ISSN:2790-1688 DOI: 10.56028/aetr.1.1.42

Denitrification performance and microbial communities of solid-phase denitrifying reactors using novel composite

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Abstract. This study explored the denitrification performance of solid-phase denitrification reactors packed with elemental sulfur/wheat straw/shell powder composites to treat nitrate-contaminated wastewater under different conditions. The sulfur-based autotrophic denitrification integrated natural-carbon-source (NCS)-based heterotrophic denitrification system was formed (SND). The fast start-up and intensified nitrogen removal performance were obtained in SND systems. The optimum denitrification performance was achieved by the SND15 reactor, with a maximum denitrification rate of 530 mg N·L-1·d-1 over 180 days operation. The co-existence of autotrophs and heterotrophs were observed in all reactors, this may contribute to better control of nitrogen in SND system. The outcomes provide a potential strategy of cost reduction to improve nitrogen removal of wastewater treatment plants (WWTPs) effluent.

Keywords: Autotrophs; Heterotrophs; Natural-carbon-source-based heterotrophic denitrification; Sulfur-based autotrophic denitrification.

1. Introduction

Nowadays, nitrate contamination in surface and ground water has become a serious worldwide issue, and the intensive use of fertilizer is the main source of nitrate contamination [1]. The accumulation of nitrate has many adverse impacts on ecological environment and human health because it can cause algae bloom and methemoglobinemia. Thus, efficient technologies to treat nitrate-contaminated wastewater are urgently needed.

Various methods have been proposed to remove nitrate from sewage, including electrodialysis, ion exchange, distillation, and biological processes. Compared to physicochemical processes, biological denitrification is a cost-effective method and widely used in sewage treatment plants to treat nitrate-polluted wastewater, and heterotrophic denitrification (HD) is the preferred biological method due to its favorable denitrification rate [2]. Nevertheless, water-soluble organic carbon is generally required for denitrification due to low carbon/nitrate (C/N) ratio of nitrate-polluted wastewater, which complicates this process and increases the operation costs.

The packed bed reactor to treat nitrate-contaminated wastewater has attracted the attention of many researchers in recent years, because it is easier to operate with low cost [2, 3]. The elemental sulfur-based autotrophic denitrification (ESAD) process is considered as a suitable process, as it has lower operating expense and decreasing the generation of sludge [4], and natural carbon sources (NCS) also have good denitrification capacity [5]. Nevertheless, few researchers focused on the ESAD/NCS composite filter e.g., the effects of influent nitrogen loading rates (NLR) on denitrification capacity of composite filter, the optimization of the chemical composition of composite filter, were not systemically explored.

Hence, the overarching goal of this research was to provide a robustness alternative for nitrate-contaminated wastewater treatment through solid-phase denitrification process. In this paper, a variety of NCS-sulfur based composite filters (NSCFs) were investigated in packed bed reactors. The goals of this paper were: 1) to comparatively assess the denitrification ability of different types of NSCFs; 2) to identify the variations in microbial communities of NSCF. The findings of this study would be useful to the solid-phase denitrification process.

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2. Materials and methods

2.1 The preparation of composite filters and experimental procedure

Three types of NSCFs (F1-F3) were prepared in this study. The elemental sulfur powder blended with shell powder and wheat straw powder according to the weight ratios of 6:3.5:0.5, 6:3:1, and 6:2.5:1.5, were respectively for F1, F2, and F3. The prepared F1, F2, and F3 were respectively stirred under 150°C-170°C, and the generated molten material was shaped in the mold to make oval-shaped composites. The SND5, SND10, and SND15 reactors were respectively filled the same weight (1 kg) of F1, F2, and F3 and operated under different NLRs for 180 days (Table 1). The reactors were made of plexiglass with effective working height of 360 mm and diameter of 60 mm (working volume 1.02 L). The prepared NSCFs were oval form with width of 10 mm and length of 15 mm on average.

Table 1. Influent parameters of SND_5-SND_{15} reactors.

Periods	1	2	3	4	5
Days	0-15	16-30	31-45	46-90	91-180
HRT (h)	6	3	2	1	0.5
NLR (mg $N\cdot L^{-1}\cdot d^{-1}$)	120	240	360	720	1440
NO_3^--N (mg L^{-1})	30				
Influent DO (mg L^{-1})	< 0.1				

Both reactors were fed with synthetic wastewater prepared with tap water supplemented with NaNO3 agent. The seed sludge obtained from the Fairy River Sewage Treatment Plant was selected as the inoculum (Shenyang, China). In SND5-SND15 reactors, each reactor was inoculated with 150 mL seed sludge (with mixed liquor suspended solids (MLSS) of about 4.3 g L−1), and then operated for 2 h in internal circulation mode at a flow rate of 10 L h−1 to ensure seed sludge was evenly attached onto the filters before continuous influent experiments. The influent DO was <0.1 mg L−1 to ensure anaerobic condition by using nitrogen gas, and the temperature of the water was maintained at 29 ± 2 °C throughout the study. The hydraulic retention times (HRT) were calculated considering the empty bed volume.

