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Competition for Single Carbon Source Between Denitrification and Phosphorus Release in Sludge under Anoxic Condition*

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Abstract In order to investigate the competition for carbon source between denitrification and phosphorus release processes, simultaneous phosphorus release and denitrification in sludge operated in anoxic, aerobic mode are investigated by varying the ratio of influent COD to nitrogenous compound concentration under anoxic condition using a lab-scale sequencing batch reactor (SBR). The results show that the nitrate reduction rate is nearly independent of the ratio of influent COD to nitrate under anoxic condition. More NO_x^--N in the influent leads to less PO_4^{3-}-P release during the feeding period. However, PO_4^{3-}-P release proceeds at a low rate simultaneously with denitrification even when the influent NO_x^--N concentration is as large as 20 mg·L^{-1} and its rate is increased obviously when NO_x^--N is denitrified to a concentration lower than 0.5 mg·L^{-1}. The variation of pH during anoxic period gives some information about the biochemical reactions of denitrification and PO_4^{3-}-P release. When more nitrate is present in the influent, more acetate uptake in feeding period is used for direct microorganism growth.
Keywords denitrification, SBR, phosphorus release, pH, organic substrate

1 INTRODUCTION

Although biological nutrient removal (BNR) processes have been successfully applied in the treatment of nutrient materials such as nitrogen and phosphorus, some problems are to be resolved, especially when the ratio of influent COD to nitrogenous compound concentration is low. In order to enrich phosphate accumulation organisms (PAOs) in a BNR system, an anaerobic-anoxic (or aerobic) sequence and the presence of short chain fatty acids (SCFA) in the anaerobic phase are necessary. Meanwhile, BNR processes usually use influent carbon source to denitrify NO_x^--N with a predenitrification configuration. It seems that there exists a competition between phosphorus release and denitrification for the limited available carbon source [1]. The presence of nitrate may reduce phosphate release rate in the anaerobic stage [2]. One of the possible reasons is that phosphorus can be used by denitrifying phosphorus removing bacteria (DPB) [3]. The nitrate present is considered to provide denitrifying bacteria organic substrate by out-competing PAOs [4]. However, the inhibitory effect of nitrate on phosphorus release may be more direct because of some denitrification intermediate products [5].

Some researchers found that phosphorus release occurred when electron donor (substrate) and electron acceptor (nitrate or oxygen) were present simultaneously [6, 7], but most researchers used oxygen as electron acceptor. Wang *et al*. [8] studied the effects of oxygen level in overlying water on phosphorus release from lake sediments. Jiang *et al*. [9] found that phosphorus release extent was affected by many factors such as temperature and DO. However, the effects of NO_x^--N concentration on phosphorus release have been seldom investigated. In this study, the competition for carbon source between phosphorus release and denitrification in sludge operated in anoxic, aerobic mode is investigated by varying the ratio of influent COD to nitrogenous compound concentration under anoxic conditions.

2 MATERIALS AND METHODS

2.1 Operation of sequencing batch reactor

The experiments with 8 stages were performed in a sequencing batch reactor with 12 L working volume. In each stage the cycle consisted of 30 min feeding (anoxic stirring), 150 min aerobic reaction, 60 min settling, 5min decanting, and idling. The influent nitrogenous compound concentration was different for different stage (as shown in Table 1). At the end of aerobic period and before the mixing was stopped, excess sludge of 100 ml was withdrawn to maintain the sludge retention time (SRT) at 15 d (assuming that no solids left the system through the effluent). The mixed liquor suspended solids (MLSS) was controlled within the range of 1600−1900 mg·L^{-1}. At the end of each settling period, 3L supernatant was decanted from the reactor, resulting the hydraulic retention time

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(HRT) 12 h. The temperature was maintained at (21±1) °C with a thermostatic heater. DO, pH and ORP probes (WTW oxi340) were placed in the reactor to monitor the parameters.

