

Title:

Effect of phosphorus load on nutrients removal and N₂O emission during low-oxygen simultaneous nitrification and denitrification process

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Abstract

Three laboratory scale anaerobic-aerobic (low-oxygen) SBRs (R1, R2 and R3) were conducted at different influent phosphorus concentration to evaluate the impacts of phosphorus load on nutrients removal and nitrous oxide (N₂O) emission during low-oxygen simultaneous nitrification and denitrification (SND) process. The results showed that TP and TN removals were enhanced simultaneously with the increase in phosphorus load. It was mainly caused by the enrichment of polyphosphate accumulating organisms (PAOs) under high phosphorus load and low COD/P ratio (<50), which could use nitrate/nitrite as electron acceptors to take up the phosphorus. N₂O emission was reduced with increasing phosphorus load. N₂O-N emission amount per cycle of R3 was 24.1% lower than that of R1. It was due to the decrease of N₂O yield by heterotrophic denitrification. When the phosphorus load increased from R1 to R3, heterotrophic denitrification (D) ranged from 42.6% to 36.6% of the N₂O yield.

Keywords: Phosphorus load; Nutrients removal; Nitrous oxide; Low-oxygen;

1. Introduction

To date, nutrients in the wastewater are enforced to be removed in order to protect the water from eutrophication. Therefore, simultaneous nitrification-denitrification (SND) under oxygen-limiting condition is widely studied, due to its high nutrients removal efficiencies and low energy consumption (Holman and Wareham, 2005; Danie et al., 2009). However, it was reported that a significant amount of N_2O may be produced during this low-oxygen process (Meyer et al., 2005; Zhu and Chen, 2011; Jia et al., 2012). N_2O is an important greenhouse gas, and is also the dominant ozone-depleting substance emitted in the 21st century (IPCC, 2007). Therefore, its control has attracted increasingly more attentions over the past decade.

N_2O emission during wastewater treatment process is affected by many factors, such as dissolved oxygen (Tallec et al., 2006), COD/N ratio (Chung and Chung, 2000; Wu et al., 2009), pH value (Thörn and Sörensson, 1996), nitrite concentration (Yang et al., 2009; Rajagopal and Béline, 2011) and consumption of internal storage compounds (Lemaire et al., 2006; Jia et al., 2012). In addition, the impact of phosphorus on N_2O emission in kinds of ecosystems has been studied by many researchers (Hall and Matson, 1999; Liu and Song, 2010; Mori et al., 2010), and the influence is still under discussion. However, no literatures have yet focused on the impact of phosphorus concentration on N_2O emission during biological wastewater treatment process.

During conventional biological nutrients removal process, N removal is accomplished by a two-stage treatment, aerobic nitrification and anoxic denitrification,

1 whereas P removal is achieved through enhanced biological phosphorus removal
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3 (EBPR) under alternating anaerobic–aerobic conditions (Zeng et al., 2003). The group
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5 of microorganisms that is largely responsible for phosphorus removal is called
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7 polyphosphate accumulating organisms (PAOs). Under anaerobic conditions, PAOs
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9 are able to take up organic substrates and store them as polyhydroxyalkanoates (PHA)
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11 using the energy obtained partly from the glycogen utilization but mostly from the
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13 hydrolysis of the intracellular stored polyphosphate (polyP), resulting in
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15 orthophosphate release into solution. Then, under aerobic or anoxic conditions PAOs
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17 oxidize the internally stored PHA for biomass growth, glycogen replenishment and
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19 polyphosphate recovery from external P uptake (Morse et al., 1998).
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28 An essential requirement for successful phosphorus removal is to only provide
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30 carbon under anaerobic conditions in order to provide PAOs with a selective
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32 advantage, as other heterotrophic organisms like glycogen accumulating organisms
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34 (GAOs), can take up carbon in the absence of an electron acceptor. The ratio of
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36 organic carbon to phosphorus in the influent (C/P ratio) has been shown to have
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38 significant impacts on the competition of microbial community for carbon (Thomas et
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40 al., 2003; Chuang et al., 2011). Mino et al. (1998) found that a low COD/P ratio (e.g.
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42 10-20) in influent tends to favor the growth of PAOs instead of GAOs, whereas a high
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44 COD/P ratio (e.g. >50) will be favorable to the growth of GAOs.
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53 Moreover, it is found that denitrification and P removal can be achieved
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55 simultaneously, which called denitrifying phosphorus removal, due to the capacity of
56
57 denitrifying phosphorus accumulating organisms (DPAOs) to use nitrate and/or nitrite
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1 as electron acceptor for P removal instead of oxygen (Kuba et al., 1996). In this
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3 simultaneous nitrification-denitrification-phosphorus removal system, carbon is
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5 supplied in an initial anaerobic period and can therefore selectively be taken up by
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7 PAOs and stored as PHA. In the following aerobic period, simultaneous nitrogen and
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9 phosphorus removal is achieved by the presence of adjacent aerobic and anoxic
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11 microzones in microbial aggregates caused by mass transport limitation of oxygen.
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13 Both N and P removal processes require COD. Therefore the ratio of COD:N:P is
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15 essential for the removal of N and P.
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22 It was reported that N_2O could accumulate during denitrifying phosphorus
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24 removal process due to the competition for electrons between the denitrifying
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26 enzymes (Kampschreur et al., 2009). The variation of phosphorus load can change the
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28 community composition of the bioreactor, leading to the different N_2O emission
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30 characteristics. Moreover, it is necessary to investigate the influence of phosphorus
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32 load on the two processes, nitrifier denitrification and heterotrophic denitrification, to
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34 investigate the mechanism of N_2O emission under different P load. However, so far,
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36 no information, to our best knowledge, is available regarding this point.
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44 This study presented an initial attempt to investigate the impacts of phosphorus
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46 load on nutrients removal and N_2O emission during low-oxygen SND process. To this
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48 end, three sequencing batch reactors (SBRs) were constructed with different influent
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50 phosphorus concentration to (1) determine the influence of phosphorus load on
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52 contaminant removal performance, and (2) investigate the impact of phosphorus load
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54 on N_2O emission characteristics during low-oxygen SND process.
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2. Materials and methods

