Journal of Hazardous Materials 172 (2009) 595–600

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Biological denitrification of high-nitrate wastewater in a modified anoxic/oxic-membrane bioreactor (A/O-MBR)

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ARTICLE INFO

Article history:
Received 18 May 2009
Received in revised form 10 July 2009
Accepted 10 July 2009
Available online 18 July 2009

Keywords:
Denitrificationn
High strength nitrate waste
Membrane bioreactor
Nitrite accumulation

ABSTRACT

A modified anoxic/oxic-membrane bioreactor has been applied to the denitrification of a high strength nitrate waste (about 3600 mg/L nitrate-N) generated from an initiating explosive factory. Nitrate removal efficiency and nitrite accumulation in the treated water were investigated under various conditions set by several factors including the type of carbon source used, ratios of carbon to nitrogen, pH and hydraulic retention times (HRTs). The results of the preliminary experiments, which were carried out in parallel CSTR systems, demonstrated that sodium acetate had shown the best performance as the external carbon source. The optimal reaction parameters in the anoxic/oxic-membrane bioreactor were pH 7.5–8.5, C/N 1.56 and HRT 30 h, with over 99.9% of nitrate removed and without accumulation of nitrite. Explicitly high average-specific denitrification rate of 324 mg NO_3^--N/g VSS/h could be attained under these conditions. The aerobic process and membrane module used subsequently could remove the residual COD, excessive biomass and soluble microbial products generated during the denitrification process.

1. Introduction

Water is of fundamental importance for life since the mechanism of metabolism and synthesis are in close relation with the specific characteristics of water [1]. However, various contaminants are introduced into the water environment because of the fast movement of humans towards urbanization, industrialization and agricultural activities. Of these various contaminants, nitrogen-containing compounds such as nitrate can create serious problems, such as eutrophication of rivers, deterioration of water quality and potential hazard to human and animal health. Nitrate is very soluble in water and is easily transported to the groundwater and surface water once discharged. As a result, nitrate contamination of water resources is becoming a serious environmental problem worldwide [2]. To avoid adverse health effects, permissible level for nitrate in drinking water is limited to 44.43 mg/L in USA and 50 mg/L in Europe.

However, conventional processes of water treatment methods including coagulation, filtration and disinfection are not effective for the elimination of nitrate from the water. In order to treat nitrate-contaminated water, supplementary treatment technologies such as chemical, physical and biological methods have been developed. Of these options, physical and chemical methods are usually suitable for low-level nitrate waste and result into secondary wastes and problematic by-products [3]. However, biological denitrification (BD) with no discharge has become popular. Biological denitrification occurs naturally when certain microorganism uses nitrate as terminal electron acceptor in its respiration process, in the absence of oxygen. The process can be described as follows:

$$\text{nitrate}\ (NO_3^-) \Rightarrow \text{nitrate}\ (NO_2^-) \Rightarrow \text{nitric oxide}\ (NO)$$
$$\Rightarrow \text{nitrous oxide}\ (N_2O) \Rightarrow \text{nitrogen}\ (N_2)$$

In the process, transformation of oxidized nitrogen compounds into harmless nitrogen gas occurs, with the accompanying carbon removal. Denitrifying bacteria, which are involved in this process, are ubiquitous in nature. Therefore, the process occurs naturally in the aquifer, the soil and surface water. As the most environmentally friendly and cost-effective method, this process has been intensively studied because it provides a promising chance for real treatment of nitrate waste.

Nitrate wastes containing greater than 1000 mg/L nitrate-N are generated in industries like cellophane, explosives, fertilizer, pectin, nuclear, metals finishing, etc. [4,5]. These high strength nitrate wastewater discharges may cause an alarming increase in the nitrate level of groundwater and water resources [3]. However, knowledge about denitrification of high strength nitrate waste is yet limited [3–6]. There are three main problems in biological denitrification for high-nitrate wastewater. Firstly, the process of biological denitrification is often slow and lasts several days particularly for industrial wastewaters containing high concentration of nitrate [4,6]. Secondly, biological denitrification may be prolonged by

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doi:10.1016/j.jhazmat.2009.07.045

Table 1
Characteristics of raw wastewater from an initiating explosive factory for denitrification.

