



# Nitrification and denitrifying phosphorus removal via nitrite pathway from domestic wastewater in a continuous MUCT process



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## HIGHLIGHTS

- Short HRT (6 h) and low DO (0.3–0.5 mg/L) were key factors to achieve nitrification.
- Number and percent of AOB had a clear correlation with nitrite accumulation rate.
- The highest percentage of AOB was 13% of the total bacterial population.
- About 90% of total phosphorus (P) removal was completed by denitrifying P removal.
- P removal under nitrification was 30% higher than that under complete nitrification.

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## ABSTRACT

Nitrification and denitrifying P removal under mode of nitrification and nitrification was investigated in continuous MUCT process treating domestic wastewater. Nitrification was established through short hydraulic retention time to 6 h and low dissolved oxygen concentration of 0.3–0.5 mg/L. Nitrification was stabilized for 95 days with average nitrite accumulation ratio over 90%. Ammonia and total nitrogen removal under nitrification reached 99% and 83%, respectively, much better than complete nitrification. Real-time quantitative PCR assays presented that cell numbers and percentages of ammonia oxidizing bacteria (AOB) population had a clear correlation with nitrite accumulation ratios. The highest percentage of AOB was 13% of total bacterial population. P removal was mainly completed by denitrifying P removal of about 90% occurring in anoxic zone. The P removal efficiency under nitrification was 30% higher than that under complete nitrification. Denitrifying P removal under nitrification was highly beneficial to the treatment of wastewater with limiting carbon source.

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## 1. Introduction

Carbon source in real domestic wastewater is typically limiting and can hardly satisfy a need for simultaneous nitrogen and phosphorus (P) removal. In order to solve the problem of carbon source, novel processes incorporating both P and nitrogen removal have been developed, such as nitrogen removal via nitrite pathway and denitrifying P removal (Oehmen et al., 2007). Nitrogen removal via nitrite pathway is defined that ammonia is oxidized to nitrite (nitrification), and then directly reduced to N<sub>2</sub> gas (denitrification) (Makinia et al., 2011). Compared with the traditional biological nitrogen removal, nitrification/denitrification can save 25% of aeration costs, 40% of carbon source demand and 50% of sludge production due to shortening of process (Fux et al., 2006; Zhu

et al., 2008). Therefore, for the treatment of domestic wastewater with low carbon to nitrogen ratio (C/N), nitrification/denitrification is especially significant since carbon source in it is typically limiting.

From a microbiological point of view, the key to achieve nitrification is to inhibit or eliminate nitrite oxidizing bacteria (NOB) while ammonia oxidizing bacteria (AOB) have to become the dominant nitrifying bacteria. The selection factors to wash out NOB such as low dissolved oxygen (DO) concentration (Blackburne et al., 2008; Guo et al., 2009; Ruiz et al., 2006), high temperature and short sludge retention time (SRT) (Hellinga et al., 1998), high free ammonia (FA) and free nitrous acid (FNA) inhibition (Park and Bae, 2009; Vadivelu et al., 2006), adding inhibitors (e.g., NaCl) (Mosquera-Corral et al., 2005), controlling of hydraulic retention time (HRT) (Zeng et al., 2010) and aeration time (Zeng et al., 2009) have been investigated. These studies about nitrification and denitrification had the following three characteristics. Firstly, most of studies used sequencing batch reactor (SBR) to achieve nitrification (Fux et al., 2006; Ganigue et al., 2012; Zeng et al., 2009).

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However, continuous-flow process is most commonly used in WWTPs. Compared with SBR, it is difficult to achieve nitrification with the continuous-flow process. Very limited studies on achieving nitrification and denitrification in a continuous-flow system were related to pre-denitrification (AO) process (Ma et al., 2009) and anaerobic–anoxic–aerobic (A<sup>2</sup>O) process (Zeng et al., 2010). Secondly, the treated wastewater mainly focused on high temperature wastewater (Hellings et al., 1998), high nitrogen loaded wastewater (Ganigue et al., 2012; Li et al., 2011; Wang et al., 2011) and synthetic wastewater (Liang et al., 2011). Natural temperatures, FA and FNA concentrations in real domestic wastewater cannot usually reach inhibitory level to NOB. Therefore, nitrification is difficult to establish in the treatment of domestic wastewater. Thirdly, most research was carried out in biological nitrogen removal systems without regard to P removal. But process incorporating both nitrogen and P removal is commonly required in WWTPs, which is more complicated to control than a sole nitrogen removal system. Consequently, achieving nitrification/denitrification in continuous-flow process treating domestic wastewater with both nitrogen and P removal should be investigated further.

Main difference of denitrifying P removal from traditional EBPR is nitrate or nitrite used as electron acceptors instead of oxygen for PHAs oxidation under anoxic conditions, which allows simultaneous nitrogen and P removal (Carvalho et al., 2007; Kuba et al., 1996; Zafiriadis et al., 2011). If denitrifying P removal is based on nitrification, i.e., nitrite used as electron acceptor during anoxic P-uptake, demand of carbon sources and aeration costs will be further reduced. That is advantageous for treatment of wastewater with low C/N ratio.