2.1 Reactor setup and operation

Experiments were carried out in three lab-scale anaerobic-aerobic SBRs (R1, R2 and R3), with effective volume of 5 L. The schematic diagram of the experiment system is shown in Fig. 1. The SBRs were operated at room temperature (25 ± 2 °C) with a cycle time of 6 h, consisted of 90 min anaerobic stage, 180 min aerobic stage, 70 min settling, and 20 min decant. In each cycle, 3 liters of synthetic municipal wastewater was fed into each SBR in the first 5 min of anaerobic stage and same amount of supernatant was withdrawn after settling, resulting in a hydraulic retention time (HRT) of 10 h.

Electromagnetic stirrers were used to keep the suspension of sludge during anaerobic and aerobic stage. During aerobic stage, air supply was regulated using an on/off control system to keep the dissolved oxygen (DO) level between 0.35-0.80 mg/L. Before settling, 0.25 L mixed liquor was wasted to control the solids retention time (SRT) at approximately 20 d. The SBRs were seeded with sludge collected from a parent SND SBR (Jia et al., 2012), and the mixed liquor suspended solid (MLSS) was maintained at approximately 3000-3500 mg/L.

After the SBRs were acclimated under specific operation condition for over 3 months and reached stable performances, indicated by stable nitrogen and phosphorus concentrations in the effluents, the effluent was sampled and analyzed every 3 days to evaluate the contaminant removal performance. The systems were gastight and off gases were collected into gas sampling bags to measure N_2O concentrations at time

1 intervals of 15 min. Meanwhile, liquid phase samples were taken to measure the water
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3 quality parameters and sludge samples were taken to measure the intracellular PHA
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5 content.
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8 9 2.2 Synthetic wastewater

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11 The three SBRs were fed with synthetic municipal wastewater containing different
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13 phosphorus concentration. The synthetic wastewater used in this study was comprised
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15 of (per liter): 289.55 mg $C_6H_{12}O_6 \cdot H_2O$; 174.58 mg CH_3COONa ; 152.86 mg NH_4Cl ;
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17 200 mg $NaHCO_3$; 11-32.93 mg KH_2PO_4 ; 18.41-55.22 mg $K_2HPO_4 \cdot 3H_2O$; 10 mg
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19 $MgSO_4 \cdot 7H_2O$; 10 mg $FeSO_4 \cdot 7H_2O$; 10 mg $CaCl_2 \cdot 2H_2O$ and 1 ml nutrient solution
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21 (Zeng et al., 2003). The influent characteristics of each SBR are shown in Table 1.
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28 2.3 Batch experiments

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30 In order to investigate the characteristics of N_2O emission under different
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32 phosphorus load, the contribution of nitrifier denitrification and heterotrophic
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34 denitrification to N_2O emission was evaluated by the batch experiments. The
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36 experiments were carried out according to the methods described by Tallec et al.
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38 (2006), with slight modification.
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44 After the SBRs were acclimated, a total of 3 liters of mixed liquor and sludge was
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46 taken from each SBR at the end of anaerobic stage and divided equally into three mini
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48 bioreactors with working volume of 1 L. Three batch experiments were
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50 simultaneously conducted: (a) no addition of nitrite and inhibitor, (b) with addition of
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52 nitrite, and (c) with addition of both nitrite and nitrification inhibitors (Allythiourea
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54 (ATU) and chlorate ($NaClO_3$)). The nitrite, ATU and $NaClO_3$ were added at the start
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1 of experiment to have a concentration of 5 mg/L, 10 mg/L, and 1 g/L, respectively.

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3 A mixture of N₂ and air was supplied into the mini bioreactors with the ratio
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6 adjusted so as to best simulate the DO variation and hydrodynamic environment of
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9 the parent reactor. The off-gas during each experiment was all collected into gasbags
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11 to quantify the emission amount of N₂O. Each experiment was conducted three times.
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14 2.4 Analysis

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17 The analysis of COD, NH₄⁺-N, NO₃⁻-N, NO₂⁻-N, TN, TP and MLSS were
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19 conducted in accordance with the standard methods (APHA, 2001). DO was
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21 measured with a DO meter (HQ30d53LDO™, Hach, USA). N₂O concentration was
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23 determined by the gas chromatography (SP-3410, China) with an electron capture
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25 detector (ECD) and a Poropak Q column. PHA was measured by the gas
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27 chromatography with a flame ionization detector (FID) and a column DB-5.
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34 The emission rate and quantity of N₂O were calculated using the equation
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36 described by Hu et al. (2010). During the batch experiments, the N₂O yields by
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38 nitrifier denitrification and heterotrophic denitrification were calculated according to
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40 the method described by Tallec et al. (2006). N₂O-N conversion rate was calculated
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42 by the following equation:
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$$46 \text{ N}_2\text{O-N conversion rate} = \text{N}_2\text{O-N/TN removed} \times 100\%.$$