Parameter	Range	Average
pH	7.5–8.0	7.8
NO_3^--N (mg/L)	3500–3700	3600
NO_2^--N (mg/L)	2–5	3
COD (mg/L)	60–80	70
2,4,6-trinitrophenol (mg/L)	1–5	3
Salinity (%)	3.1–3.3	3.2

Table 2
Running parameters at the different stages of the experiment. Stage 1, start-up period; stage 2, effect of influent C/N ratio; stage 3, effect of pHs; stage 4, effect of HRT; stage 5, performance of the A/O-MBR.

Stage	Carbon sources	HRTs	C/N ratios	pHs
Stage 1	Sodium acetate	44 h	2	8.5
Stage 2	Sodium acetate	44 h	1.25–2	8.5
Stage 3	Sodium acetate	44 h	1.56	6.5–9.5
Stage 4	Sodium acetate	22–89 h	1.56	8.5
Stage 5	Sodium acetate	30 h	1.56	8.5

accumulation of nitrite. Complete nitrate removal may be hampered by nitrite accumulation during the biological denitrification process, because nitrite is an inhibitor of bacterial growth [6,7]. Thirdly, biological denitrification, however, can produce excessive biomass and soluble microbial products which require subsequent treatment, especially when heterotrophic bacteria are used [1].

In recent years, in order to optimize the biological denitrification process, many different types of denitrification reactors have been used, such as membrane bioreactors [8], submerged filters [9] and fixed-film reactors [10]. Among them, membrane bioreactors (MBRs in short) have attracted considerable interest, because the separation devices can solve some of problems associated with traditional biological denitrification processes, such as excessive biomass and soluble microbial products in the treated effluent [10,11]. With the microfiltration or ultrafiltration units, MBRs may overcome the inconvenience of microbiological contamination of treated water, reducing the post-treatment steps. In addition, the entire biomass is confined within the system, providing exact control of the residence time for the microorganisms in the reactor (solids retention time) [12]. However, membrane bioreactors have been limited to the denitrification of drinking water [8,11,13,14]. Reports about the denitrification of high strength industrial nitrate waste using membrane bioreactor are rare [15].

In this study, a bench-scale continuous flow anoxic/oxic-membrane bioreactor (A/O-MBR) has been developed for biological removal of high strength nitrate waste from an explosive industry. The influence of operational parameters, such as the type of the external carbon sources, ratios of carbon to nitrogen, reaction pH, and hydraulic retention times (HRTs), was investigated.

2. Materials and methods

2.1. Characteristics of denitrifying culture and raw wastewater from an initiating explosive factory

The feed wastewater was taken from a wastewater pilot plant of an initiating explosive factory. The wastewater was pretreated by a combined process of chemical precipitation and biological aerated filter in the wastewater pilot plant, with the high strength nitrate remained. Details of the wastewater after pretreatment were included in Table 1. The aim of this paper was to denitrify this high strength nitrate waste. In addition, phosphate was supplied to avoid nutrient limitations (0.022 P/COD mass ratio). Sodium acetate or other carbon sources were added as external carbon source to give certain C/N molar ratio.

The seed sludge for denitrification was collected from the anaerobic sludge of a pharmaceutical industry wastewater treatment plant. The fresh sludge was incubated and acclimatized to the diluted industrial wastewater in a 20 L sequencing batch reactor, using sodium acetate as the external carbon source (C/N molar ratio = 2.0). The industrial wastewater was diluted by the tap water. The proportion of the industrial wastewater increased step by step from about 20% to finally 100%, at an interval of 20%. 1 month later, the acclimatized denitrification sludge was added into the anoxic tank of the A/O-MBR. Aerobic sludge taken from a municipal wastewater treatment plant was added into the aerobic tank of the A/O-MBR directly without acclimatization.

