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Comparison of heterotrophic and autotrophic denitrification processes for nitrate removal from phosphorus-limited surface water☆

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ABSTRACT

Phosphorus (P) limitation has been demonstrated for micro-polluted surface water denitrification treatment in previous study. In this paper, a lab-scale comparative study of autotrophic denitrification (ADN) and heterotrophic denitrification (HDN) in phosphorus-limited surface water was investigated, aiming to find out the optimal nitrogen/phosphorus (N/P) ratio and the mechanism of the effect of P limitation on ADN and HDN. Furthermore, the optimal denitrification process was applied to the West Lake denitrification project, aiming to improve the water quality of the West Lake from worse than grade V to grade IV (GB3838-2006). The lab-scale study showed that the lack of P indeed inhibited HDN more greatly than ADN. The optimal N/P ratio for ADN and HDN was 25 and a 0.15 mg PO_4^{3-}-P L^{-1} of microbial available phosphorus (MAP) was observed. P additions could greatly enhance the resistance of ADN and HDN to hydraulic loading shock. Besides, The P addition could effectively stimulate the HDN performance via enriching the heterotrophic denitrifiers and the denitrifying phosphate-accumulating organisms (DNPAOs). Additionally, HDN was more effective and cost-effective than ADN for treating P-limited surface water. The study of the full-scale HDBF (heterotrophic denitrification biofilter) indicated that the denitrification performance was periodically impacted by P limitation, particularly at low water temperatures.

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1. Introduction

Many important lakes in China suffer from eutrophication due to agricultural non-point source pollution. For example, the eutrophic water body of the West Lake is caused by nitrogen (N) fertilizers which are widely used in agricultural industry. Thus, it has greatly affected the landscape effect of the West Lake. The predominant nutrient in the West Lake is nitrogen (~3.0 mg TN L^{-1}) rather than phosphorus (~0.04 mg PO_4^{3-}-P L^{-1}). Therefore, nitrogen removal becomes a very urgent task. On the other hand, all forms of nitrogen tend to transform into nitrate nitrogen (NO_3^--N) by nitrifiers and the NO_3^--N is difficult to be removed under natural conditions. NO_3^--N in the West Lake water accounts for as high as 60%–70% of the total nitrogen (TN). Consequently, denitrification becomes a pressing problem to prevent eutrophication of water body and control nitrogen pollution.

Nowadays heterotrophic denitrification (HDN) is the most frequently-used denitrification process. External organic carbon (C) sources are provided as electron donors for the reduction of nitrate due to low COD/NO_3^--N (C/N) ratios (He et al., 2013; Karanasios et al., 2016). Nevertheless, there are some shortcomings in HDN, including excess external C sources which may cause secondary pollution, and large amounts of excess sludge which needs suitable treatment and disposal. Because of no more organic C source supplied and a lower sludge yield coefficient, autotrophic denitrification (ADN) seems to be a useful alternative to HDN (Moon et al., 2008; Sierra-Alvarez et al., 2007). Nitrate removal from synthetic micro-polluted surface water using the ADN process in a lab-scale has been investigated in previous studies (Wang et al., 2017a; Zhou et al., 2016). These studies proved that applying the ADN process to micro-polluted surface water was feasible, and high nitrate removal efficiencies could be obtained.

Initiatively, a comparative study of the HDN and ADN processes for treating nitrate-contaminated surface water was firstly

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investigated in our previous study. The results of the comparative study suggested that higher nitrogen removal rates were achieved in an autotrophic denitrification biofilter (ADBF) compared to a heterotrophic denitrification biofilter (HDBF) (Wang et al., 2017b). However, the finding is contrary to conventional understanding that the denitrification rate of HDN is generally much higher than that of ADN. The reasons and mechanisms have not been figured out yet. Noteworthily, phosphorus (P) was not detected in the synthetic surface water (below 0.01 mg PO_4^{3-}-P L^{-1}), which might limit the bacterial growth due to nutrient deficiency and lead to a different nitrogen removal performance between ADN and HDN (Wang et al., 2017b).

The effect of phosphorus content (P-content) on biological nitrogen removal has been reported in previous studies. de Vet et al. (2012) found that nitrification was almost totally prevented by limitation of phosphorus and the nitrification process could be effectively stimulated by adding phosphoric acid. Hunter (2003) observed that the removal efficiency of nitrate was very low and the nitrite accumulation rate reached up to as high as 52%–88% when a sand column was fed with groundwater which contained ~17 mg NO_3^--N L^{-1} and 0.009 mg PO_4^{3-}-P L^{-1}. Besides, they found that the removal efficiency of nitrate increased significantly with few accumulations of nitrite when an additional 0.16 mg PO_4^{3-}-P L^{-1} was supplied to the groundwater. However, the effect of P-content on ADN and its difference compared to it on HDN have not been investigated so far.

