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High nitrate removal by autohydrogenotrophic bacteria in a biofilm-electrode reactor

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ABSTRACT

A biofilm-electrode reactor has been developed to effectively treat high nitrate-polluted wastewater. The novelty of this system is that the biological degradation of NO_3^--N (electron acceptor) was enhanced by the generation of H_2 (electron donor) and direct immobilization of autohydrogenotrophic bacteria on the surface of the cathode. Nitrate degradation rate increased as current increased from 10 to 120 mA. When current was increased above 120 mA, the degradation rate was decreased. Meanwhile, nitrite accumulation decreased as current (below 120 mA) increased. If current was increased further to higher than 120 mA, nitrite was observed to accumulate again. Denitrification rate increased with initial nitrate loading below 350 mg NO_3^--N L^{-1}. However, the denitrification was found to be inhibited at high nitrate loading (350–500 mg NO_3^--N L^{-1}). Meanwhile, nitrite accumulation increased as nitrate loading increased and the highest accumulated nitrite level reached 16.07 mg NO_2^--N L^{-1} at nitrate loading of 500 mg NO_3^--N L^{-1}. The reactor obtained optimum denitrification when C/N, temperature, and pH were 1.0, 25–30 °C, and 6.0–7.0, respectively.

Keywords: Biofilm-electrode reactor; High nitrate; Autohydrogenotrophic bacteria; Current; Nitrite

1. Introduction

Nitrate and nitrite in drinking water can threaten human health and other life forms, due to the fact that the uninterrupted consumption of water containing high levels of nitrate can be the source of certain diseases, such as methemoglobinemia in the fetus and stomach cancer [1–4]. According to some reports [5–7], different industrial wastewater contained more than 200 mg NO_3^--N L^{-1}, and their biological denitrification usually took a few days. Therefore, some researchers such as Zayed and Winter [7] investigated nitrate removal approach from dairy wastewater, which used activated sludge to treat 250 mg NO_3^--N L^{-1} nitrate-polluted wastewater.

Denitrification was reported as one of the most common and effective methods applied by researchers [8–10] in both freshwater and seawater. Researchers also reported that biological denitrification was the most favorable approach to treat nitrate-contaminated water [11–13].

Although autohydrogenotrophic denitrification is effective for the treatment of nitrate-polluted water, the denitrification rate may be controlled by mass transfer rate of H_2 from gas phase to liquid phase because of low solubility of H_2 in water, so that

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bio-reactors always need large volumes in order to increase the contact time, which may be required to achieve the complete denitrification. Moreover, the explosive nature of hydrogen gas may also cause safety problems during the autotrophic denitrification process. These drawbacks would result in higher operating costs in comparison with other methods.

To overcome the above mentioned problems, it is essential to develop an appropriate treatment method for nitrate-polluted water. Biofilm-electrode reactor (BER), consisting of a couple of electrodes has been first proposed by Sakakibara and Kuroda [14], in which autohydrogenotrophic bacteria was cultured on the cathode surface. This process was easy for operation and maintenance.

The term BER commonly refers to the process where an electric current passes through a proper electrode system, which enhances the biological degradation of the pollutants (such as nitrate), due to the generation of appropriate H_2 (energy source) and direct immobilization of autohydrogenotrophic bacteria on the surface of the electrode [12,15,16].

To put it simply, BER was developed on the basis of internal production of H_2, performed by the electrolysis of water. Sakakibara [14] and Feleke [17] successfully immobilized denitrifying microorganisms on the cathode, and hydrogen was simultaneously produced by the cathode. Ghafari [12] used an upflow BER to accommodate hydrogenotrophic denitrifying bacteria employing palm shell granular activated carbon as the bio-carrier and cathode material. Zhao et al. [18] developed an intensified biofilm-electrode reactor (IBER) for the treatment of nitrate-contaminated groundwater. Prosnansky et al. [19] developed a multiple-GAC BER system combined with microfiltration for the treatment of nitrate-contaminated wastewater. Grommen et al. [20] designed a hydrogenotrophic denitrification reactor for the removal of nitrate from aquaria and the reactor performed well. From the research of Kiss et al. [21], a two-reactor system, which used sodium bicarbonate as carbon source, was first enriched with hydrogen in an electrolysis cell prior to enter a packed-bed bio-reactor. Cast and Flora [22] applied denitrifying bacteria to compare the denitrification efficiency of two cathode materials in water treatment.