2.2. Experiment equipment and operating conditions

Fig. 1 shows the schematic diagram of the bench-scale modified anoxic/oxic-membrane bioreactor. The bioreactor was designed in two parts: an anoxic tank where nitrate was biologically reduced to nitrogen, followed by an aerobic tank for excessive carbon removal. The effective volumes of the anoxic and aerobic compartment were 10 and 26 L, respectively. The polyvinylidene fluoride (PVDF) hollow-fiber membrane module (Tianjin Motianmo, China) was installed vertically in the aerobic zone, with pore size of 0.2 μm and filtration area of 1 m^2.

A peristaltic pump drove the flow of wastewater through the reactor, with influent entering at the base of the anoxic tank. The effluent of the anoxic tank entered the aerobic tank through a gravity drain. Air was continuously supplied from an aeration pipe located directly below the membrane module for biomass growth and scouring the membrane surface. The sludge of the anoxic tank was mixed by a submersible pump. The mixed liquor was pumped through the membrane tube and the permeation was collected from the membrane tube. The membrane unit timely controlled the suction process or relaxation process (no permeate extraction) by a control panel. The recirculation was turned off and the effluent recirculation rate was zero. To keep the cultivation temperature stable at 30 °C, a thermostatic bath was used. The recirculated stream was previously heated in the thermostatic bath, and driven by a submersible pump. The pH of the reaction mixture was monitored and kept constant through probes linked to the controller, with the addition of H_2SO_4 solution.

2.3. Experimental procedure

Before our study could be carried out, it was necessary to investigate the effect of different carbon sources (sodium acetate, methanol and glucose) on the denitrification process. Three parallel continuous stirred tank reactors (CSTR in short, the working volume was 5 L, inoculated with the acclimated sludge) were used, operating for the same time and under the same conditions, except for the external carbon source used. The initial denitrification sludge concentrations were 2.0 g VSS/L. This procedure consisted of a batch culture lasting 1 day. 1 day later, the continuous flow was started under the conditions of pH = 8.5, HRT = 44 h and C/N molar ratio = 2. The nitrate and nitrite concentrations of the effluent were monitored. The external carbon source which showed the best denitrification performance was chosen for the subsequent stage of research.

Thereafter, the study aiming to achieve the optimal operation conditions of the anoxic/oxic-membrane bioreactor was carried out. Running conditions involving a combination of the parameters were shown in Table 2. The initial anoxic and aerobic sludge concentrations were 2.0 and 0.5 g VSS/L, respectively. The test started as a batch and when nitrate was completely reduced, continuous flow of feed solution was started (corresponding to the stage 1 in