47 3. Results and discussion

48 3.1 Effect of phosphorus load on contaminant removal performance

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51 After running for about three months, the effluent contaminant concentration
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54 tended to be stable and the SBRs were in steady-state. The contaminants removal
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1 efficiencies of each SBR were evaluated and the results are shown in Table 1. The
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3 COD removal efficiency of all three SBRs was high ($> 92\%$) and there was no
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5 significant difference of three SBRs. The high COD removal efficiency was due to the
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7 easy degradation of the influent organic compounds (glucose and sodium acetate).
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9 The influent carbon was consumed quickly for denitrification and hydrolysis of
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11 intracellular stored polyphosphate.
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17 The TP removal was enhanced with the increase of phosphorus load. The TP
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19 removal efficiency of R1 was only 85.7%. R3 gained the highest TP removal
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21 efficiency (89.3%) although the phosphorus load was much higher than R1 and R2.
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23 The influent COD/TP ratio of R1, R2 and R3 was 91.6, 40.8 and 27.6 on average.
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25 With the decrease of COD/P, the phosphorus removals exhibited an upward trend. It
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27 has been reported that the influent COD/TP ratio of wastewater has great impact on
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29 phosphorus removal (Wang et al., 2009; Kapagiannidis et al., 2012). Numerous
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31 studies have found that a high COD/P ratio (e.g. >50 mg COD/mg P) in influent tends
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33 to favor the growth of GAOs instead of PAOs, which outcompete PAOs for organic
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35 substrate. The metabolism of GAOs is similar to that of PAOs, except that no P
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37 transformations are taking place (Liu et al., 1997). Thus, a low COD/P ratio will be
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39 more favorable to the growth of PAOs and phosphorus removal (Mino et al., 1998).
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50 The TN removal efficiency presented an upward trend with the increase of
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52 phosphorus load, although there was no significant difference for R1 and R2. The
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54 ammonium was almost completely removed in all SBRs, indicating that the nitrifier
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56 was not affected by the C/P ratio. The higher TN removal efficiency of R3 was due to
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1 the little accumulation of nitrate. The effluent nitrate concentration of R3 was only
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3 5.7±1.3 mg/L, which was lower than that of R1 and R2. It was known that the
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5 nitrogen removal during SND process was achieved by the coupled denitrification
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7 during aerobic stage, i.e., nitrifier denitrification and heterotrophic denitrification
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9 (Chiu et al., 2007). The autotrophic nitrifiers were not affected by the phosphorus
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11 concentration under P-rich condition. Therefore, the variation of nitrogen removal
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13 was mainly caused by the activity of heterotrophic denitrification.
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20 To further investigate the influence of phosphorus load on contaminant removal,
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22 the characteristics of C, P and N transformation during one cycle in each SBR were
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24 studied. Fig. 2 shows the time profiles of COD and TP concentrations in the liquor as
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26 well as intracellular PHA content in the sludge during one cycle in each SBR. All of
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28 the three SBRs had similar time course of COD depletion. During the anaerobic stage,
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30 the COD was consumed quickly in the first 30 min, with the increase of TP
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32 concentration and intracellular PHA content. Part of the organic substrates was used
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34 as carbon source for denitrification and another part was stored as intracellular PHA.
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36 Then the COD concentration was stabilized at low level during the rest period of one
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38 cycle in each SBR. With the consumption of COD, the PHA content in the sludge
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40 increased during the anaerobic stage using the energy supplied by hydrolysis of
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42 intracellular stored polyphosphate (polyP) (Mino et al., 1998), resulting in the release
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44 of orthophosphate into solution and the rise of TP concentration in the SBR. During
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46 the following low-oxygen aerobic stage, the stored PHA was consumed for biomass
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48 growth and polyphosphate recovery from external phosphorus uptake as well as
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1 possible denitrification driven by PHA (Chuang et al., 2011), leading to the gradually
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3 decrease of intracellular PHA and TP concentration in the liquid in each SBR. Similar
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5 phenomena were also found in other literatures about simultaneous nitrogen and
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7 phosphorus removal (Zeng et al., 2003; Meyer et al., 2005; Lemaire et al., 2006).
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11 However, it was noteworthy that the amount of released phosphorus and stored
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13 intracellular PHA was different with the variation of phosphorus load. For R1, the TP
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15 concentration at the end of anaerobic stage reached to 12.1 mg/L. Then during the
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17 aerobic stage the phosphorus was taken up by PAOs and the effluent phosphorus
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19 concentration was only about 0.5 mg/L (Fig. 2a). For R2 and R3, the TP concentration
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21 was 17.3 mg/L and 27.5 mg/L at the end of anaerobic stage, and the effluent
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23 concentration was 0.8 mg/L and 1.5 mg/L, respectively (Fig. 2b and 2c). The results
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25 indicated that more phosphorus would be released during anaerobic stage and
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27 absorbed during aerobic stage when the influent phosphorus load was higher.
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36 Meanwhile, the synthetic intracellular PHA during the anaerobic stage was
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38 enhanced with the increase of phosphorus load, although the amount of organic
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40 carbon input was same in each SBR. At the end of anaerobic stage, the PHA content
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42 in the sludge of R1 and R2 was 64.0 mg/gSS and 73.8 mg/gSS (Fig. 2a and 2b). For
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44 R3, the synthetic PHA was as high as 175.2 mg/gSS (Fig. 2c). It was mainly caused
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46 by the enrichment of PAOs under high phosphorus load. Seviour et al. (2003)
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48 indicated that more PAOs could be accumulated and activated in the reactor with
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50 higher P loading. More P was available for PAO to accumulate as internal poly-P
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52 under higher phosphorus load, despite the similar COD loading adopted in all SBRs,
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1 resulting in more PHA synthesized and stored during anaerobic stage.
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3 Fig. 3a shows the time course of nitrogen concentration during one cycle of R1.
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6 During the feeding period of the anaerobic stage, the TN and $\text{NH}_4^+\text{-N}$ concentrations
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8 decreased sharply due to the dilution of the residual water of the previous cycle.
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10 Meanwhile, the nitrite concentration increased transitorily for the reduction of nitrate,
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12 and then decreased to about zero quickly due to the fast denitrification. The TN
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14 removal mainly occurred during the aerobic stage and the removal rate was high in
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16 the first 120 min of aerobic stage. During this period, the ammonium concentration
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18 decreased fast for nitrification and nearly no nitrate accumulated. Although there was
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20 slight accumulation of nitrite, it was decreased quickly to about zero. It showed that
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22 the simultaneous denitrification in this period was enhanced, leading to the high
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24 removal rate of TN. Then in the last 60 min of aerobic stage, the ammonium was
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26 almost completely removed, and the nitrate concentration increased gradually to about
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28 7.7 mg N/L. During this period, the TN removal rate was very low, and the effluent
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30 TN concentration was about 8.6 mg N/L.
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42 For R2 and R3, the time course of nitrogen transformation was similar with that of
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44 Run 1 (Fig. 3b and 3c). However, with the increase of influent phosphorus load, the
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46 accumulation of nitrate was eased. The effluent nitrate concentration of R3 was lower
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48 than that of R1 and R2. Meanwhile, the accumulation of nitrite of R3 was also lower
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50 than that of R1 and R2. The maximum concentration of nitrite was 1.1 and 2.1 mg/L
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53 at 180 min in Run 1 and Run 2, while the peak value in Run 3 was only 0.7 mg/L.
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58 The above results indicated that the increase of phosphorus load enhanced the
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1 simultaneous denitrification during the aerobic stage. During this period, the COD
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3 concentration in each SBR was very low (Fig. 2) and the carbon source for
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5 denitrification was insufficient. Therefore, the simultaneous heterotrophic
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7 denitrification was carried out using internal stored carbon source. It was reported that
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9 under the low C/N ratio condition, some heterotrophic denitrifiers, such as PAOs and
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11 GAOs, could use the intracellular PHA as carbon source to reduce the nitrate/nitrite
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13 (Zeng et al., 2003). The higher phosphorus load could synthesize more PHA during
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15 anaerobic stage and supply more intracellular carbon as electron donor for
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17 denitrification during aerobic stage. That may be one reason for the higher TN
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19 removal efficiency of R3.
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28 It was noteworthy that simultaneous high nitrogen and phosphorus removal
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30 efficiency was achieved in R3, due to the enrichment of PAOs/DPAOs under high
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32 phosphorus load. The results were consistent with the other researchers' studies that
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34 the simultaneous nitrification, denitrification and phosphorus removal was achieved
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36 by coupling nitrification with denitrification by PAOs using PHA stored in the
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38 anaerobic period as carbon source in one anaerobic-aerobic stage (Zeng et al., 2003;
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40 Meyer et al., 2005; Lemaire et al., 2006). This combination could offer substantial
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42 savings on carbon for the overall nutrient removal process.
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50 3.2 Effect of phosphorus load on N₂O emission