As a result, the BER might be a solution to the problem of high cost of hydrogen supply during the autohydrogenotrophic denitrification. However, the longer hydraulic retention time (HRT), the lower nitrate removal rate and the escaping biomass as main disadvantages, limit its applicability [23].

In this work, a BER has been developed. In this denitrification system, biological denitrification assisted by electricity would theoretically be performed through *in situ* hydrogen production on the cathode surface (graphite felt). Hydrogen and a lower oxidation-reduction potential environment produced through the cathodic reactions would be utilized by autohydrogenotrophic bacteria to reduce NO_3^--N into N_2 [2,24]. The objective of this work was to investigate the effect of electric current, nitrate loading, C/N, temperature, and pH on nitrate remediation and to optimize the operating parameters of this bio-electrochemical system for autotrophic denitrification.

2. Materials and methods

2.1. Experimental apparatus

A schematic of the reactor used in this study is shown in Fig. 1. The main reactor compartments consisted of a closed plexiglass cylinders (diameter 100 mm, height 300 mm), a carbon rod (diameter 6 mm, height 350 mm) as anode, and a piece of graphite felt (specific surface area 4,860 m^2) which closed to the interior walls of the plexiglass cylinders as cathode. A DC regulated power supply (PS-A203D, 0–20 V, 0–2 A) was used to provide the current. This system was sealed so that the reactor could be under anaerobic condition [25]. The dissolved oxygen (DO) in the BER was found to be between 0.1 and 0.3 mg L^{-1} during the operation.

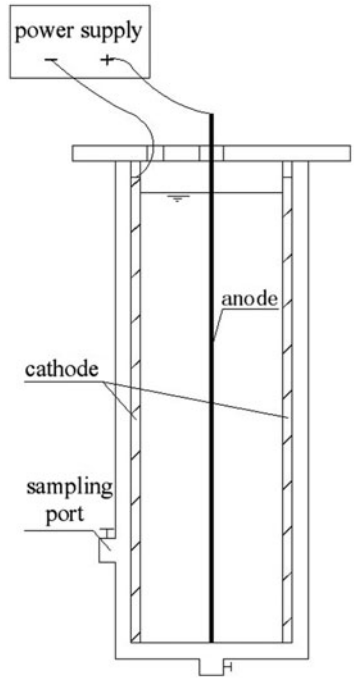

Fig. 1. Schematic representation of the biofilm-electrode denitrification system.

2.2. Synthetic wastewater

The composition of synthetic wastewater was (mg L^{-1}): $MgSO_4$ 20, $MgCl_2$ 300, K_2HPO_4 250, $NiCl_2{\cdot}6H_2O$ 16, $MnSO_4{\cdot}7H_2O$ 15, $CoCl_2$ 25, KH_2PO_4 250, $NaHCO_3$ (inorganic carbon source) 210, $CaCl_2$ 30, $FeSO_4{\cdot}7H_2O$ 20, $ZnSO_4$ 20, and $CuCl_2{\cdot}2H_2O$ 10. $NaNO_3$ (nitrate loading) ranged from 30 to 500 mg NO_3^--N L^{-1}, pH 7.0–7.5.

2.3. Microorganisms

Denitrifying bacteria which formed biofilm on the surface of the cathode (graphite felt) was collected from the anaerobic tank of Erlangmiao Municipal Wastewater Treatment Plant (Wuhan, China). About 2 L of anaerobic sludge (mixed liquor volatile suspended solids (MLVSS) = 3,039 mg L^{-1}) was placed into the reactor with nutritive material at 25°C. $NaHCO_3$ and $NaNO_3$ were added into the anaerobic sludge according to C/N = 1.0. Then the current was added to the system. About 30 d later, nitrate degradation rate could be stable because a dark gray color covered the graphite felt, indicating that the biofilm had formed well. Initial nitrate loading was maintained around 35 mg NO_3^--N L^{-1}. The system was started up when more than 80% of the nitrate was eliminated. DO was kept below 0.3 mg L^{-1}, HRT was kept at 10 h, and pH was automatically maintained at 7.0–7.5 in the BER. At this time, the population of denitrifying bacteria was about 45×10^6 cfu mL.