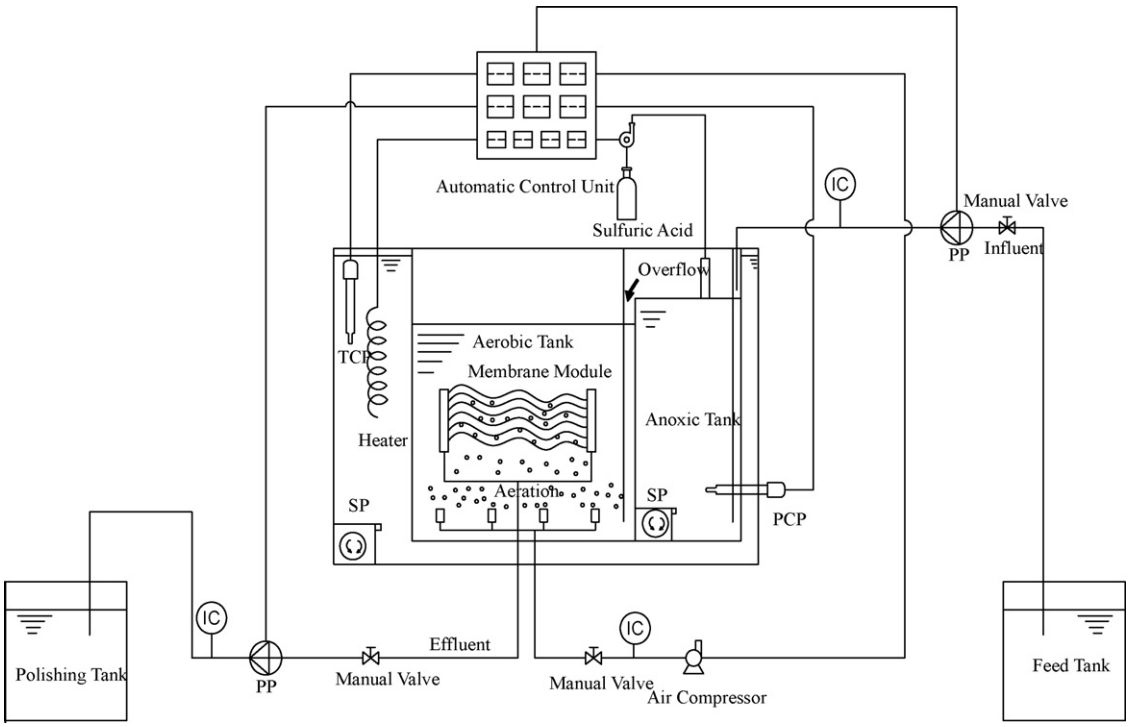

Fig. 1. Schematic representation of the experimental setup. PCP, pH control probe; TCP, temperature control probe; PP, peristaltic pump; SP, submersible pump; IC, rotameter or water flowmeter.

Table 2). 10 days later, the concentration of nitrate and nitrite in the effluent had stabilized, indicating that the steady state conditions achieved. Then, three groups of experiments were performed at different C/N molar ratios (stage 2 in Table 2), pHs (stage 3 in Table 2), and HRTs (stage 4 in Table 2). The system was kept running under each condition set by the above parameters for 6 or 7 days, due to its reaching steady state in 3 days. At last, the modified A/O-MBR was operated under the optimal conditions for about 2 months, in order to investigate the performance of the A/O-MBR system for the denitrification of this high strength nitrate waste.

2.4. Analytical methods

Wastewater parameters, including COD, NO_3^--N, NO_2^--N, were analyzed according to China NEPA standard methods (1997). The total-N was based on the sum of NO_3^--N and NO_2^--N, rather than an independent TN test. The volatile suspend solids (VSS) were measured by calculating the weight loss of sludge between drying 24 h at 105 °C and burning 2 h at 550 °C. Total bacterial counts were performed on R2A agar at 20 °C for 7 days. The turbidity was measured using a turbidimeter (WGZ-200, Shanke, China).

3. Results and discussion

3.1. Batch test: effect of different external carbon sources on denitrification process

External carbon sources play an important role in the denitrification process. The effectiveness of three different carbon sources (methanol, sodium acetate and glucose) for the removal of high strength nitrate waste from an initiating explosive factory, was studied.

In the reactor using sodium acetate as the external carbon source, the residual nitrate-N concentrations in the effluent were well below 3 mg/L, with the removal rates always above 99.9%, except for the first day (batch culture mode). Nitrite concentrations in the treated effluent, which represented an undesired by-product due to its direct toxicity, were not remarkable (Fig. 2). The high efficiency of acetate was also observed by Reyes-Avila et al. [16]. In the anaerobic continuous stirred tank reactor using acetate as the carbon and energy source, the nitrate consumption efficiency remained constant and close to 100%. However, in the reactor using glucose as an external carbon source, as high as 2100 mg/L of nitrite-N was accumulated in the reactor (Fig. 2), probably because the biocommunity was enriched for facultative anaerobes reducing nitrate only to nitrite at the expense of true denitrifiers, causing decreased nitrite reduction rates and thus nitrite accumulation [17,18]. Nitrate and nitrite profiles confirmed that when methanol was used as external carbon source, no significant denitrification