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52 The time course of N₂O emission rate under each phosphorus load is shown in Fig.
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55 4a. During the feeding period of anaerobic stage, it presented transitory high N₂O
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57 emission rate in all SBRs and then decreased to about zero. The high N₂O emission
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1 was due to the NO_2^- accumulation at the beginning of new cycle (Fig. 3). During the
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3 following anaerobic stage, the N_2O emission rate was very low in each SBR. The
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5 majority of N_2O emission occurred in the aerobic stage. The N_2O emission rate
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7 increased rapidly at the beginning of aeration and reached to the peak at about 165
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9 min in each SBR. The emission rate was then decreased with the accumulation of
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11 nitrate. At the beginning of aerobic stage, the TN and $\text{NH}_4^+\text{-N}$ removal rates were
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13 high with no nitrate/nitrite accumulation (Fig. 3). Nitrifier denitrification and
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15 heterotrophic denitrification, which were the two main processes responsible for most
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17 N_2O emission during low-oxygen SND process (Meyer et al., 2005), were carried out
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19 quickly during this period, leading to the high N_2O emission rate. The accumulation
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21 of nitrate indicated that the two processes have been finished, resulting in the decrease
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23 of N_2O emission rate. It was found that the profile of N_2O emission rate in each SBR
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25 was accordance to the change of nitrite concentration (Fig. 3 and Fig. 4a), which was
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27 also observed in other literature (Hu et al., 2010). The NO_2^- could stimulate the
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29 emission of N_2O (Colliver and Stephenson, 2000).
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42 With the increase of phosphorus load, the maximum N_2O emission rate was
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44 decreased. The peak average N_2O emission rate was 8.9 and 8.5 $\mu\text{g/gMLSS}/\text{min}$ for
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46 R1 and R2, respectively. The maximum N_2O emission rate of R3 was 6.9
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48 $\mu\text{g/gMLSS}/\text{min}$, which was much lower than that of R1 and R2. The $\text{N}_2\text{O-N}$ emission
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50 amount and conversion rate in each SBR were calculated and the results are shown in
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52 Table 2. The emission amount during anaerobic stage was negligible compared to the
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54 amount during aerobic stage. It was found that N_2O emission amount was reduced
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1 with the increase of phosphorus load. The N_2O -N emission amount per cycle of R3
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3 was 24.1% lower than that of R1. Only 6.22% of removed nitrogen of R3 was
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5 converted to N_2O -N, which was also much lower than that of R1 and R2.
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9 The time course of ammonium concentration in each SBR showed that the
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11 community of ammonia-oxidizing bacteria (AOB) was not affected significantly by
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13 the phosphorous load (Fig. 3). Therefore, the variation of N_2O emission in three SBRs
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15 was not caused by the nitrifier denitrification. The respective contribution of
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17 heterotrophic denitrification and nitrifier denitrification in each SBR was evaluated by
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19 the batch experiments and the results are shown in Fig. 5. In all three SBRs, nitrifier
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21 denitrification (ND) appeared to be the major process responsible for the N_2O
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23 emission during low-oxygen SND process, which was also observed in other literature
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25 (Tallec et al., 2006). N_2O emission from ND was stimulated by a nitrite addition, but
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27 there was no significant difference of three SBRs. However, the contribution of
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29 heterotrophic denitrification was decreased with the increase in phosphorus load.
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31 When the phosphorus load increased from R1 to R3, heterotrophic denitrification (D)
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33 ranged from 42.6% to 36.6% of the N_2O yield.
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44 The above results indicated that the reduction of N_2O emission of R3 was mainly
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46 caused by the decrease of N_2O yield by heterotrophic denitrification. Some
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48 heterotrophic bacteria, mainly GAOs and PAOs in the present study, could use nitrate
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50 or/and nitrite as electron acceptors and thereby carry out denitrification using
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52 intracellular PHB, the main part of PHA, as carbon source under low C/N ratio
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54 condition (Zeng et al., 2003). The slower nature of PHB degradation can produce
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1 competition for electrons between denitrifying enzymes, resulting in a higher NO
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3 reduction rate compared to the N₂O reduction rate, causing the accumulation of N₂O
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5 (Kampschreur et al., 2009). Compared with R1, R3 could synthesize more PHB
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7 supplied as electron donor for denitrifying enzymes and eased the competition for
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9 electrons. That may be one reason for the low N₂O yield of R3.
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14 In addition, for R1, the low phosphorus load and high COD/TP ratio favored the
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16 growth of GAOs instead of PAOs. On the contrary, the PAOs were enriched under
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18 high phosphorus load and low COD/TP ratio condition of R3. It was reported that
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20 N₂O was the main denitrification end-product of GAOs (Zeng et al., 2003; Lemaire et
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22 al., 2006). Zhu and Chen (2011) also found that N₂O emission was much higher when
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24 the number of GAOs was more than PAOs. The enrichment of PAOs instead of GAOs
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26 of R3 was another main reason for low N₂O yield.
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33 It was found that the high phosphorus load of R3 in the present study achieved
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35 higher nutrients removal as well as lower N₂O yield, which makes this approach
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37 attractive. However, balancing three different processes (nitrification, denitrification
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39 and phosphorus removal) simultaneously in a single sludge system requires skilful
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41 management of the bacterial populations, because that the successful enrichment of
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43 PAOs can fail due to the proliferation of GAOs.
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50 **4. Conclusions**