Kuba et al. (1996) observed that one facultative anaerobe could use oxygen or nitrate as an electron acceptor to carry out both denitrification and P-uptake. Saito et al. (2004) reported that nitrite could also be used as an electron acceptor for P-uptake. These bacteria are named the denitrifying phosphorus removal bacteria (DPBs). Studies about denitrifying P removal mainly focused on denitrifying capabilities of PAOs using different types of electron acceptors, i.e., oxygen, nitrite and nitrate (Lanham et al., 2011; Saito et al., 2004; Zhou et al., 2010), and control method of denitrifying P removal process (Wang et al., 2009, 2011). These studies had the following three characteristics. Firstly, most of studies were undertaken in SBR treating synthetic wastewater (Carvalho et al., 2007; Kuba et al., 1996; Lanham et al., 2011). According to mechanism of denitrifying P removal, alternating anaerobic/anoxic condition favors the growth of DPBs, and thus SBR becomes popular operational mode. However, studies also indicated that long-term operation under alternating anaerobic/anoxic conditions led to gradual decline in biomass, and finally caused failure of EBPR (Yang et al., 2003). Secondly, most researches were carried out in EBPR systems without involving nitrification (Carvalho et al., 2007; Lanham et al., 2011; Zhou et al., 2010). Electron acceptor such as nitrite or nitrate was externally added into batch reactor at the beginning of anoxic phase rather than generated from nitrification process, which was different from real wastewater treatment system. Thirdly, denitrifying P removal has not been fully understood in real wastewater treatment system although it was reported in the WWTPs with MUCT or UCT process (Kuba et al., 1997). Due to the restrictions resulted from operation and management of WWTPs, influencing factors cannot be optionally changed to gain a good insight into denitrifying P removal. Very limited research has been conducted about denitrifying P removal in a continuous-flow process treating real domestic wastewater under nitrification and nitrification mode.

Therefore, this study aims to (1) develop a method to achieve nitrification in MUCT process treating real domestic wastewater, (2) determine the mechanism of nitrification by correlating the dynamics of AOB population with biological nutrient removal

and an analysis of selection factors and (3) analyze denitrifying P removal under nitrification and nitrification mode, and provide an effectively operational method to achieve denitrifying P removal on the basis of nitrification.

## 2. Methods

### 2.1. Experimental set-up and operation

Fig. 1 shows the experimental system consisting of a MUCT reactor with a working volume of 70 L and a secondary settler with a working volume of 24 L. The MUCT reactor was divided into seven chambers. The first four chambers with mechanical mixers were used as anaerobic or anoxic zones and the following three with air diffusers were used as aerobic zones. The first chamber provided an anaerobic zone for P-release and for influent. Sludge from secondary settler was firstly recycled to anoxic zone I with a recycle ratio of  $R_1$  for the removal of  $\text{NO}_2^-$ -N or  $\text{NO}_3^-$ -N in the returned sludge by denitrification, and then was recycled to anaerobic zone with a recycle ratio of  $R_2$  for P-release. Recycling of sludge in the secondary settler from anoxic zone I to anaerobic zone effectively eliminates the negative impact of  $\text{NO}_2^-$ -N or  $\text{NO}_3^-$ -N on anaerobic P-release. The third and fourth chambers were anoxic zone II for denitrification of recycled nitrification liquid from the last aerobic chamber with a recycle ratio of  $R_3$ . The volume ratio of the anaerobic zone, anoxic zone I, anoxic zone II and the aerobic zone was 1:1:2:3. The flow rates of feeding, returned sludge, nitrification liquid recycle and anoxic recycle were controlled by peristaltic pumps.

### 2.2. Seed sludge and wastewater

The seed sludge was taken from one municipal wastewater treatment plant using an anaerobic–anoxic–aerobic process in Beijing. This WWTP performs nitrification/denitrification and P removal well without nitrite accumulation. The composition of municipal wastewater treated in this WWTP is similar with that used in this study.

Raw wastewater from a campus sewer line was pumped into a storing tank for sedimentation, and then fed into the reactor. Table 1 shows the influent characteristics. Mean influent COD to nitrogen ratio (C/N) was about 2.0 and the minimum was as low as 0.86, and thus the organic carbon source was typically limiting.

### 2.3. Experimental procedure

The MUCT reactor was operated for 240 days including eight successive phases. Table 2 shows the operational conditions over experimental period. DO concentration in aerobic zone was controlled at 0.3–0.5 mg/L. Wastewater temperature was maintained at ambient temperature. Sludge retention time (SRT) and concentration of mixed liquor suspended solid (MLSS) was controlled by discharging excess sludge from the bottom of settler. The experimental purpose of phases I–IV was to investigate the effect of HRT control on establishment of nitrification. During the phases V–VI, nitrification was stably operated. During the phases VII–VIII, nitrification was broken down and converted into nitrification. Moreover, denitrifying P removal under the nitrification and nitrification mode was also investigated during the phases I–VIII.

The batch tests of denitrifying P removal: the tested sludge was taken from the MUCT reactor on day 171 and day 227, respectively. The ratio of the maximal anoxic P-uptake rate to the maximal aerobic P-uptake rate ( $K_{\text{axmax}}/K_{\text{omax}}$ ) was used to characterize the relative P removal activity of DPBs to PAOs (Meinhold et al., 1999). The detailed procedures are as follows:

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