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Behavior of autotrophic denitrification and heterotrophic denitrification in an intensified biofilm-electrode reactor for nitrate-contaminated drinking water treatment

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ABSTRACT

An intensified biofilm-electrode reactor (IBER) was developed to treat nitrate-contaminated drinking water. Different running conditions were conducted to investigate the behavior of autotrophic denitrification (AD) and heterotrophic denitrification (HD) in the IBER. In AD process, the nitrate nitrogen coulomb-reduction rate was used to evaluate the performance of the reactor. The maximum NO_3^--N removal efficiency was 6.8% at the current of 60 mA, while nitrate nitrogen coulomb-reduction rate was 0.024 mg C^{-1}. The optimum conditions for HD process were C/N = 0.8 and HRT = 8 h, under which complete NO_3^--N removal and no NO_2^--N accumulation were observed. With the cooperative effect of AD and HD in the heterotrophic and autotrophic denitrification (HAD) process, large treatment capacity, high denitrification efficiency, and low nitrite and ammonia accumulation were achieved. The results proved that HAD process was superior to single AD and HD for nitrate removal in the IBER.

1. Introduction

Nitrate pollution in groundwater has become more and more serious with extensive use of nitrogenous fertilizers in agriculture as well as discharge of domestic and industrial wastewater (Wang et al., 2009). Human health damage from methaemoglobinemia syndrome and gastric cancer caused by exceeded nitrate in drinking water has attracted worldwide attention (Ghafari et al., 2009a). The maximum contaminant levels (MCLs) for nitrate mandated by USEPA are 10 mg N L^{-1}, and the same values are also proposed by China (Standards for Drinking Water Quality, GB5749-2006) to prevent negative health effects.

Biological denitrification including autotrophic and heterotrophic denitrification is considered to be the most promising approach for nitrate removal among other conventional techniques like physicochemical, since nitrate can be reduced to harmless nitrogen gas by bacteria (Karanasios et al., 2010). External organic carbon sources such as methanol (Wang et al., 2009), ethanol (Gomez et al., 2003), glucose, glycerol, acetic acid, and lactic acid (Akunna et al., 1993), and starch (Kim et al., 2002) have been employed in heterotrophic denitrifying processes for water treatment. The efficiency of heterotrophic denitrification is relatively higher, however, most researches neglect the problems of fast growth of bacteria causing clogging in reactors and difficulties in sludge disposal, and toxic residuals and formation of by-products in the treated water caused by organic carbon sources (Ghafari et al., 2008). On the other hand, autotrophic denitrification avoids these shortcomings by employing carbon dioxide or bicarbonate (Ghafari et al., 2009b) as the carbon source, and sulfur (Soares, 2002), iron (Jha and Bose, 2005) or hydrogen (Karanasios et al., 2010; Vasiliadou et al., 2006; Zhou et al., 2009) as electron sources for biosynthesis. The most attractive research among autotrophic denitrification is hydrogenotrophic denitrification, which appears to have high selectivity for nitrate removal, lower cell yield, and the lack of harmful by-product, in contrast to the use of sulfur and iron (Karanasios et al., 2010).

Biofilm-electrode reactors (BER), developed on the basis of the hydrogenotrophic denitrification, has attracted considerable interests to remove nitrate from polluted water (Zhao et al., 2011; Ghafari et al., 2008; Zhou et al., 2009). In the BERs, hydrogen gas is internal produced by the electrolysis of water, thus avoiding the waste of excessive hydrogen gas by external addition. Nevertheless, limited and low solubility of hydrogen in water results in comparatively lower denitrification efficiency than heterotrophic denitrification (Zhao et al., 2011).

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In order to achieve higher nitrate removal efficiency, some researchers added a part of organic carbon source (C/N $\geqslant$ 1) into AD process (Wu et al., 2005; Bao and Hao, 2006). Hence, heterotrophic/autotrophic denitrification (HAD) coexisted and played roles simultaneously. Della Rocca et al. (2007) developed an approach of heterotrophic/autotrophic denitrification (HAD) by symbiotic relation of HD and AD processes supported by cotton (carbon source) and zero valent iron (for hydrogen production), and they proposed that CO_2 generated by HD could be used as inorganic carbon source in AD. Liu et al. (2009) combined two-step process of heterotrophic denitrification in a fluidized reactor and sulfur autotrophic denitrification processes (CHSAD) for the removal of nitrate in drinking water. However, the reactors used by Della Rocca et al. (2007) and Liu et al. (2009) were not constituted by BERs. Moreover, the autotrophic bacteria and heterotrophic bacteria were separated to two independent parts in their reactors. To the best of our knowledge, HAD of autotrophic bacteria and heterotrophic bacteria mingled coexisting in BER was few reported by other researches.