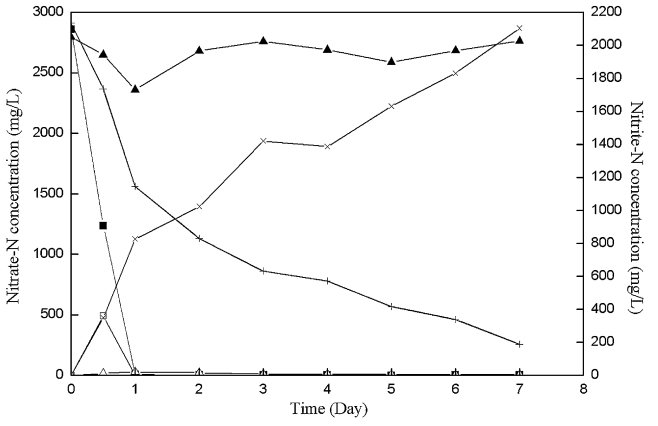

Fig. 2. Continuous denitrification of the explosive wastewater using different external carbon sources. (■) nitrate-N concentration using sodium acetate as external carbon source; (□) Nitrite-N concentration using sodium acetate as external carbon source; (+) nitrate-N concentration using glucose as external carbon source; (×) nitrite-N concentration using glucose as external carbon source; (▲) nitrate-N concentration using methanol as external carbon source; (△) nitrite-N concentration using methanol as external carbon source.

occurred, as shown in Fig. 2. This was very likely due to the toxicity of excess methanol towards the microorganisms, especially at high concentrations.

In addition to the heterotrophic denitrification (HD), autotrophic denitrification (AD), based on an inorganic carbon source, involves sulfur or hydrogen gas as the electron donor for the bacterial metabolic chain [16,19,20]. However, the heterotrophic denitrification is thought to be a high-rate process [6,16,21]. Reyes-Avila et al. [16] developed an anaerobic continuous stirred tank reactor for the simultaneous biological removal of nitrogen, carbon and sulfur by denitrification. In this system, sulfide drove autotrophic denitrification (AD) at a rate over two orders of magnitude less than acetate (HD). The presence of acetate in addition to sulfide increased both sulfide oxidation and denitrification rate by roughly 55% and an order of magnitude, respectively.

The results indicated that sodium acetate was the optimal external carbon source for the denitrification of this high strength nitrate waste (3600 mg/L nitrate-N). In fact, for the denitrification of high strength nitrate waste, sodium acetate was most widely used [3–6]. In the subsequent stage of the research, sodium acetate was used as the external carbon source throughout the study.

3.2. Continuous flow A/O-MBR reactor

3.2.1. Start-up of the A/O-MBR reactor

The start-up process started as a batch. When nitrate was completely removed, the influent and effluent run continuously and the denitrification process started (corresponding to the stage 1 in Table 2). The nitrate, nitrite concentration was measured in effluent solution of the anoxic tank and the biomass concentration (g VSS/L) was measured in the anoxic tank. However, the biomass washout was obvious, the VSS in the anoxic tank decreased from 2 to 0.51 g/L, and after the sixth day reached steady state. Nevertheless, our results showed that the biomass washout did not affect nitrate removal. During the start-up process, the nitrate was completely removed in the reactor and nitrite accumulation was not observed. Correspondingly, average-specific denitrification rates increased from 40 to 160 mg NO_3^--N/g VSS/h at the HRT of 44 h, with the decrease of the VSS.

3.2.2. Effect of influent C/N ratio on denitrification process

In order to determine the optimal C/N ratio, the amount of sodium acetate decreased step-wise (from C/N molar ratio of 2.0 to 1.25). The theoretical stoichiometric equation for the denitrification with sodium acetate as carbon source was as follows [4]:

$$0.625CH_3COO^- + 1NO_3^- + 0.375H^+ \rightarrow 1.25HCO_3^- + 0.5N_2 + 0.5H_2O \quad (1)$$

The theoretical C/N molar ratio for denitrification using sodium acetate as external carbon sources was established as 1.25. However, the C/N of 1.25 was found to be insufficient for the bacteria to grow, accompanied by incomplete denitrification and accumulation of nitrite (Fig. 3). The practical C/N ratios were higher than the theoretical one because some carbon was used in new biomass formation.