2.4. Analytical methods

On a UV–visible spectrophotometer (nanbeijt, China), nitrate (NO_3^--N), nitrite (NO_2^--N), and ammonia NH_4^+-N were measured using ultraviolet spectrophotometry, spectrophotometry based on N-(1-naphthyl) ethylenediamine dihydrochloride, Nessler's reagent spectrophotometry, respectively. The pH measured by pH meter (PC-320). DO and temperature in the reactor was measured by YSI550A DO meter. The bacterial population was counted by Acridine orange direct count.

3. Results and discussion

3.1. Effect of current on BER

It can be seen from Figs. 2 and 3 that the concentrations of NO_3^--N and NO_2^--N of the BER were changed with various current as a function of time. The applied current varied from 10 to 150 mA, with pH, initial nitrate loading, C/N, and temperature at steady state values of 7.0–7.5, 30 mg NO_3^--N L^{-1}, 1.0, and 25°C, respectively.

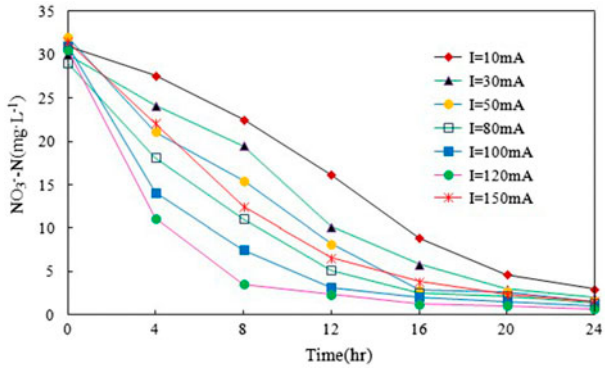

Fig. 2. Concentration of NO_3^--N in the BER as a function of time at different current conditions (pH 7.0–7.5, nitrate loading 30 mg NO_3^--N L^{-1}, C/N 1.0, temperature 25°C).

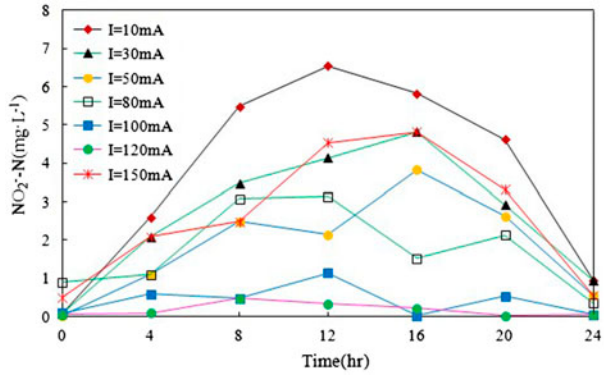

Fig. 3. Concentration of NO_2^--N in the BER as a function of time at different current conditions (pH 7.0–7.5, nitrate loading 30 mg NO_3^--N L^{-1}, C/N 1.0, and temperature 25°C).

As shown in Fig. 2, nitrate degradation rate increased as electric current intensity increased from 10 to 120 mA. A marked effect of current was between 80 and 100 mA, when the denitrification rate was more than doubled, and a further increase (up to 4.85 mg NO_3^--N L^{-1} h^{-1}) occurred at 120 mA. Meanwhile, when current was varied from 100 to 120 mA, NO_3^--N was rapidly reduced down to below 10 mg NO_3^--N L^{-1} in 8 h. However, there was a decline in nitrate removal when the current was increased to 150 mA.

Fig. 3 shows the comparison of nitrite curves obtained in the reactor. At the initial current of 10 mA, the highest nitrite level (6.54 mg NO_2^--N L^{-1}) was achieved. While, the accumulated nitrite started to decrease as current increased from 10 to 120 mA.

