 and 400 mA/m^2 at initial pH 7.25 ± 0.02, and initial pH was adjusted to 7.25, 8.25, 9.25, 10.25 and 11.25 by 1 mol/L NaOH solution at the observed optimum current density. This pH set-point was based on the terminative pH (i.e. pH at the end of the experiment) which was observed under the different current densities. The reactor was operated for 14 days for each experimental condition, and 8 mL of sample was taken from the sampling port every 24 h.

To ascertain the potential electrochemical nitrate reduction, electrochemical experiments were carried out without bacteria inoculation. The applied current densities were also gradually increased from 50 to 400 mA/m^2 at an interval of 50 mA/m^2.

2.5. Analytical methods

NO_3^-–N, NO_2^-–N and NH_4^+–N were determined by ultraviolet spectrophotometer (DR6000, HACH, US) according to the Water and Wastewater Monitoring Analysis Method (SEPA, 2002). Adenosine triphosphate (ATP) content was measured by ATP meter (AF-100, TOA-DKK, Japan). The standard deviations were analyzed at a confidence level of 90%, and all analyses were carried out in Origin 8.0 (OriginLab, trial version). The pH was determined by pH meter (Seven Multi S40, Mettler Toledo, Switzerland).

Scanning electron microscope (SEM) was performed to observe the induced changes in cell shape and extracellular polymers under 0, 50, 200 and 400 mA/m^2. At the end of each experiment, bacterial samples were washed gently with a saline (0.9% NaCl, v/v) and fixed for 2 h with 2.5% glutaraldehyde. The samples were dehydrated by using sequential ethanol concentrations from 30%, 50%, 75%, 90% to 100%, and exposure for 15–20 min per concentration.