2.2 Analytical methods and DNA extraction and Illumina MiSeq sequencing

Water samples of inlet and outlet were daily tested. The samples were filtered using 0.45 μm membrane filters and analyzed for ammonium, chemical oxygen demand (COD), MLSS, mixed liquid volatile suspended solids (MLVSS), nitrite, nitrate, alkalinity, and sulfate according to standard methods [6]. DO and pH were detected with a digital DO probe (JPB-607A, LeiCi, China) and a digital pH probe (PH-700, YouTe, China), respectively.

In order to examine the distribution characteristics of microbial community under various conditions, bio-samples on the surface of NSCFs were collected from reactors, respectively. A sample was collected from the top area of reactors on day 180. The DNA extraction was performed using a PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA). The primers 338F (5’-ACTCCTACGGGAGGCAGCAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’) were used to target the V3-V4 hypervariable regions of the bacteria 16S rRNA gene, and according

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to the standard protocols (Majorbio, Shanghai, China), all the purified amplicons were pooled in equimolar, and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, USA).

3. Results and Discussion

3.1 Performance of lab-scale bioreactors

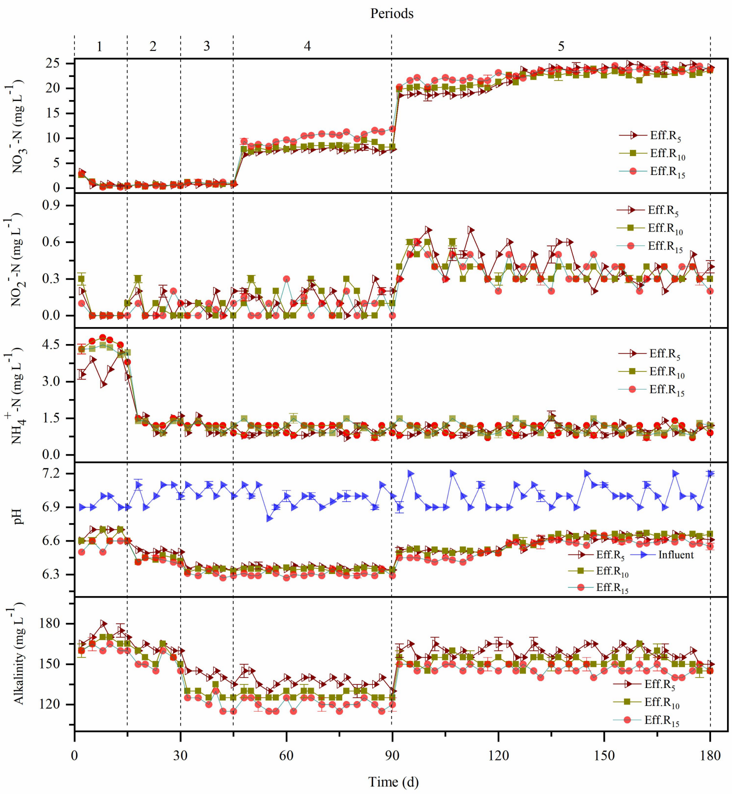

Fig. 1. The performance of the SND5-SND15 reactors throughout the study.

The performance of the SND5-SND15 reactors is depicted in Fig. 1. In all reactors, the nitrate was almost disappeared within three days. This indicates that the NSCFs could fast start-up with promising denitrification performance. All three reactors achieved almost complete denitrification until period 4. During period 4, the average nitrate removal efficiency (NRE) of SND5, SND10, and SND15 reactors was 64.5 ± 3.1%, 61.9 ± 3.3%, and 74.1 ± 2.8%, respectively. The higher NRE in SND15 than that in SND5 and SND10 may be caused by a relatively high COD concentration. Taking the relatively high denitrification rate into account, the higher content of NCS of NSCF may lead to enhanced denitrification effect. However, the NRE of all reactors showed a decreasing trend during the rest days of period 5 (days 91-180), with the average NRE of SND5, SND10, and SND15 reactors was 19.5 ± 3.7%, 23.8 ± 2.6%, and 31.1 ± 3.9%, respectively. The optimum denitrification performance was achieved by the SND15 reactor, with a maximum denitrification rate of 530 mg N·L-1·d-1 over 180 days operation. In general, results from our study showed that the F3 filter in reactor SND15 can potentially intensify the effluent water quality than F1 and F2 filters.