Specifically, nitrite level remained below 1.00 mg NO_2^--N L^{-1} at the applied current range of 100–120 mA. Nevertheless, when the applied current was increased further to higher than 120 mA, nitrite was observed to accumulate again. Moreover, the concentration of NH_4^+-N fluctuated slightly and the level ranged of 0.16–1.25 mg NH_4^+-N L^{-1}.

The bio-electrode denitrification process is totally related to the electrical current. Adjustment of the current plays an important role in operating BERs, in providing suitable conditions for autohydrogenotrophic bacteria and achieving a high rate of denitrification. The reason might be that the value of pH and concentration of H_2 (energy source) are dependent on current intensity [26]. Apparently, apart from the slight electrochemical effect, the promoting effect of current on autohydrogenotrophic denitrification was tremendous in this work. Nitrate degradation rate increased as current increased from 10 to 120 mA, which was attributed to H_2 (energy source) for biofilm-electrode denitrification process, increased along with the current (10–120 mA). Moreover, the highest denitrification rate (4.85 mg NO_3^--N L^{-1} h^{-1}) was observed in the reactor at an applied current of 120 mA, the reason could be that the presence of current was more conducive to the growth of autohydrogenotrophic bacteria and that the autotrophic denitrification rate was accelerated as denitrifying bacteria adapted to this environment.

However, there was a decline in nitrate removal when the current was increased further to higher than 120 mA, the reason for the decline in denitrification rate at the highest value of current tested was not clear. There were two main reasons for this phenomenon according to many researchers. Firstly, in the reactor with the biomass attached to the cathode, the decline of denitrification rate at relatively high level of current was attributed to the forced migration of nitrate to the bulk liquid, brought about by the potential gradient [27]. Secondly, the nitrate degradation rate enormously decreased when the current was increased to 150 mA, which was attributed to hydrogen inhibition. In the present research, the reason that caused this phenomenon might be the latter. Excess hydrogen started to appear when the current was beyond 150 mA, this indicated that increased current accelerated electrolyticaction. An excessively high concentration of hydrogen has been reported as an inhibitor of denitrification and a cause of nitrite accumulation [12,28,29]. However, the effect was not sustained for a long time, due to the excessive amounts of nitrite would be converted to nitrogen at the end of the experiment. This outcome showed that such a high current was not suitable for the bio-electrode denitrification system. Otherwise, nitrite was also accumulated at lower current (10–50 mA), due to the fact that lower current could inhibit the activity of microorganism and nitrate reductase, leading to a decline in nitrate removal. Therefore, it is necessary to control current at about 120 mA in this reactor.

3.2. Effect of nitrate loading on BER

Figs. 4 and 5 show the evolution of the concentrations of NO_3^--N and NO_2^--N of the denitrification reactor as a function of time, respectively. Prior to investigating the experiments for optimization of nitrate loading, a preliminary study was carried out with different concentrations of nitrate loading to find out an efficient range for further investigations. As such, applying pH 7.0–7.5, current 120 mA, C/N 1.0, and temperature 25°C, experiments were carried out using concentrations from 30 (≈stoichiometry) up to 500 mg NO_3^--N L^{-1}.

Fig. 4 demonstrates nitrate degradation rates for these runs. Denitrification rate increased with initial nitrate loading below 350 mg NO_3^--N L^{-1}. When nitrate loading was less than 120 mg NO_3^--N L^{-1}, nearly all NO_3^--N was rapidly reduced down to below 10 mg NO_3^--N L^{-1} in 24 h. The highest denitrification rate (44.82 mg NO_3^--N L^{-1} h^{-1}) was observed in the reactor at an applied nitrate loading of 350 mg NO_3^--N L^{-1}. When the applied nitrate loading was increased further to higher than 350 mg NO_3^--N L^{-1}, nitrate degradation rate would be decreased enormously.

Fig. 5 shows the comparison of nitrite curves obtained in the reactor. The nitrite concentration always remained below 5.00 mg NO_2^--N L^{-1} when nitrate loading was less than 200 mg NO_3^--N L^{-1}.

