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Effects of important factors on hydrogen-based autotrophic denitrification in a bioreactor

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ABSTRACT

Effects of biomass, pH, temperature, nitrate loading, C/N on autohydrogenotrophic denitrification were investigated in a lab-scale bioreactor. Nitrate degradation rate increased as biomass increased. When OD_{600} was 0.173, NO_3^--N was rapidly reduced down to zero in 3.0 h. The optimum pH for the reactor was 6.0–7.0, high pH values caused accumulated nitrite and decrease of denitrification rate. The average nitrate reduction rate increased from 4.50 to 17.15 mg NO_3^--N L^{-1} h^{-1} as temperature increased from 20 to 35°C. However, there was a slight decline in denitrification rate at temperature of 40°C. High nitrate loading of 155 mg NO_3^--N L^{-1} aroused decrease of denitrification rate. Although C/N value was not directly influence the nitrate reduction rate, it could contribute to increase pH value in order to inhibit the reductase's activity to hinder the denitrification process.

Keywords: Effects; Autohydrogenotrophic denitrification; Nitrate degradation rate; Accumulated nitrite

1. Introduction

Removing nitrate and nitrite from water has gained great attention in recent years due to some serious human health risks such as methemoglobinemia, gastric cancer and non-Hodgkin's lymphoma [1–4], which are induced from ingestion of nitrate-polluted surface water and groundwater. In view of these problems, many countries promulgate specific regulations to set the maximum contaminating levels of nitrate in groundwater. The value of nitrate nitrogen is proposed by China is 10 mg NO_3^--N L^{-1} [5], which is in accordance with that proposed by the World Health Organization [6].

The traditional physicochemical methods used to eliminate nitrate from water are ion exchange, electrodialysis and reverse osmosis [7–9]. However, these approaches have been found to be cost-ineffective due to high installation or maintenance costs and the concentrated waste brines require further treatment or disposal [6]. Moreover, as energy consumption is becoming a major issue for the modern human society, energy saving from wastewater treatment naturally becomes the interest of wastewater industry.

Biological denitrification process is widely applied in nitrate removal from groundwater attributing to its high energy efficiency [10]. Although their application can also present some limitations, the low cost of these inorganic substrates and low formation of biomass are important advantages [11]. For instance, autohydrogenotrophic denitrification is an excellent method because H_2 is clean nature [12], and the process is low biomass yield and relatively low cost [13], as well as

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because it does not persist in the treated water to create biological instability and no further steps are required to remove either excess substrate or its derivatives [14].

Previous studies on autohydrogenotrophic denitrification of drinking water are limited [14–23], due to the fact that H_2 is flammable and explosive when it mixtures with air, is poorly soluble in water and results in lower denitrification rates compared to heterotrophic denitrification [19]. The heterotrophic denitrification process is applied extensively because of its high efficiency and the simplicity of the reactors required. However, more bacterial growth may cause increase of the effluent turbidity [24], and excessive organic carbon resulting in secondary pollution [25] make it unfavorable during heterotrophic denitrification process. For this reason, many researchers developed new natural materials (such as wheat straw, plant prunings etc.) as organic carbon sources for use in heterotrophic denitrification [26,27]. Although this method was cost-effective, the pretreatment process was complicated and lengthy [28]. Furthermore, the production of CO_2 was wasted during heterotrophic denitrification. Compared with heterotrophic denitrification, hydrogenotrophic denitrification offers two major advantages. First, almost no denitrification byproducts are present in the treated water. Second, hydrogen costs less time than the common organic supplements for removal of the same amount of nitrate [16].

In this work, a hydrogen-based bioreactor was developed to treat nitrate-polluted wastewater. This autohydrogenotrophic denitrification process based on inorganic carbon source [29], involved hydrogen as the electron donor and nitrate as the electron accepter for the bacterial metabolic chain. The objective of this work was to investigate the effects of biomass, pH, temperature, nitrate loading and C/N on denitrification process. The results of this research will offer theory supports to parameters optimization of autohydrogenotrophic denitrification.