In this study, when C/N molar ratio was set at 1.25 (within 'carbon-limited' conditions), the nitrite-N concentration of 949 mg/L was recorded, corresponding to 26% of the initial nitrate concentration (Fig. 3). The same phenomenon of nitrite accumulation when carbon source was not enough was also observed by other researchers [10,11]. Nitrate was only reduced to nitrite which brought to nitrite accumulation. Once operating under the conditions of C/N ≥ 1.56, however, the impact of C/N ratios on nitrate and nitrite removal was negligible and the total-N concentrations were well below 4 mg/L (Fig. 3). At this stage, sodium acetate was

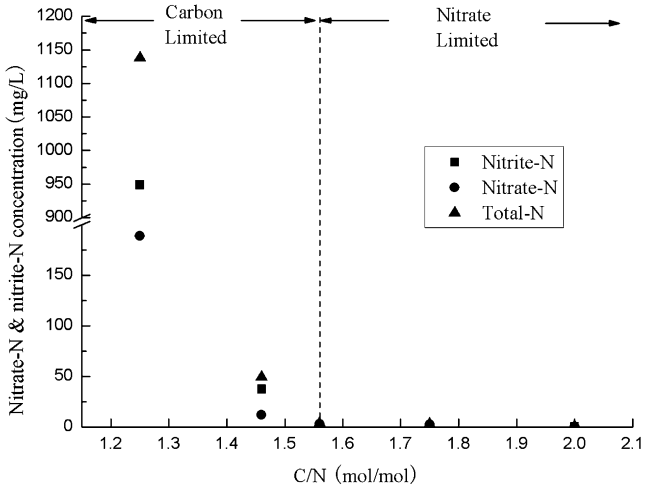

Fig. 3. Continuous denitrification of the explosive wastewater with different C/N molar ratios at influent nitrate-N concentration of about 3600 mg/L.

in excess. This stage can be described as nitrate-limited conditions [11]. However, nitrite in effluent increased from 0.78 to 37.5 mg/L, when C/N decreased from 1.56 to 1.46. Correspondingly, the concentration of nitrate in effluent increased to 11.9 mg/L.

The aim of this study, was to reach an effective nitrate removal strategy without the accumulation of nitrite. When the C/N ratio was set at 1.56, an optimum was found. At this dosing point, the sum of the nitrate and the nitrite concentration was rather low. In addition, the dosing of sodium acetate was minimized, with relatively low feed cost.

For the denitrification of high strength nitrate waste (greater than 1000 mg/L nitrate-N), C/N molar ratios ranged from 1.46 to 2, with acetate as carbon source [3,5,22]. While in the present study, it was suggested that optimum C/N molar ratios was 1.56. It was postulated that variation in C/N ratio corresponded to metabolic pathway diversity, although this relationship could also differ with competing physical parameters [11].

3.2.3. Effect of pHs on denitrification process

The values of pH play an important role in the denitrification process. In order to investigate the effect of pH values on denitrification, different pH conditions for denitrification were tested. As presented in Fig. 4, at high pH level (pH of 9.5), nitrite accumulation

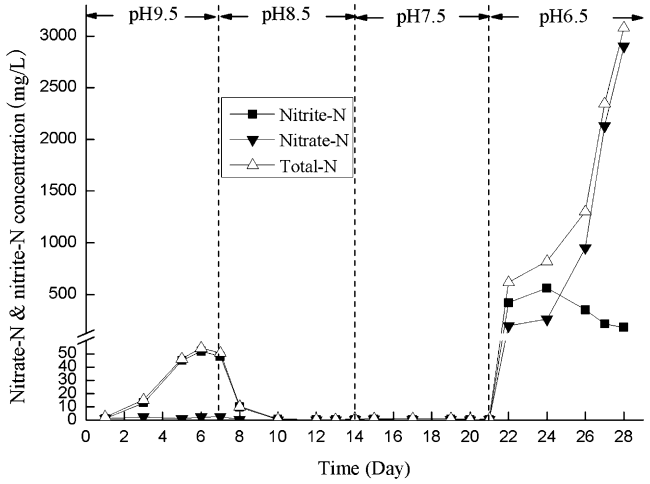

Fig. 4. Continuous denitrification of the explosive wastewater under different pH conditions at influent nitrate-N concentration of about 3600 mg/L.