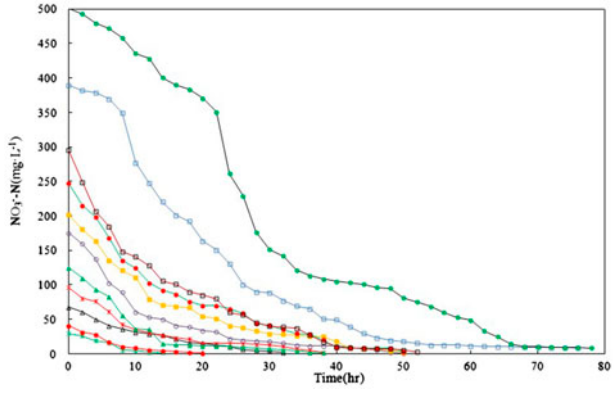

Fig. 4. Concentration of NO_3^--N in the BER as a function of time at different nitrate loading conditions (pH 7.0–7.5, current 120 mA, C/N 1.0, and temperature 25°C).

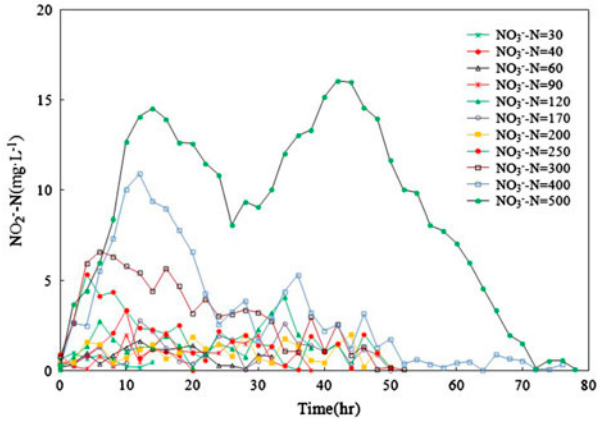

Fig. 5. Concentration of NO_2^--N in the BER as a function of time at different nitrate loading conditions (pH 7.0–7.5, current 120 mA, C/N 1.0, and temperature 25°C).

Nitrite gradually accumulated as nitrate loading increased above 200 mg NO_3^--N L^{-1} during the nitrate reduction. The maximum nitrite level increased to 5.37, 6.61, and 10.89 mg NO_2^--N L^{-1} when initial nitrate concentration was 250, 300, and 400 mg NO_3^--N L^{-1}, respectively. The highest NO_2^--N level (16.07 mg NO_2^--N L^{-1}) was observed in the reactor at an applied nitrate loading of 500 mg NO_3^--N L^{-1}. Moreover, the concentration of NH_4^+-N fluctuated slightly and the level ranged of 0.23–1.08 mg NH_4^+-N L^{-1}.

In previous researches on autotrophic nitrate removal using hydrogen as an electron donor, experiments were always conducted with low concentrations of nitrate, such as 50 mg NO_3^--N L^{-1} [30–34]. However, in this work, initial nitrate concentration increased up to 500 mg NO_3^--N L^{-1}. This is meaningful because nitrate levels are usually high in wastewater up to as much as 500–1,000 mg NO_3^--N L^{-1}. Park et al. [35] varied the initial nitrate concentration in a range from 20 to 492 mg NO_3^--N L^{-1} in order to investigate the nitrate reduction rate. Their data showed that the nitrate removal rate increased as the initial nitrate loading increased, while nitrite accumulation was observed. In this work, nitrate degradation rate increased as initial nitrate loading (below 350 mg NO_3^--N L^{-1}) increased. This environment was quite fit for autohydrogenotrophic bacteria to grown on. Therefore, more bacteria could contact with nitrate by the big surface area of biofilm under this condition.