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52 With the increase of phosphorus load, TP and TN removal was enhanced by the
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54 enrichment of PAOs. Under low COD/P ratio condition (<50), PAOs instead of GAOs
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56 were enriched. Some PAOs can use nitrate/nitrite as electron acceptors to take up the
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1 phosphorus, achieving simultaneous nitrogen and phosphorus removal. N₂O emission
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3 was reduced by the increase of phosphorus load, due to the decrease of N₂O yield by
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5 heterotrophic denitrification. Under high phosphorus load, more PHB was synthesized,
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7 easing the competition of denitrification enzymes for electrons. The enrichment of
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9 PAOs instead of GAOs was another reason for low N₂O yield.
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Figure captions:

1
2 Fig. 1 Schematic description of the experiment system.
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5 Fig. 2 Time profiles of COD, TP concentration and PHA content during one cycle in
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7 each SBR. (a) R1; (b) R2; (c) R3. Each data point is the mean of at least three
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9 repeated experiments.
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13 Fig. 3 Time profiles of nitrogen and N₂O emission rate during one cycle in each SBR.
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15 (a) R1; (b) R2; (c) R3. Each data point is the mean of at least three repeated
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17 experiments.
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21 Fig. 4 Time profiles of N₂O emission rate during one cycle in each SBR. Each data
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23 point is the mean of at least three repeated experiments.
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27 Fig. 5 N₂O emission rate of each batch experiment for three SBRs. Regarding the
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29 treatment “with nitrite addition”, white is the N₂O emission rate by nitrifier
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31 denitrification (ND), and grey is the N₂O emission rate by denitrification (D). All the
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33 data are the mean of at least three repeated experiments.
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Table 1 Mean influent and effluent contaminant concentrations with standard deviations (in brackets) and removal efficiencies in each SBR. All the data are mean values of at least 15 experiments.

Parameters	R1			R2			R3		
	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)	Influent (mg/L)	Effluent (mg/L)	Removal efficiency (%)
COD	393.9 (17.6)	28.2 (1.4)	92.8	395.9 (12.4)	20.3(2.5)	94.9	389.2 (15.3)	22.3 (3.8)	94.3
TP	4.2 (0.2)	0.6 (0.3)	85.7	9.7 (0.6)	1.3 (0.3)	86.6	14.1 (1.5)	1.5 (0.4)	89.3
TN	40.8 (2.0)	8.6 (3.4)	78.9	42.1 (2.4)	8.7 (3.6)	79.3	41.5 (2.4)	6.8 (3.2)	83.6
NH ₄ ⁺ -N	39.7 (1.7)	0.6 (0.1)	98.5	41.2 (2.1)	0.4 (0.1)	99.0	40.3 (1.8)	0.4 (0.1)	99.0
NO ₃ ⁻ -N	1.1 (0.1)	7.6 (2.3)	/	1.2 (0.3)	7.9 (3.0)	/	1.4 (0.5)	5.7 (3.1)	/
NO ₂ ⁻ -N	0	0	/	0	0	/	0	0	/

Table 2 N₂O-N emission during one cycle in each SBR. All the data are mean values with standard deviations in brackets of at least three repeated experiments.