Therefore, a comparative study of nitrate removal rate between ADN and HDN for treating phosphorus-limited surface water was investigated in this study, which aimed to find out an optimal nitrogen/phosphorus (N/P) ratio (mg/mg) by adjusting the influent P-content. Moreover, the study also tried to figure out the mechanism of the effect of P-content on nitrate removal in the ADBF and the HDBF by microbial community analyses. Significantly, this work could provide scientific directions for making an optimal choice between ADN and HDN, thus finding the feasibility of practical application to nitrate removal from real phosphorus-limited surface water.

2. Materials and methods

2.1. Experimental setup, start-up and operation of the ADBF and the HDBF

Two lab-scale up-flow biofilters (ADBF and HDBF) were set up at Shanghai Jiao Tong University in Shanghai, China. The introduction to the ADBF and the HDBF was shown in our previous study (Wang et al., 2017b). Tap water added with ~2.5 mg $NaNO_3$-N L^{-1} was used as synthetic nitrate-contaminated surface water (SNCSW) (Wang et al., 2017b). The ADBF was fed with SNCSW and supplied with sulfur (S) and P sources by using tap water mixed with $Na_2S_2O_3 \cdot 5H_2O$ and KH_2PO_4. HDBF was fed with SNCSW and supplied with C and P sources by using tap water mixed with CH_3COONa and KH_2PO_4.

The ADBF and the HDBF were started up without the inoculation of seed sludge and operated in a continuous-flow mode for over 6 months. The whole operation period could be divided into five phases (phase I, phase II, phase III, phase IV and phase V), and the detailed operation conditions are shown in Table 1. Besides, according to the optimal dose of electron donors which were obtained in our previous study (Wang et al., 2017b), $S_2O_3^{2-}$ and NO_3^- molar ratio (S/N ratio) of the ADBF was adjusted to 1.2 and C/N ratio of the HDBF was adjusted to 7 during the whole operation period.

Table 1
Operation conditions of the ADBF and the HDBF in each phase.

Phase	Time (d)	T[a] (°C)	HRT[b] (h)	S/N[c] (C/N)	PO_4^{3-}-P[d] (mg L^{-1})
I	22–69	12 ± 2	4	1.2 (7)	0.80
II	105–124	15 ± 2	4	1.2 (7)	0.20
III	126–145	18 ± 2	4	1.2 (7)	0.05
IV	157–172	22 ± 2	1	1.2 (7)	0.20
V	175–185	24 ± 2	0.5	1.2 (7)	0.20

[a] Water temperature (mean ± standard deviation).
[b] Hydraulic retention time (HRT) in the effective empty bed (working volume of 5.5 L).
[c] S/N represented for the molar ratio of $S_2O_3^{2-}$ and NO_3^- (mol/mol).
[d] Phosphate-P.

2.2. Water sampling and chemical analysis

Water samples from the lab-scale experiment were collected and monitored every day from day 22 to day 185. NO_3^--N, nitrite nitrogen (NO_2^--N) and PO_4^{3-}-P levels were monitored according to Standard Methods (APHA, 2005). TN was analysed by means of Muti N/C 3000 (Analytik Jena AG, Germany).

2.3. Sludge sampling and microbial community analysis

In order to investigate the variation of microbial community composition in the ADBF and the HDBF, the sludge samples of ADBF (Non-P) and HDBF (Non-P) were collected from the ADBF and the HDBF, respectively, when they were operated with no addition of the phosphorus source (Wang et al., 2017b). The sludge samples of ADBF (P) and HDBF (P) were collected from the ADBF and the HDBF, respectively, when they were operated with an addition of 0.20 mg PO_4^{3-}-P L^{-1}. Microbial DNA was extracted from sludge samples using the E. Z.N.A. ® Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The 16S rRNA genes were amplified by PCR (95 °C for 2 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using primers 515F 5′-barcode- GTGCCAGCMGCCGCGG)-3′ and 907R 5′-CCGTCAATTCMTTTRAGTTT-3'.

Purified PCR products were quantified by Qubit ® 3.0 (Life Invitrogen) and every twenty-four amplicons whose barcodes were different were mixed equally. The pooled DNA product was used to construct Illumina Pair-End library and then the amplicon library was paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Shanghai BIOZERON Co., Ltd).

Operational Units (OTUs) were clustered with 97% similarity and chimeric sequences were identified and removed using UCHIME. The rarefaction analysis based on Mothur version 1.21.1 was conducted to reveal the alpha diversity, which could be characterized by the diversity indexes of Chao1, ACE, Simpson and Shannon (Schloss et al., 2009).

2.4. Calculation of the nitrogen removal rate and efficiency

NO_3^--N and TN removal rates were calculated using the following equations (1) and (2):

$$NO_3^- - N \text{ removal rate } \left(mg\ L^{-1}\ h^{-1}\right) = \left[(NO_3^- - N)_{influent} - (NO_3^- - N)_{effluent}\right] / t \quad (1)$$

$$TN \text{ removal rate } \left(mg\ L^{-1}\ h^{-1}\right) = \left(TN_{influent} - TN_{effulent}\right) / t \quad (2)$$

NO_3^--N and TN removal efficiencies were calculated using the

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