However, the denitrification was found to be inhibited at high nitrate loading (350–500 mg NO_3^--N L^{-1}) in the present research, most likely due to incomplete inhibition of nitrate reductase activity during the bacterial metabolic process. Polcyn and Lucinski [36] argued that under high nitrate additions, the competition for electrons between nitrate and nitrite reductase was responsible for nitrite accumulation since nitrate reduction was energetically more advantageous, and hence, the limited electron flow was preferentially directed to nitrate reductase. High level of nitrate could result in high level of nitrite accumulated during the nitrate reduction [35]. Denitrification is a biological process that reduces NO_3^--N to NO_2^--N, and finally to N_2. Since there were two steps to convert NO_3^--N into N_2, the reaction rate of each step would have tremendous effects on the biological process. Although nitrite was not added into the reactor, NO_2^--N was detected and its concentration increased by the increasing of initial nitrate loading. The reason could be that partial NO_3^--N was transformed to NO_2^--N by some bacteria existing in the denitrification system. Even though nitrite could quickly rise to its highest level (16.07 mg NO_2^--N L^{-1}) in the BER at an applied nitrate loading of 500 mg NO_3^--N L^{-1}, the nitrite gradually reduced to below 1.00 mg NO_2^--N L^{-1} along with the increase of run time (above 72 h), due to the fact that nitrite was produced as an intermediate product and then reduced further to nitrogen.

3.3. Effect of C/N on BER

Figs. 6 and 7 show the evolution of the concentrations of NO_3^--N and NO_2^--N of the denitrification reactor as a function of time, respectively. Prior to investigating the experiments for optimization of C/N, a preliminary study was carried out with different concentrations of C/N to find out an efficient range for further investigations. As such, applying pH 7.0–7.5, current 120 mA, nitrate loading 30 mg NO_3^--N L^{-1}, and temperature 25°C, experiments were carried out using C/N from 0.2 to 5.0.

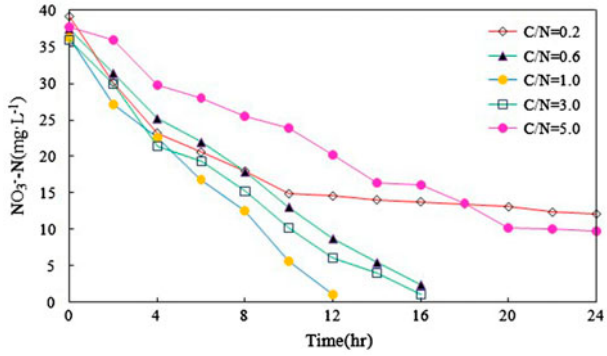

Fig. 6. Concentration of NO_3^--N in the BER as a function of time at different C/N conditions (pH 7.0–7.5, nitrate loading 30 mg NO_3^--N L^{-1}, current 120 mA, and temperature 25°C).

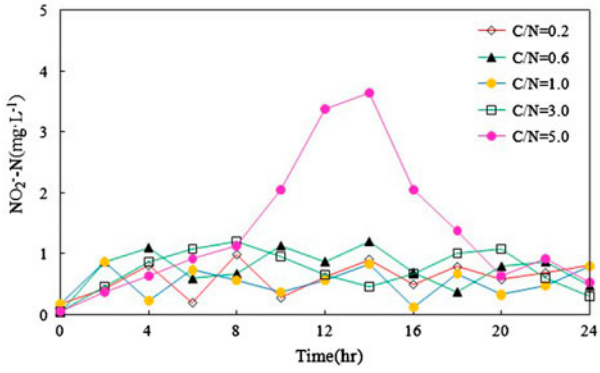

Fig. 7. Concentration of NO_2^--N in the BER as a function of time at different C/N conditions (pH 7.0–7.5, nitrate loading 30 mg NO_3^--N L^{-1}, current 120 mA, and temperature 25 ℃).

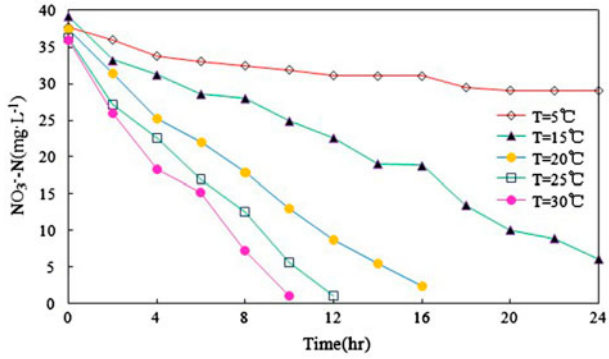

Fig. 8. Concentration of NO_3^--N in the BER as a function of time at different temperature conditions (pH 7.0–7.5, nitrate loading 30 mg NO_3^--N L^{-1}, current 120 mA, and C/N 1.0).