2.2 Wastewater and sludge

The simulated wastewater with the following composition was used as the feeding solution: 663.8 mg of $CH_3COONa{\cdot}3H_2O$ (326.9 $mg{\cdot}L^{-1}$ as COD basis), 166.9 mg NH_4Cl (43.7 $mg{\cdot}L^{-1}$ as NH_4^+-N basis), (0−150 mg) $NaNO_2$ (0−30 $mg{\cdot}L^{-1}$ as NO_2^--N basis), (0−145 mg) KNO_3 (0−20 $mg{\cdot}L^{-1}$ as NO_3^--N basis), 18.8 mg of KH_2PO_4 (4.3 $mg{\cdot}L^{-1}$ as PO_4^{3-}-P basis), 375 mg of $NaHCO_3$ (400 $mg{\cdot}L^{-1}$ as alkalinity), 40 mg of $CaCl_2{\cdot}2H_2O$, 80 mg of $MgSO_4$ and 0.3 ml of nutrient solution per liter. One liter of nutrient solution consisted of 1.5 g of $FeCl_3{\cdot}6H_2O$, 0.15 g of H_3BO_3, 0.03 g of $CuSO_4{\cdot}5H_2O$, 0.18 g of KI, 0.12 g of $MnCl_2{\cdot}4H_2O$, 0.06 g of $Na_2MoO_4{\cdot}2H_2O$, 0.12 g of $ZnSO_4{\cdot}7H_2O$, 0.15 g of $CoCl_2{\cdot}6H_2O$, and 10 g of EDTA [10]. The seeded sludge was from Wenchang wastewater treatment plant (Harbin, China). Two months after the seeding, the experiments began when the system reached steady state as indicated by the reactor performance [11].

Table 1 Operating stages and influent nitrogenous compound concentration

Operating stages	NO_2^--N concentration /$mg{\cdot}L^{-1}$	NO_3^--N concentration /$mg{\cdot}L^{-1}$
SBR1	0	0
SBR2	0	20
SBR3	0	10
SBR4	0	5
SBR5	5	0
SBR6	10	0
SBR7	20	0
SBR8	30	0

2.3 Analytical methods

Samples taken from the reactor for analysis were immediately filtered using 0.45 mm filter papers to separate the bacterial cells from the liquid. COD_{Cr}, MLSS, MLVSS, SV, SVI, NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P were measured according to standard methods [12]. DO, pH and ORP were monitored by a WTW Multi 340i DO meter.

3 RESULTS AND DISCUSSION

3.1 Effects of nitrate on acetate uptake competition between denitrification and PO_4^{3-}-P release

Both denitrification and PO_4^{3-}-P release need organic substrate as the electron donor. According to the half reaction Eq. (1), 2.86 g COD is needed to reduce 1 g NO_3^--N to N_2. Considering the heterotrophic bacteria assimilation effect, $2.86/(1-Y_H)$ g COD will be consumed to deoxidize 1 g NO_3^--N to N_2. Y_H is the yielding coefficient of heterotrophic bacteria. According to ASM1, Y_H can be taken as 0.67 [13]. Then 8.67 g COD will be consumed to deoxidize 1 g NO_3^--N to N_2 theoretically. However, a single equation can not give how much COD is taken to release per unit mass of PO_4^{3-}-P. Some equations were obtained with different experimental conditions and sludges. Filipe *et al*. got Eq. (3) [14].

$$0.20NO_3^- + 1.2H^+ + e^- \longrightarrow 0.1N_2 + 0.6H_2O \quad (1)$$

$$0.33NO_2^- + 1.33H^+ + e^- \longrightarrow 0.17N_2 + 0.67H_2O \quad (2)$$

$$\underset{\text{Acetate}}{CH_2O} + \underset{\text{Glycogen}}{0.5CH_{10/6}O_{5/6}} + \underset{\text{Poly-P}}{\left(0.25+\alpha_{PAO}\right)HPO_3} + \left(\alpha_{PAO}-0.617\right)H_2O \longrightarrow \underset{\text{Phosphate}}{\left(0.25+\alpha_{PAO}\right)H_3PO_4} + \underset{\text{PHB}}{1.33CH_{1.5}O_{0.5}} + 0.167CO_2 \quad (3)$$