2. Materials and methods

2.1. Experimental apparatus

A schematic of the reactor used in this work is shown in Fig. 1. The main reactor compartments consisted of four same airtight flasks (250 mL). During the experiment, the domesticated anaerobic sludge (200 mL, mixed liquor suspended solids 77.9 g/L, mixed liquor volatile suspended solids 35.1 g/L) and bacterial culture media were poured into flasks. After enough hydrogen was introduced to the flasks, the flasks were placed into Oven Controlled Crystal Oscillator in order to proceed the denitrification reaction well. During the experiments, the hydrogen pressure was remained at 0.05 MPa.

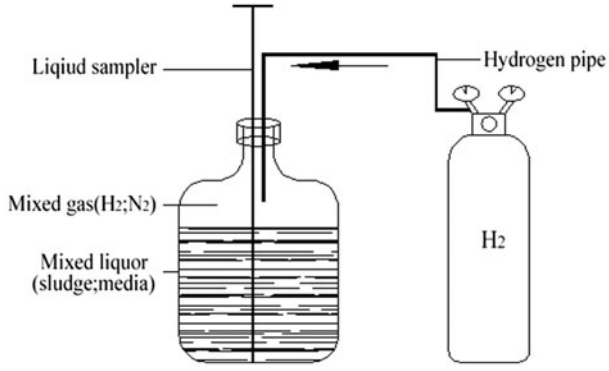

Fig. 1. Schematic diagram of the laboratory-scale bioreactor for hydrogenotrophic denitrification.

2.2. Microorganisms and culture media

Autohydrogenotrophic denitrifying bacteria was collected from the anaerobic tank of Erlangmiao Municipal Wastewater Treatment Plant in Wuhan, China. The synthetic sludge as bacterial seed was cultured for 30 d in an airtight container. Enough hydrogen and nutrients were provided during the acclimation stage.

The composition of culture media were (mg L^{-1}): $ZnCl_2$ (CAS number 7646-85-7) 0.68, $CoCl_2{\cdot}6H_2O$ (7791-13-1) 0.19, $MnSO_4{\cdot}7H_2O$ (10034-99-8) 0.12, $NiCl_2{\cdot}6H_2O$ (7791-20-0) 0.27, $CuCl_2{\cdot}2H_2O$ (10125-13-0) 0.32, $Na_2MoO_4{\cdot}2H_2O$ (7631-95-0) 0.36, $MgCl_2{\cdot}6H_2O$ (7791-18-6) 0.28, H_3BO_3 (11113-50-1) 0.35, $NaHCO_3$ (inorganic carbon source) (144-55-8) 7500, KH_2PO_4 (7778-77-0) 0.975, $NaNO_3$ (7631-99-4) 425, pH 7.0–7.5.

2.3. Analytical methods

Nitrate (NO_3^--N), nitrite (NO_2^--N), ammonia (NH_4^+-N) were measured according to the standard methods [30]. The pH was measured by pH meter (PC-320). Temperature in the reactor was measured by YSI550A meter. The microbial biomass was monitored by OD_{600}. The cell count was counted by Acridine orange direct count [31].

3. Results and discussion

3.1. Denitrification effect under different biomass

Fig. 2 shows the effect of biomass on denitrification rate was investigated by a short-term operation for

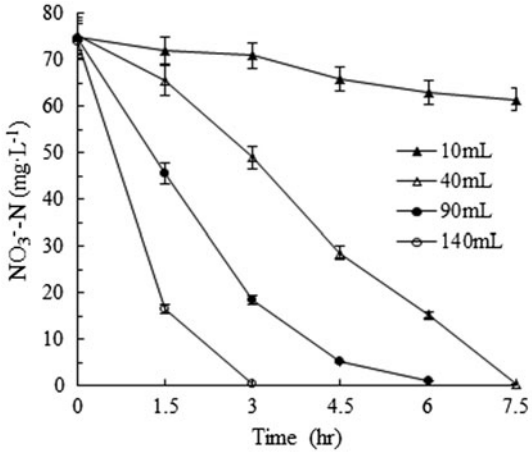

Fig. 2. Concentration of NO_3^--N in the reactor at different biomass conditions (pH 7.0, nitrate loading 75 mg NO_3^--N L^{-1}, C/N 30, temperature 30°C).