As shown in Fig. 6, nitrate degradation rate increased as C/N increased from 0.2 to 1.0. However, when C/N was varied from 1.0 to 5.0, there was a decline in nitrate removal. Fig. 7 shows the comparison of nitrite curves obtained in the reactor. The concentration of nitrite in effluent was lower than 1.13 mg NO_2^--N L^{-1} when C/N was increased from 0.2 to 3.0, while the highest accumulated nitrite level (3.38 mg NO_2^--N L^{-1}) was achieved at C/N of 5.0. Moreover, the concentration of NH_4^+-N fluctuated slightly and the level ranged of 0.07–0.95 mg NH_4^+-N L^{-1}.

A low ratio of C/N caused incomplete denitrification and a high ratio of C/N led to nitrite accumulation, which provided high concentration of nitrous instead of N_2 [26]. This could explain why nitrate degradation rate was very low at C/N of 0.2 and nitrite was accumulated when C/N was 5.0 in the present study. Some researches showed that completed denitrification at C/N = 1.0 has outperformed many other systems [37]. In this work, results indicated that the BER obtained optimum denitrification when C/N was 1.0, due to the fact that the bacteria and reductase performed high activity under this environment.

3.4. Effect of temperature on BER

Figs. 8 and 9 show the evolution of the concentrations of NO_3^--N and NO_2^--N of the denitrification reactor as a function of time, respectively. The applied temperature varied from 5 to 30 ℃, with pH, initial nitrate loading, C/N, and current at steady state values of 7.0–7.5, 30 mg NO_3^--N L^{-1}, 1.0, and 120 mA, respectively.

It was observed from Fig. 8 that denitrification rate increased from 1.10 to 5.02 mg NO_3^--N L^{-1} h^{-1} when

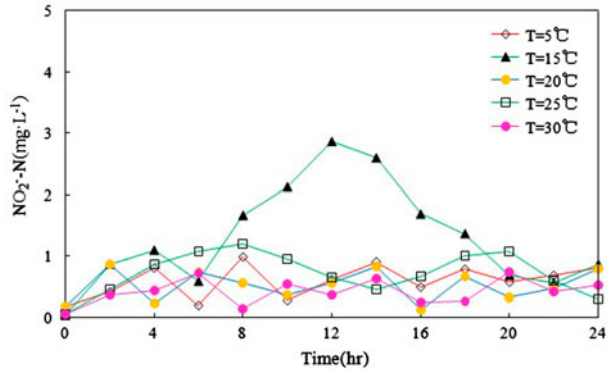

Fig. 9. Concentration of NO_2^--N in the BER as a function of time at different temperature conditions (pH 7.0–7.5, nitrate loading 30 mg NO_3^--N L^{-1}, current 120 mA, and C/N 1.0).

temperature increased from 5 to 30 ℃. As shown in Fig. 9, nitrite was always below 1.21 mg NO_2^--N L^{-1} except at temperature of 15 ℃. Moreover, the concentration of NH_4^+-N fluctuated slightly and the level ranged of 0.05–0.73 mg NH_4^+-N L^{-1}.

The results illustrated that denitrification effect was excellent when temperature was 25–30 ℃, due to the fact that bacteria and reductase could perform high activity. This result was in accordance with some similar researches [23]. Some researchers reported that temperature affected the denitrification process by affecting bacteria behavior [31]. In the present study, the BER was inhibited at temperature of 5 ℃, which was attributed to low activity of the autohydrogenotrophic bacteria and reductase under this circumstance. However, nitrite was not accumulated since the process of denitrification was very slow at

temperature of 5℃. When temperature was 15℃, there was an incomplete denitrification so that nitrite was accumulated.

3.5. Effect of pH on BER

Figs. 10 and 11 show the evolution of the concentrations of NO_3^--N and NO_2^--N of the denitrification reactor as a function of time, respectively. The applied pH varied from 4.0 to 9.0, with temperature, initial nitrate loading, C/N, and current at steady-state values of 25℃, 30 mg NO_3^--N L^{-1}, 1.0, and 120 mA, respectively.