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3.2 Evolution of microbial community

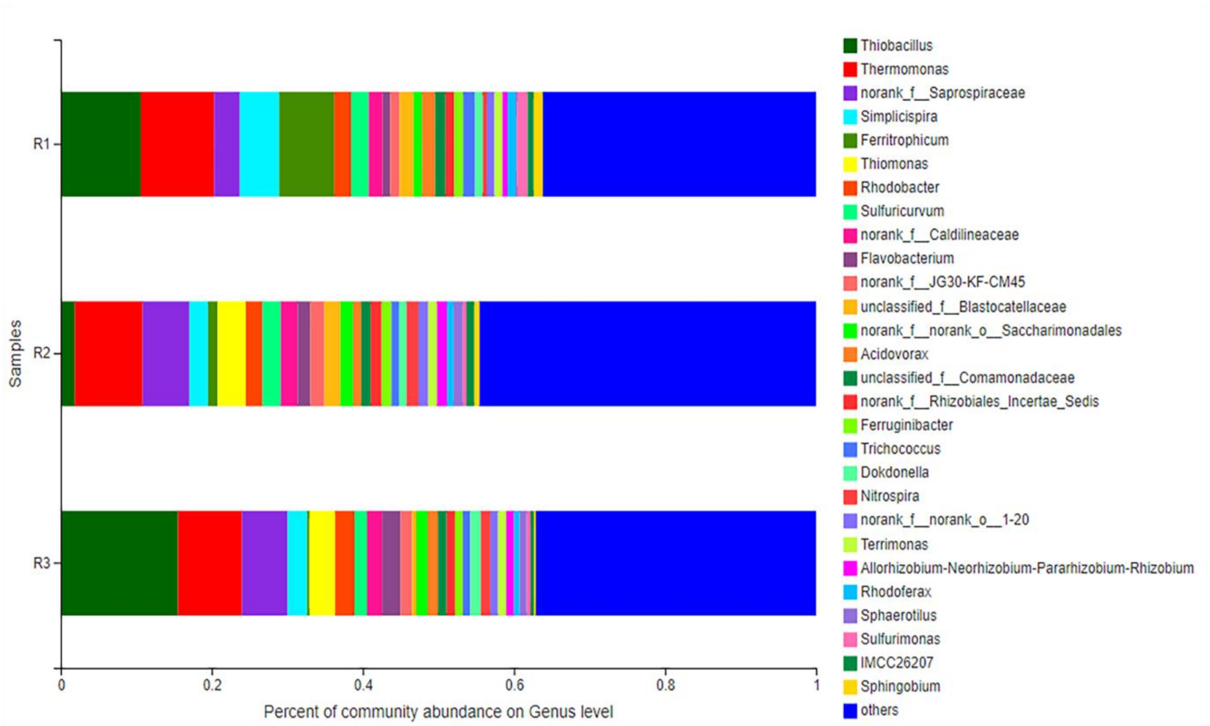

Fig. 2. Microbial community of the reactors classified at genus level. (R_1, R_2, and R_3: microbial communities on day 180 of SND_5, SND_{10}, and SND_{15} reactors, respectively)

The results of the taxonomic classification at the genus levels are shown in Fig. 2, with the relative abundance that greater than 1% were illustrated. In general, the most dominant genus was Thiobacillus. Other dominant genera were Sulfurimonas, Thermomonas, Ferruginibacter, Dokdonella, Simplicispira, Ferritrophicum, and Terrimonas. Sulfurimonas, Thermomonas, and Ferritrophicum have been identified as autotrophs in previous studies [7, 8], and Ferruginibacter, Terrimonas, and Dokdonella are considered as heterotrophs [9, 10]. The co-existence of autotrophs and heterotrophs were observed in all reactors, this may contribute to better control of nitrogen in SND system. The NSCF F3 enhanced the ability of nitrate removal, and represents an emerging opportunity for engineering application.

4. Conclusion

Based on the results and discussions presented above, the conclusions are obtained as below:

(1) The SND systems were successfully operated in packed bed denitrification reactors, with the optimal denitrification rate of 530 mg N·L-1·d-1 was obtained in SND15 system when the HRT of 1 h.

(2) Heterotrophs and autotrophs are co-existence in the reactors.

In general, compared with other reactors, the enhanced denitrification performance was observed in the SND15 reactor. This study confirmed the overall performance of the SND system, with F3 as a promising filter for the purification of nitrogen-contaminated wastewater.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (Grant No. 2020YFC1806402), Major key and core technology research project (direction of water pollution control industry chain), Science and Technology Plan of Shenyang in 2020（Grant No.

ISSN:2790-1688 DOI: 10.56028/aetr.1.1.42
20-202-4-37 ）, and the Ningxia provincial Natural Science Foundation of China (Grant No. 2020AAC03272).

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