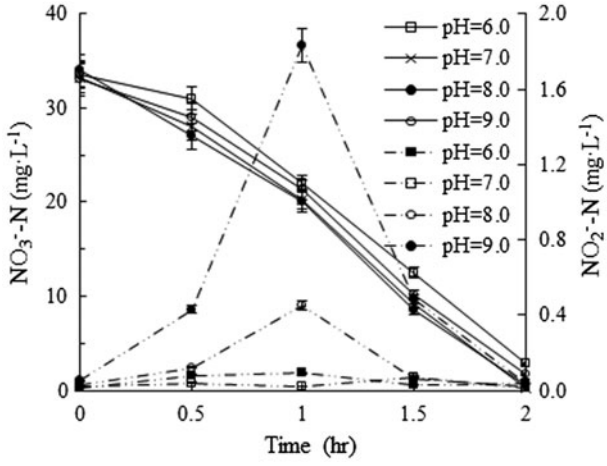

Fig. 3. Concentrations of NO_3^--N and NO_2^--N in the reactor at different pH conditions (sludge 140 mL, nitrate loading 33 mg NO_3^--N L^{-1}, C/N 30, temperature 30°C).

one day at each operating biomass of 10, 40, 90, and 140 mL sludge (OD_{600} 0.011, 0.034, 0.092, 0.173) in four airtight flasks. As such, applying pH 7.0, nitrate loading 75 mg NO_3^--N L^{-1}, C/N 30, and temperature 30°C.

As shown in Fig. 2, nitrate degradation rate increased as biomass increased. When OD_{600} was 0.173, NO_3^--N was rapidly reduced down to zero in 3.0 h. Meanwhile, the highest average denitrification rate (37.89 mg NO_3^--N L^{-1} h^{-1}) was observed during this phase. The completed denitrification times were increased to 6.0 and 7.5 h at OD_{600} of 0.092 and 0.034, and also the average denitrification rates were decreased to 12.30 and 9.91 mg NO_3^--N L^{-1} h^{-1}, respectively. The reduction rate of nitrate was significantly reduced down to 2.99 mg NH_3^--N L^{-1} h^{-1} when OD_{600} was further reduced to 0.011. However, the concentrations of NO_2^--N and NH_4^+-N were always closed to zero during the experiment.

Increased biomass indicated that more bacteria could participate in the denitrification process. In the present study, the highest average denitrification rate was observed at OD_{600} of 0.173 due to the fact that larger amount of autohydrogenotrophic denitrifying bacteria (cell count 98×10^6 cfu/mL) caused a mass of biological metabolism in the limited volume of the reactor. Therefore, it is not difficult to explain the phenomenon that the denitrification reaction was barely occurred when OD_{600} was 0.011.

3.2. Denitrification effect under different pH

Fig. 3 shows the effect of pH on denitrification rate was investigated by a short-term operation for one day at each operating pH of 6.0, 7.0, 8.0, and 9.0 in four airtight flasks. As such, applying sludge 140 mL, nitrate loading 33 mg NO_3^--N L^{-1}, C/N 30, and temperature 30°C.

It was observed from Fig. 3 that nitrate could be completely degraded at pH ranged from 6.0 to 9.0. However, the denitrification rates were different and there were different degrees of nitrite accumulations under different pH conditions. The highest accumulated nitrite level was increased to 1.92 mg NO_2^--N L^{-1} at pH of 9.0 in this phase. Moreover, the concentration of NH_4^+-N was always closed to zero during the experiment.