It can be seen from Fig. 10 that denitrification rate increased from 2.32 to 4.03 mg NO_3^--N L^{-1} h^{-1} when pH increased from 4.0 to 7.0, while pH further increased from 7.0 to 9.0, when nitrate reduction rate dropped significantly. Fig. 11 showed that nitrite level was always fluctuated slightly when pH was 6.0–7.0. However, there were different degrees of nitrite accumulation at pH of 4.0, 8.0 and 9.0. Moreover, the concentration of NH_4^+-N fluctuated slightly and the level ranged of 0.08–0.51 mg NH_4^+-N L^{-1}.

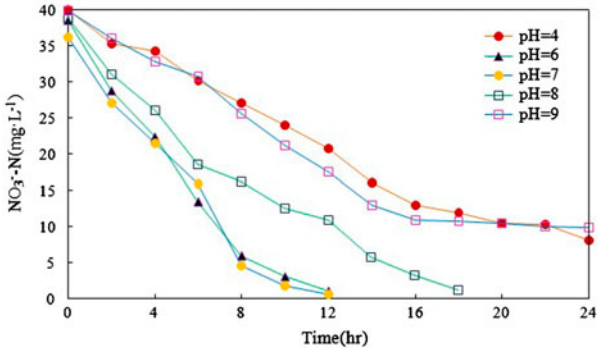

Fig. 10. Concentration of NO_3^--N in the BER as a function of time at different pH conditions (temperature 25℃, nitrate loading 30 mg NO_3^--N L^{-1}, current 120 mA, and C/N 1.0).

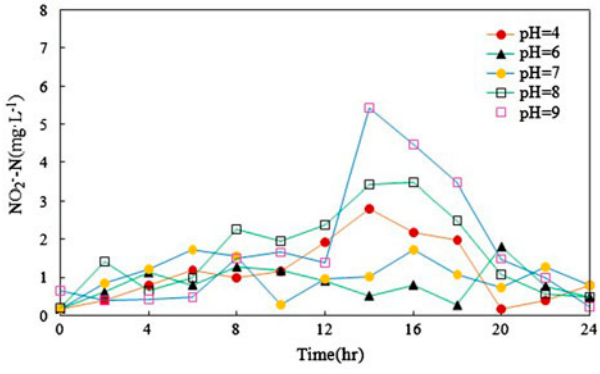

Fig. 11. Concentration of NO_2^--N in the BER as a function of time at different pH conditions (temperature 25℃, nitrate loading 30 mg NO_3^--N L^{-1}, current 120 mA, and C/N 1.0).

pH is known to be one of the main controlling factors for the denitrification process. Culture pH control can be effective on the efficiency of nitrate removal, while uncontrolled pH can lead to incomplete denitrification [38]. The optimum pH for the BER was 6.0–7.0 in this research. This result was accordance with the research of Ho et al. [39], whose experiment demonstrated that when the pH of the bio-reactor remained at 7.0, nitrate could be reduced effectively with no nitrite accumulation. Denitrification rate decreased at pH of 4.0, 8.0 and 9.0, the reason was that pH influenced the enzyme activity of bacteria. Nitrite was accumulated under this environment due to the fact that the activities of microorganism and nitrate reductase were inhibited.

4. Conclusions

In this work, a BER (anode carbon, cathode, graphite felt) has been developed to effectively treat high nitrate-polluted wastewater. The performance of this system was investigated using a laboratory-scale apparatus.

The optimum electric current intensity for the reactor was 120 mA. This reactor could cope with high nitrate of 500 mg NO_3^--N L^{-1}. The highest denitrification rate increased to 44.82 mg NO_3^--N L^{-1} h^{-1} at nitrate loading of 350 mg NO_3^--N L^{-1}. Nitrite level increased as nitrate loading increased and the highest accumulated nitrite level reached 16.07 mg NO_2^--N L^{-1} at nitrate loading of 500 mg NO_3^--N L^{-1}.

The BER obtained optimum denitrification when C/N was 1.0. The optimum temperature and pH for the reactor was 25–30℃ and 6.0–7.0, respectively.

It was demonstrated that this BER was effective for treatment of high nitrate-polluted wastewater.

Acknowledgments

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