Table 3
Effluent turbidity, effluent COD, effluent nitrate, effluent nitrite and effluent total bacteria counts.

Operation time (day)	VSS (g/L)	Turbidity (NTU)	Total bacterial counts (CFU/ml)	COD (mg/L)	Nitrate-N (mg/L)	Nitrite-N (mg/L)
7	0.36	0.7	23	108	0.69	0.43
14	0.38	0.8	15	112	0.87	0.25
21	0.35	0.3	36	89	0.53	n.d.
28	0.41	0.5	12	103	n.d.	0.38
35	0.35	0.6	15	120	0.25	0.19
42	0.34	0.9	13	98	n.d.	n.d.
49	0.38	0.6	24	76	0.66	0.41
56	0.36	0.7	18	102	0.32	0.34

n.d.: not determined.

was observed. The nitrite-N peak concentration in the anoxic tank was observed to be 52 mg/L at pH 9.5. However, this nitrite accumulation was not remarkable compared with the influent nitrate concentration (about 3600 mg/L nitrate-N). At pH of 7.5 and 8.5, complete denitrification occurred without significant nitrite accumulation (Fig. 4). However, when pH decreased to 6.5, the nitrite-N and nitrate-N peak concentration was as high as 560 and 2900 mg/L, respectively. On day 28, total-N in the anoxic tank was as high as 3080, denitrification was severely inhibited (Fig. 4).

The results illustrated that denitrification effect was excellent when pH was neutral and alkalescent, as was reported by other researchers [4,6,10,23]. Acidic and alkaline conditions were not fit for denitrification. Glass and Silverstein [4] found that at pH of 6.5 and 7.0, no significant denitrification occurred for the denitrification of 1350 mg/L nitrate-N. Poor performance of denitrification at acid conditions was also observed by Wang et al. [10]. At alkaline condition, nitrite reductase was repressed due to simultaneous lack of protons in the periplasmic space and competition for the flow of electrons to nitrate reductase [4]. As a result, nitrite accumulation occurred and too much nitrite accumulation would bring toxicity to microorganism.

As presented in Fig. 4, denitrification of the wastewater was optimal at pH of 7.5–8.5, which was chosen as optimal parameter for further investigation.

3.2.4. Effect of HRTs on denitrification process

It is very important to determine the appropriate HRT for the reactor because the performance of denitrification is associated with HRT obviously. The efficiency would decrease and the construction cost would increase if HRT was too long.

In Fig. 5, the variations of the concentration in nitrate and nitrite at different HRTs are shown. The dinitrification was almost complete during all the phases, and there was no nitrite accumulation in the reactor, except at the HRT of 22 h. The concentration of nitrate-N in effluent was always below 2.0 mg/L with the denitrification efficiency more than 99.9%. There was a transient nitrate accumulation due to the new flow rate corresponding to the HRT of 30 h. But 2 days later, the denitrification was complete again. At the HRT of 22 h, when the accumulation was greater, the denitrification started to be incomplete (Fig. 5). The results indicated that, under the conditions of C/N molar ratio of 1.56 and pH of 8.5, the appropriate HRT was 30 h with sodium acetate as external carbon source.

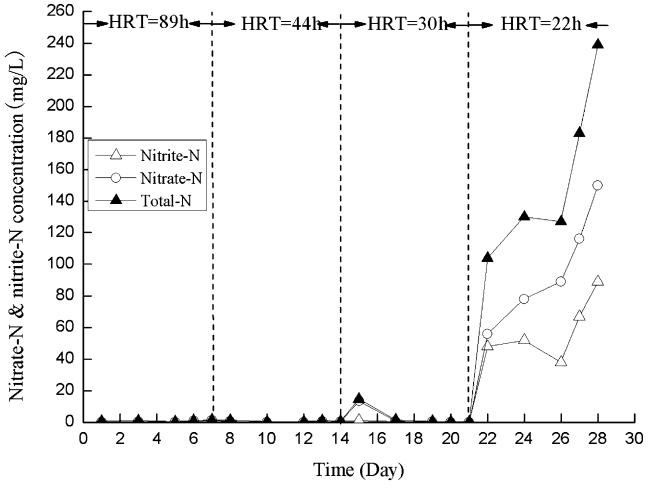

Fig. 5. Continuous denitrification of the explosive wastewater under different HRT conditions at influent nitrate-N concentration of about 3600 mg/L.