Culture pH control can be effective on the efficiency of nitrate reduction, while uncontrolled pH can lead to incomplete denitrification [32]. Ho et al. [33] proved that nitrate could be effectively reduced with no nitrite accumulation when the pH of the bioreactor remained at 7. Xia et al. [34] demonstrated that the optimum pH for autotrophic denitrification was 7.2–8.2, with the maximum efficiency at pH 7.7. Lee and Rittmann [35] reported that the hydrogenotrophic denitrification process was positively related to pH, with an optimum value range of 7.6–8.6. In the present study, the optimum pH for the reactor was 6.0–7.0. When pH was 8.0–9.0, the accumulated nitrite was detected, and the nitrite level was increased with pH increased from 8.0 to 9.0, the reason for this phenomenon was that high pH value could inhibit the nitrite reductase activity of bacteria [36] and the denitrification reaction was not completed under this environment.

3.3. Denitrification effect under different temperature

The effect of temperature on nitrate reduction is apparent in Fig. 4. The each operating temperature

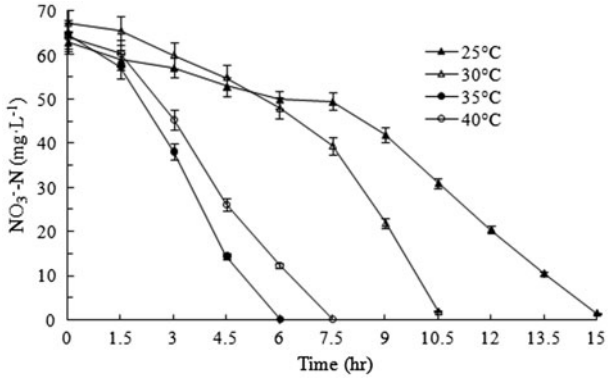

Fig. 4. Concentration of NO_3^--N in the reactor at different temperature conditions (sludge 100 mL, nitrate loading 65 mg NO_3^--N L^{-1}, C/N 30, pH 7.0).

were 25, 30, 35 and 40℃ in four airtight flasks. As such, applying sludge 100 mL, nitrate loading 65 mg NO_3^--N L^{-1}, C/N 30, and pH 7.0.

It can be seen from Fig. 4 that the average nitrate reduction rate increased from 4.50 to 17.15 mg NO_3^--N L^{-1} h^{-1} as temperature increased from 20 to 35℃. However, there was a slight decline in denitrification rate at temperature of 40℃. The concentrations of NO_2^--N and NH_4^+-N was always closed to zero during the test.

The results demonstrated that the rector could get best denitrification effect at temperature of 35℃. All metabolic activities of organisms could be quickly proceed under the participation of enzyme, whose activity might be easily influenced by variety of the temperature [16], owing to that enzyme is protein in chemical nature so that any conditions which change protein's character can lead to enzyme's activity declined or lost. Furthermore, different kinds of enzyme have its optimum temperature. Enzyme's activity will be subdued and even irreversible damage when the test temperature is above or below the optimum temperature. Rezania et al. [36] reported that denitrification rate increased as temperature increased from 12 to 25℃. Zhou et al. [37] suggested that the suitable temperature range was 30–35℃. Karanasios et al. showed that the temperature values applied in studies on hydrogenotrophic denitrification varied between 10 and 30℃ [13]. Kurt et al. [16] proved that temperature affected the denitrification process by affecting bacteria behavior. In the present study, the optimum temperature for the enzyme of the bacteria in the reactor was 35℃ which was in accordance with previous researches, higher or lower than 35℃ could arouse decrease of the denitrification rate.

3.4. Denitrification effect under different nitrate loading

It is apparent from Fig. 5 that the effect of nitrate loading on nitrate reduction. The each operating nitrate loading were 23, 70, 105, and 155 mg NO_3^--N L^{-1} in four airtight flasks. As such, applying sludge 100 mL, temperature 35℃, C/N 30, and pH 7.0.