	N ₂ O-N emission during anaerobic stage (mg/gMLSS)	N ₂ O-N emission during aerobic stage (mg/gMLSS)	Total N ₂ O-N emission (mg/gMLSS)	N ₂ O-N conversion rate (%)*
R1	0.0030 (0.00)	0.53 (0.04)	0.54 (0.04)	8.41
R2	0.0054 (0.00)	0.52 (0.04)	0.53 (0.04)	7.24
R3	0.0035 (0.00)	0.41 (0.06)	0.41 (0.06)	6.22

* N₂O-N conversion rate = N₂O-N/N removed × 100%.

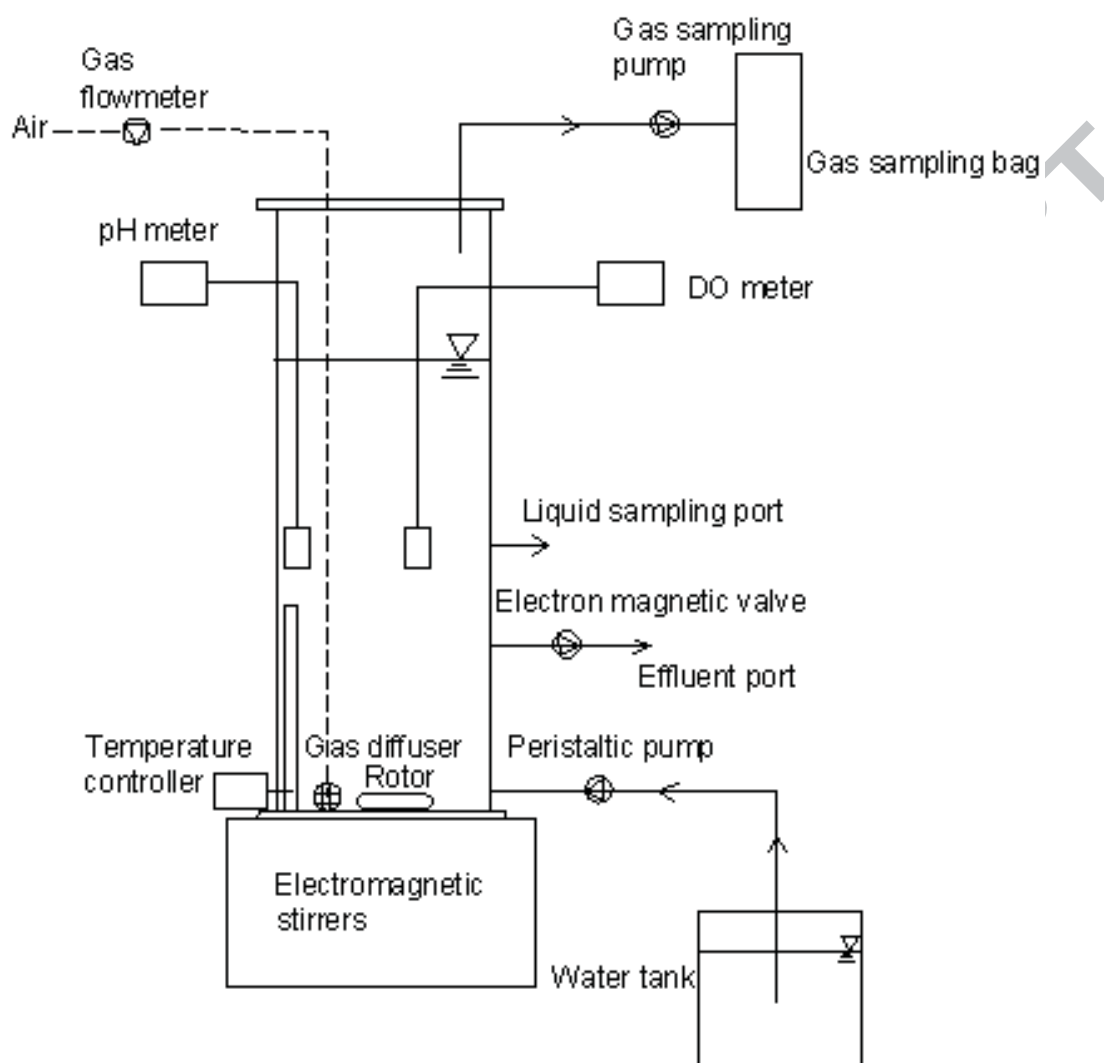


Fig. 1

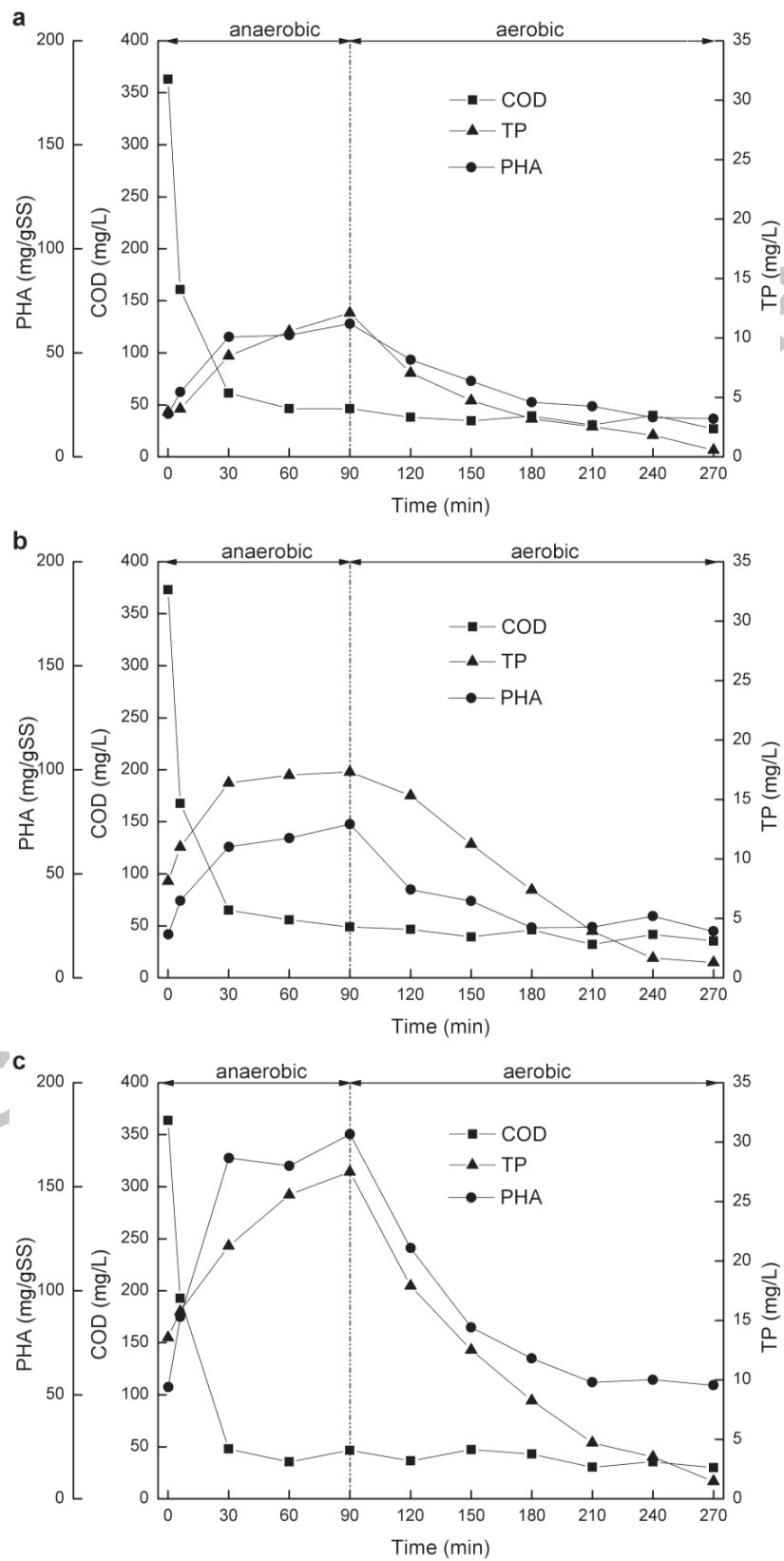


Fig. 2

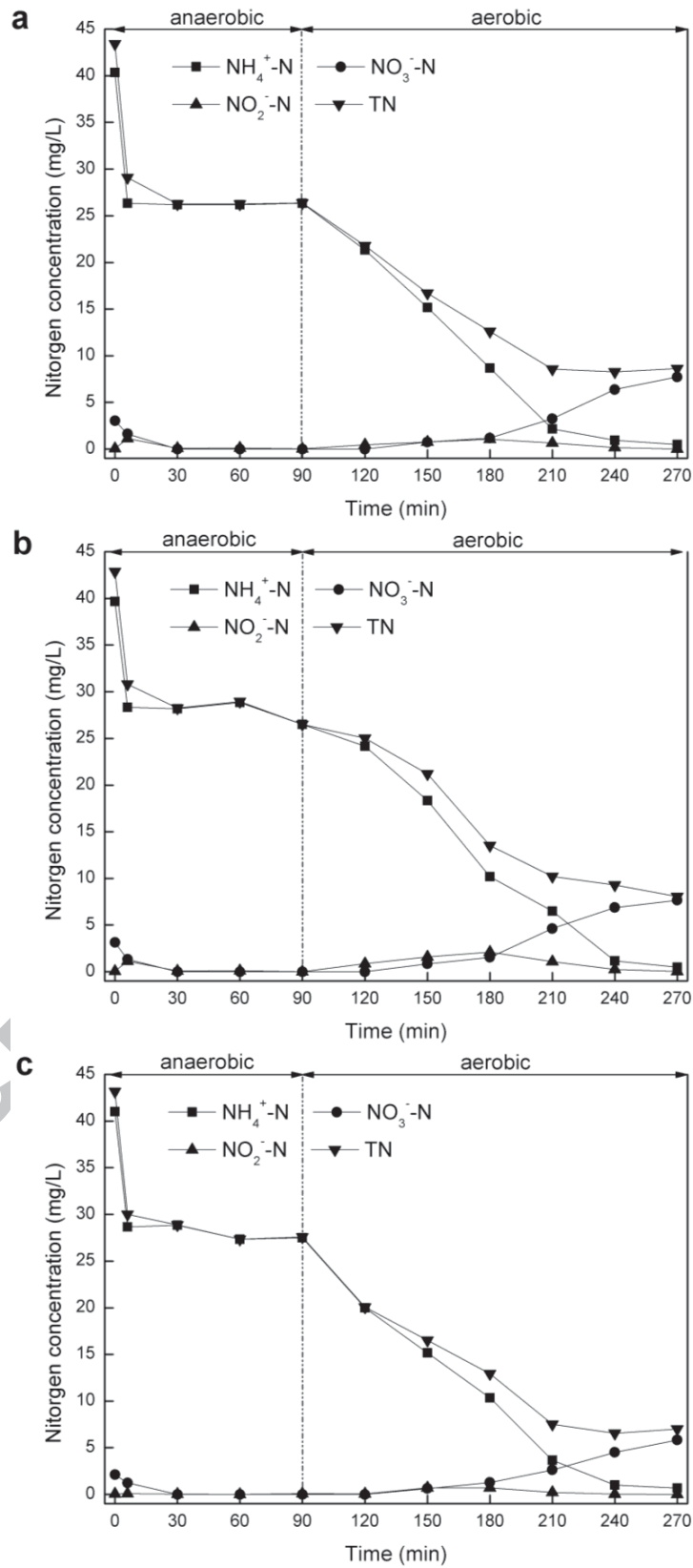


Fig. 3

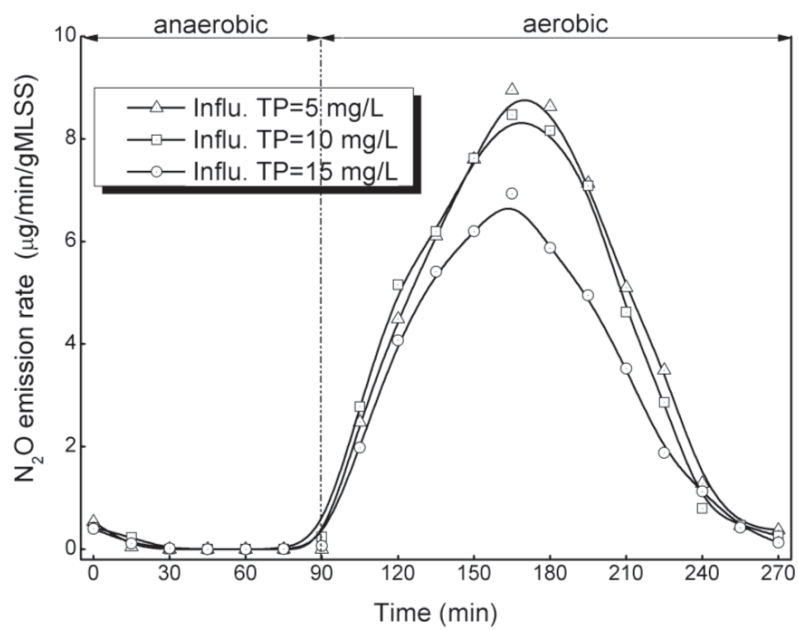


Fig. 4

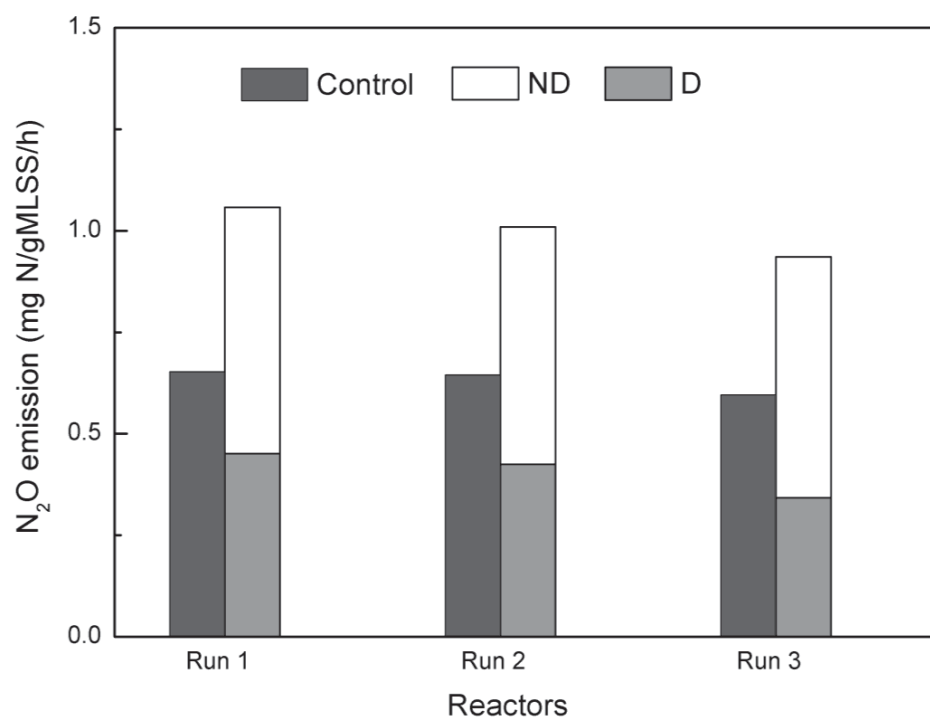


Fig. 5

Highlights

- TP and TN removal was enhanced simultaneously with the increase of phosphorus load.
- y Simultaneous N and P removal was due to denitrification of PAOs under high P load.
- y N₂O emission was reduced with the increase of P load during low-oxygen SND process.
- y N₂O yield by heterotrophic denitrification was reduced under high P load.
- y The enrichment of PAOs instead of GAOs led to low N₂O yield under high P load.