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The influence of phosphorus on the autotrophic and mixotrophic denitrification

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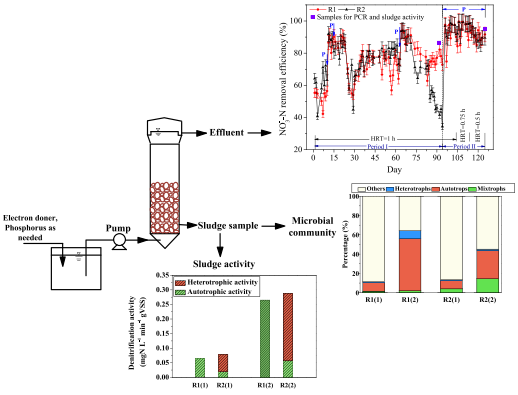
HIGHLIGHTS

- The denitrification efficiency increased by 40 and 35% after phosphorus addition.
- Both autotrophic and heterotrophic sludge activity enhanced remarkably.
- Phosphorus addition improved the proportion of denitrifying bacteria obviously.

GRAPHICAL ABSTRACT



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ABSTRACT

Autotrophic and mixotrophic denitrification, two approaches of biological denitrification, have drawn more and more attention among the techniques to remove nitrogen from the aquatic environment. This study investigated the influence of phosphorus on the denitrification performance and bacterial community structure in the autotrophic and mixotrophic denitrification reactors. The activity test was applied to evaluate the variation of denitrification activity of autotrophic and mixotrophic sludge before and after phosphorus addition. High-throughput sequencing was used to analyze the change of bacterial community structure. The results showed that NO_3^--N removal efficiency of autotrophic and mixotrophic denitrification process increased by 40 and 35%, respectively, after phosphorus addition. The sludge denitrification activity of autotrophic and mixotrophic sludge was enhanced significantly. And phosphorus addition could greatly improve the proportion of denitrifying bacteria in both autotrophic (from 11.83 to 64.31%) and mixotrophic denitrifying sludge (from 13.59 to 45.12%). Overall, phosphorus addition could greatly improve the autotrophic and mixotrophic denitrification ability in the phosphorus deficient surface water.

1. Introduction

With the acceleration of urbanization and industrialization process, the population and economy are experiencing rapid growth. The worldwide water pollution caused by intensive human activities is becoming

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increasingly serious. Among the various water pollution events, eutrophication and water bloom are frequently reported all over the world. Phosphorus and nitrogen are the major nutrients causing eutrophication. Among all the chemical forms of nitrogen in aqueous environment, nitrate is the most stable form and generally accounts for more than 60% of total nitrogen (TN) in the lake and river water. Therefore, nitrate reduction, i.e., denitrification, has become the most important step to remove the nitrogen from water.

Among the many denitrification techniques (Anabela et al., 2000; Schoeman and Steyn, 2003; Sakakibara and Nakayama, 2001; Zhou et al., 2017), biological denitrification is an effective and economic approach with the least secondary contamination. At present, the research on biological denitrification mainly focuses on heterotrophic and autotrophic denitrification with single type of electron donor. Few studies have explored mixotrophic denitrification using mixed electron donors. However, many researchers have found that mixotrophic denitrification is a common and natural phenomenon under the real circumstances. For example, Oh et al. found that simultaneous autotrophic and heterotrophic denitrification had occurred in the same reactor with sulfur source (sulfur particle), methanol and landfill leachate as the mixed electron donors. Moreover, mixotrophic denitrification had higher NO_3^--N removal efficiency than the autotrophic denitrification (Oh et al., 2001). Xu et al. (2015) found that nearly 43% of the microbes were facultative denitrifying bacteria in mixotrophic denitrification process, indicating that autotrophic, heterotrophic and mixotrophic denitrifying bacteria functioned together in the polyculture reactor. Due to its advantage of higher denitrification rate, less sludge production, lower sulfate production and alkalinity requirement (Liu et al., 2009; Oh et al., 2001; Sahinkaya et al., 2013), the mixotrophic denitrification has attracted more and more attention. At present, the studies of mixotrophic denitrification mostly focus on optimizing the running parameters and enhancing the denitrification efficiency.