With the decrease of HRT, the volumetric nitrate loading rates increased. When HRT decreased from 89 to 30 h, the volumetric nitrate loading rates increased from 1 to 3 g NO_3^--N/L/day. Under the loading conditions of 1–3 g NO_3^--N/L/day (HRT = 30–89 h), the volumetric nitrate removal rates essentially equalled to the volumetric nitrate loading rates, with removal efficiencies close to 100%. It was indicated that the influent nitrate loading did not influence nitrate removal under the load conditions of 1–3 g NO_3^--N/L/day.

For the autotrophic denitrification (AD), the reported volumetric nitrate removal rates were in the range of 0.006–0.5 g NO_3^--N/L/day [19], the value of 1–3 g NO_3^--N/L/day was high compared with the published values, indicating the high efficiency of the heterotrophic denitrification.

3.2.5. Performance of the modified anoxic/oxic-membrane bioreactor

In this study, the aerobic and the membrane separation processes after the denitrification process were used to remove the residual COD, excessive biomass and soluble microbial products. Under the optimal operation conditions (pH = 8.5, C/N molar ratio = 1.56 and HRT = 30 h), the performance of the hybrid system was investigated in terms of the VSS concentration of the anoxic tank, the effluent COD, the effluent nitrate concentration, the effluent turbidity and the effluent total bacterial counts (Table 3).

Based on the data presented in Table 3, the polyvinylidene fluoride (PVDF) hollow-fiber membrane used in this study was an effective barrier for preventing microbial contamination of the reactor effluent. The average total bacterial count was as low as 19.5 (±8.0) CFU/mL and the turbidity was well below 1 NTU (average turbidity was 0.64 (±0.18) NTU). In addition, the average COD concentration in the permeate was 101 (±13.7) mg/L, which was lower than the requirement of 150 mg/L stipulated by Discharge Standard for Water Pollutants from Ordnance Industry (GB 14470.2-2002, China). The nitrate-N and the nitrite-N concentrations were always below 1 and 0.5 mg/L, respectively.

The VSS concentration of the anoxic tank was 0.37 (±0.02) g/L at this stage, with the average-specific denitrification rate as high as 324 mg NO_3^--N/g VSS h. However, the specific denitrification rates reported in literature, were in the range of 10–142 mg NO_3^--N/g VSS h [6], the value of 324 mg NO_3^--N/g VSS h was higher than the published values. The obtained results indicated to the superiority of the acclimated culture and additional carbon sources (sodium acetate) for the denitrification at high load.

It could be concluded that under the optimal operation conditions, the performance of the modified anoxic/oxic-membrane bioreactor was excellent, with low residual COD concentration and with complete removal of nitrate waste. Microbial contamination of the effluent was not remarkable in the presence of the membrane module.

4. Conclusions

The paper describes a modified anoxic/oxic-membrane bioreactor for the treatment of a high strength nitrate waste. The following conclusions were extracted from the study.

Sodium acetate had shown the best performance as an external carbon source, compared with glucose and methanol.

Under the running conditions of C/N molar ratio = 1.56, pH = 7.5–8.5 and HRT = 30 h, nitrate-N removal for this high strength nitrate waste was almost complete, with the average-specific denitrification rate as high as 324 mg NO_3^--N/g VSS h and without nitrite-N accumulated.

The aerobic process and membrane module used subsequently could remove the residual COD, excessive biomass and soluble microbial products generated in the denitrification process.

The modified anoxic/oxic-membrane bioreactor offers bright prospects for the treatment of high strength nitrate waste.

Acknowledgements

This research is financed by Foundational Research Program of the Civilian Blasting and Research Innovation Grant for Graduate of Common High School of Jiangsu Province (AD20246).

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