As shown in Fig. 5, the average nitrate degradation rate increased from 6.31 to 22.44 mg NO_3^--N L^{-1} h^{-1} by the increase of initial nitrate loading from 23 to 105 mg NO_3^--N L^{-1}. When nitrate loading was further increased to 150 mg NO_3^--N L^{-1}, denitrification rate decreased to 20.02 mg NO_3^--N L^{-1}·h^{-1}, also the accumulated nitrite level reached 9.85 mg NO_2^--N L^{-1} under this environment. Furthermore, the concentrations of NH_4^+-N in the reactor was always closed to zero.

It was illustrated that the reactor could perform well if initial nitrate loading was less than 105 mg NO_3^--N L^{-1}. When nitrate loading was higher than 105 mg NO_3^--N L^{-1}, denitrification was suppressed due to the fact that high nitrate inhibited the activity of reductase so that the denitrification process was blocked. This results was accordance with some similar researches such as Vasiliadou's [38] experiment. Vasiliadou et al. [38] also reported that nitrite might be accumulated during the autohydrogenotrophic denitrification process and the highest accumulated nitrite level was less than 10 mg NO_2^--N L^{-1}. Park et al. [39] showed that the nitrate removal rate increased as the initial nitrate loading increased, while nitrite accumulation was observed. Similar results were observed by Park et al. [40] with the initial nitrate concentration ranged from 20 to 150 mg NO_3^--N L^{-1}. In the present experiment, the highest nitrite concentration was 9.85 mg NO_2^--N L^{-1} at nitrate loading of 150 mg NO_3^--N L^{-1}, the reason was that bacteria's lower activity caused incomplete denitrification reaction.

3.5. Denitrification effect under different C/N

Fig. 6 shows the various nitrate reduction curve of different C/N, and Fig. 7 shows the variation of pH before and after denitrification reaction in the reactor at different C/N conditions. The operating C/N was 15, 30, 45, and 60 in four airtight flasks. As such, applying sludge 100 mL, temperature 35℃, nitrate loading 30 mg NO_3^--N L^{-1}, and pH 7.0.

It can be seen from Fig. 6 that there were almost the same average denitrification rates under different C/N conditions. Nitrate could be completely converted to N_2 in 5.0 h and nitrite was not detected at C/N varied from 15 to 60. Also, ammonia was not detected during the test. As shown in Fig. 7, pH values were higher than the initial state after the

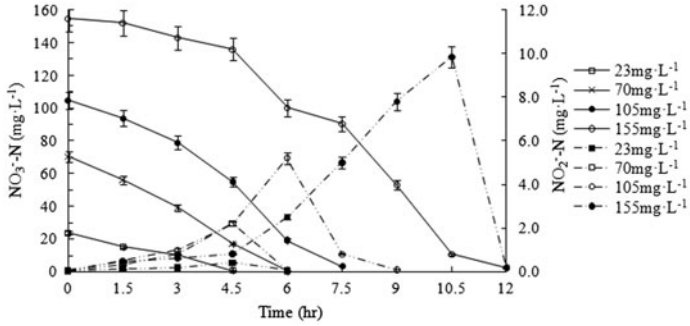

Fig. 5. Concentrations of NO_3^--N and NO_2^--N in the reactor at different nitrate loading conditions (sludge 100 mL, temperature 35°C, C/N 30, pH 7.0).