Although both nitrogen and phosphorus contribute to eutrophication, phosphorus could be more easily removed in the primary treatment process by precipitation, while nitrogen remains in water/wastewater due to its high solubility and hydrophilicity (Pehlivanoglu-Mantas and Sedlak, 2008), causing the treated water rich in nitrogen but relatively deficient in phosphorus. For example, the research of Hai et al. (2015) found that the sequencing batch biofilm reactor achieved good performance in treating the swine wastewater, and that the effluent concentrations of total nitrogen (TN) and total phosphorus (TP) were 44.6 and 1.13 mg L^{-1}, respectively. Chen et al. (2015) used bioaugmentation system to treat the municipal wastewater, the averaged effluent concentrations of TN and TP were 14.1 and 0.40 mg L^{-1}, respectively. Through the past ten years' monitoring of the water quality in West Lake, China, the authors also detected the total nitrogen (nitrate accounting for about 80%) in the water 2.5–3.5 mg L^{-1} and total phosphorus, 0.03–0.08 mg L^{-1}. The imbalance of nitrogen and phosphorus may hinder the biological denitrification process. As it is well known, phosphorus plays an important role in the growth and metabolism of microorganisms. Researches have shown that phosphorus deficiency affected heterotrophic and autotrophic denitrification remarkably (Wang et al., 2018). However, it is still not known how it affects the mixotrophic denitrification process, and whether it hinders the bacterial multiplication or only the bacterial activity. On the other hand, the pattern of phosphorus addition is also to be discussed.

In this study, the effect of phosphorus deficiency on the mixotrophic and autotrophic denitrification processes was explored and compared, targeting at the high N/P ratio surface water. The nitrate removal efficiency, the nitrite accumulation, the sulfate production as well as the sludge denitrification activity were compared before and after phosphorus addition. In addition, the influence of phosphorus on microbial community structure was investigated according to the analysis of high-throughput sequencing.

2. Materials and methods

2.1. Materials

All the chemical reagents (analytical grade) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China) and used in this study without further purification.

2.2. Experimental set-up

2.2.1. Biofilter experiments

Two identical lab-scale up-flow biofilters were applied for autotrophic and mixotrophic denitrification process, respectively. The polypropylene reactors packed with ceramics (diameter of 5–10 mm) as media had the inner diameter of 8.8 cm, height of 0.5 m and effective volume of 3.0 L. Both reactors were seeded with digested sludge obtained from the local municipal wastewater treatment plant. Tap water was used to simulate the micro-polluted surface water, which contained about 3 mg/L nitrate and phosphorus lower than 0.05 mg L^{-1}. $Na_2S_2O_3$ was added into the autotrophic denitrification reactor (R1) as electron donor. The mixotrophic denitrification reactor (R2) was operated with $Na_2S_2O_3$ and NaAc as mixed electron donor, each type responsible for 50% of the nitrogen load. Phosphorus (KH_2PO_4, about 2.2 mg L^{-1}) was added into the two reactors occasionally during period I, and continuously during period II. The operational parameters of the two reactors were listed in Table 1. Meanwhile, the experiments were performed around the year without temperature or dissolved oxygen control in order to simulate the natural condition. During the whole experiment, the water samples were taken regularly to detect NO_3^--N concentration in the effluent.

2.2.2. Sludge activity measurement

After the reactors entered into stable running stage, the denitrification sludge was sampled from the sampling ports, which were at the height of 10 cm from the bottom of the reactors, for the analysis of sludge activity. The sludge samples taken from R1 before and after phosphorus addition were labelled as sludge R1(1) and R1(2), respectively, and those from R2, as sludge R2(1) and R2(2). 10 mL sludge samples were put into transparent glass vials (100 mL) and diluted to 90 mL, then all the vials were placed in a shaker for acclimation for 24 h at 30 °C with the rotating speed of 180 rpm. Different substrates were prepared to evaluate the denitrification activity. Autotrophic denitrification activity of R1 samples were tested with thiosulfate as electron donor. While the denitrification activity of the mixotrophic sludge samples (R2) was divided into two parts: autotrophic and heterotrophic. When thiosulfate and acetate were applied as the respective sole electron donor, nitrate reduction rate represented the autotrophic and heterotrophic denitrification activity of the R2 samples. In order to simulate the substrate conditions, phosphorus was prepared for the sample R1(2) and R2(2). The respective substrate components of the sludge denitrification activity tests were listed in Table S1.

10 mL prepared substrates was injected quickly into the corresponding vial, and the activity test started timing. The shaker kept shaking at 180 rpm and 30 °C. 2 mL mixture was taken from the vial at each scheduled time, followed by immediate centrifugation at 8000 rpm and 7 °C. The ion (Ac^-, NO_3^-, NO_2^- and SO_4^{2-}) concentration in the supernatant

Table 1
Operational parameters of the two reactors.

Reactor	Periods	Days	NO_3^--N (mg L^{-1})	$Na_2S_2O_3/NO_3^-$-N (mass ratio)	NaAc/NO_3^--N (mass ratio)
R1	I	1–94		11.3:1	—
R2			About 3.0	5.65:1	3.21:1
R1	II	95–125		11.3:1	—
R2				5.65:1	3.21:1

"—" indicates no addition.