An intensified biofilm-electrode reactor (IBER) combining heterotrophic and hydrogenotrophic denitrification was developed and performed effectively for nitrate removal in our previous study (Zhao et al., 2011), in which cathode (stainless steel wire) embedded in independent carrier (fiber threads) made the reactor structure distinguish from other researches. To evaluate the general performance of the HAD, optimum running conditions for IBER were obtained by setting different hydraulic retention times (HRTs), carbon to nitrogen ratios (C/N) and electric currents (I) in the previous study (Zhao et al., 2011). However, the electrochemical effect, the behavior of single AD and HD, the superiority of HAD over single AD and HD, and how the AD and HD contributing to HAD for removal of nitrate using the developed IBER are still unknown. Therefore, the objective of this research is to: (1) analyze the electrochemical reduction effect; (2) investigate the performance of the AD and HD in the IBER; (3) find out the superiority of HAD over single AD and HD, and make clear the favorable contribution of AD and HD to HAD for nitrate removal in the IBER.

2. Methods

2.1. Chemicals and materials

All chemical reagents used were analytical grade without further purification. The cathode was stainless steel wire which could improve resistance against corrosion in the wastewater, and used to generate H_2 by electrolysis of water. The anode was carbon rods providing inorganic carbon source for autotrophic denitrification bacteria through carbon oxygenation. The fiber threads containing about 80% cotton and 20% terylene, were used as independent carriers for biofilm formation.

Activated sludge for inoculation of denitrifying bacteria was collected from the Qinghe wastewater treatment plant (Beijing, China).

The synthetic water was prepared by dissolving 0.304 $NaNO_3\,g\,L^{-1}$, 0.044 $KH_2PO_4\,g\,L^{-1}$, and CH_3OH according to different C/Ns in tap water. The original pH of the influent solution was normally about 7.5 and needed no further adjustment.

2.2. Experimental apparatus

Two identical sets of closed plexiglass cylinders (diameter 300 mm, height 300 mm) with an effective volume of 12 L, as illustrated in Fig. 1, were used in this study. The apparatus was similar to our previous studies (Zhao et al., 2011). The main reactor compartments consisted of a water collector, fiber threads carries, eight carbon rods as anode and stainless steel wire as cathode. The biofilm adhering to the fiber threads carries worked as a filter preventing suspended solids (SS) and other impurities into the effluent, thus saved a sedimentation process. Stainless steel wire was spirally inserted in the fiber threads. Carbon rods encircled the collector, and were equispaced along the cylinder wall. The design ensured that the produced hydrogen gas could spread from the inside biofilm to the outside under electrical field force, and be utilized by the bacteria effectively.

To keep the synthetic water at a constant temperature (30 ± 2 °C) in this study, length 3 m of the rubber pipe was spirally immersed in a constant temperature circulating water bath (HX-201, YKKY, Beijing, China), the synthetic water went through the pipe to be warmed up and then flowed into the reactor. To enhance the warming effect, 10 m of recirculating pipe was also spirally immersed in the water bath, as shown in Fig. 1. A DC regulated power supply (PS-302D, Shenzhen, China) was applied to provide current.

2.3. Experiment procedure

2.3.1. Electrochemical reduction

In order to ascertain the contribution of the electrochemical reduction effect in the IBER, an electrochemical experiment was carried out without inoculation of bacteria. Two groups of continuous experiments were run in the absence and the presence of organic carbon, respectively. In the absence of organic carbon, applied current was ranged from 0 to 500 mA. In the presence of organic carbon, C/N ratios were changed from 1 to 3 under 100 mA.

2.3.2. Autotrophic denitrification

The collected anaerobic sludge (4 L) was placed into the reactor and 8 L of tap water was added, with a total volume of 12 L. Then CH_3OH, $NaNO_3$ and KH_2PO_4 were added into the sludge-water according to C/N/P = 3:1:0.2, and the concentration of NO_3^--N was 50.00 $mg\,L^{-1}$. The applied current was adjusted to 10 mA with the initial voltage of 2.5 V. The sludge-water was circulated by the magnetic pump. C/N ratios were gradually reduced from 3, 2, 1.5, 1, and 0.5 to 0 when the NO_3^--N removal was considered to be steady. The influent was renewed every 24 h during this process. One month later, almost all the sludge was attached on the fiber threads and formed a biofilm. Then the continuous experiments were conducted to evaluate the performance of the AD reactor.

2.3.3. Heterotrophic denitrification

The collected anaerobic sludge (4 L) and tap water (8 L) were added to the reactor forming a mixture of 12 L. At the start-up stage, CH_3OH, $NaNO_3$ and KH_2PO_4 were added into the sludge-water according to C/N/P = 3:1:0.2. The influent (NO_3^--N = 50.00 $mg\,L^{-1}$) was continuously pumped to the reactor with HRT of 24 h for the first 2 days. The HRTs were then shortened from 24, 20, 16, 12 to 8 h at C/N = 3. The heterotrophic denitrification experiment was started up when more than 90% of the nitrate was eliminated and the biofilm was considered to be formed well. The whole cultivated process took 15 days, which was much less than the previous culture period of 25 days (Zhao et al., 2011). This might be attributed to the sufficient organic carbon and keeping a constant temperature in the reactor.

2.3.4. Cooperative heterotrophic and autotrophic denitrification

In the presence of organic carbon, the denitrification bacteria have lag effect on the nitrate removal efficiency. In order to eliminate the effect of residual organic carbon in the former step, the addition of methanol was decreased gradually till none and held for one week. Then, a C/N value was selected, at which organic carbon was insufficient and both the HD and AD process could be responsible for nitrate removal. Electric current was increased from 0 to 80 mA at the selected C/N value. The performance of