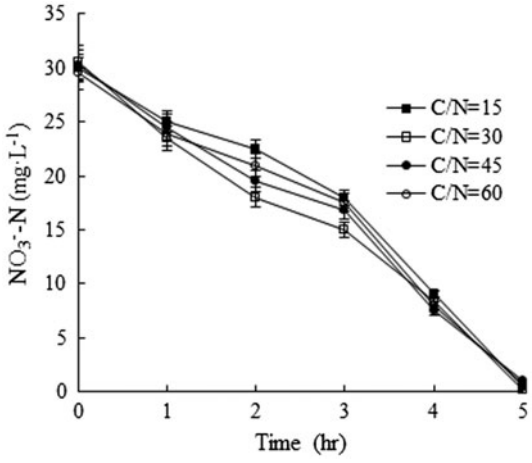

Fig. 6. Concentration of NO_3^--N in the reactor at different C/N conditions (sludge 100 mL, temperature 35°C, nitrate loading 30 mg NO_3^--N L^{-1}, pH 7.0).

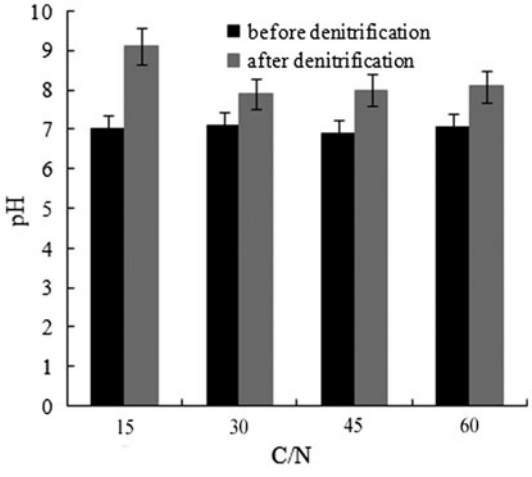

Fig. 7. Various pH values in the reactor at different C/N conditions (sludge 100 mL, temperature 35°C, nitrate loading 30 mg NO_3^--N L^{-1}).

denitrification reaction, especially at C/N = 15 (pH was increased to 9.0).

Cheng and Lin [41] defined the theoretical stoichiometric equations for denitrification, the theoretical C/N ratios were established as 0.71. Gómez et al. [42] reported that the most optimal C/N ratio was 1.1 for denitrification. Fan et al. [43] proved that optimum denitrification was attained at C/N = 2.2. In the present study, the optimal C/N for the reactor was 30, which was different from these previous studies probably because the characteristics of these autohydrogenotrophic denitrifying bacteria were different from conventional denitrifying bacteria. Although C/N value was not directly influence the nitrate reduction rate, it could contribute to increase pH value, which caused by increasing alkalinity (alkalinity increased from 140 to 185, 153, 160, 171 mg $CaCO_3$ L^{-1} after denitrification reaction) during the experiment in order to inhibit the reductase's activity to hinder the denitrification process [13]. Otherwise, more carbon source would increase costs and even make waste. Therefore, the best C/N for the reactor was 30.

Biological processes were cost effective, and showed high stability and reliability compared to physic-chemical technologies (ion exchange, reverse osmosis, electro-dialysis, etc.) for the elimination of nitrate [11,44]. The main costs of the autohydrogenotrophic denitrification process were organic carbon amount and hydrogen amount. Therefore, the key to minimizing the costs was to reasonably reduce the amounts of carbon and hydrogen, while guaranteeing high efficiency of the denitrification rate through large amounts of experiments.

4. Conclusions

The aim of this work was to investigate the effects of important parameters like biomass, pH, temperature, nitrate loading, C/N on autohydrogenotrophic

denitrification in a bioreactor. The results showed that the highest average denitrification rate was 37.89 mg NO_3^--N L^{-1}·h^{-1} at OD_{600} of 0.173. The optimum pH and temperature for the reactor were 6.0–7.0 and 35°C, also higher or lower than the value aroused decrease of the denitrification rate. The reactor could perform well if initial nitrate loading was less than 105 mg NO_3^--N L^{-1}. When nitrate loading was higher than 105 mg NO_3^--N L^{-1}, nitrite was accumulated and then denitrification was suppressed. There were almost the same average denitrification rates at C/N varied from 15 to 60.

Acknowledgements

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