where the parameter α_{PAO} represents the energy necessary to transport one C-mol of acetate across the membrane, α_{PAO} is linearly dependent on pH and is determined experimentally. In their experiment, α_{PAO} can be represented by [14]

$$\alpha_{PAO} = 0.16 \times pH - 0.7985 \quad (4)$$

In this experiment, pH lies in the range of 7.5−8.5 during anoxic period, so we obtain that 0.67−0.84 g COD will be taken to release 1 g PO_4^{3-}-P into the bulk liquid. According to this calculation, the COD consumed to reduce per unit mass of NO_3^--N to N_2 will be 3.40−4.27 times larger than that consumed to release per unit mass of PO_4^{3-}-P. Thus the denitrification process is prone to organic substrate as the electron donor, which may be the main reason that the presence of nitrate has inhibition effect on PO_4^{3-}-P release.

Although the influent nitrate concentration was different in SBR1, SBR2, SBR3 and SBR4, the initial nitrate reduction rates (mg NO_3^--N per minute) were almost the same, as shown by Fig. 1. The reason may be that denitrification process follows 0 order reaction [15]. When nitrate concentration was all excessive at the initial period of each cycle, the limiting factor for denitrification process was the amount of enzyme participating the reaction, while the mixed liquid volatile suspended solid (MLVSS) was almost the same in all SBR stages. The initial nitrate reduction rate was all around 8 $mg{\cdot}min^{-1}$.

Nitrate has obvious negative impact on PO_4^{3-}-P release in anoxic period. When the influent nitrate concentration is 5 or 10 $mg{\cdot}L^{-1}$, PO_4^{3-}-P release rate reaches a certain value and maintains at that level in the rest of feeding period, as shown in Figs. 1 (a) and 1 (b), in which the maximum PO_4^{3-}-P release rate is

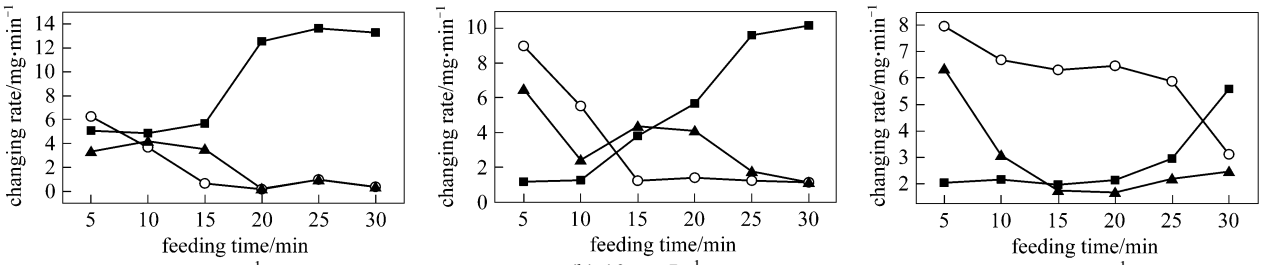

Figure 1 Polyphosphate release and nitrogenous compound deoxidize rates at different influent NO_3^--N concentration

13.28 and 10.16 mg·min^{-1}, respectively. This suggests that although nitrate is present, PO_4^{3-}-P release can proceed if the substrate is enough. No maximum PO_4^{3-}-P release rate is found when the influent nitrate concentration is as high as 20 mg·L^{-1} [Fig.1 (c)]. Interestingly, although PO_4^{3-}-P release is inhibited severely with the influent NO_3^--N concentration of 20 mg·L^{-1}, it still proceeds, because denitrifying bacteria do not have an absolute priority over PAOs with organic substrate as the electron donor, though the denitrification would produce more ATP than polyphosphate cleavage under anoxic condition [16].

The conventional enhanced biological phosphorus removal (EBPR) system can be divided into two parts: PO_4^{3-}-P release and PO_4^{3-}-P uptake. In order to achieve a satisfactory PO_4^{3-}-P removal efficiency, both parts must be operated under proper conditions. Because denitrification process competes with PO_4^{3-}-P release process to take organic substrate as electron donor, when more nitrate is present in the influent, less PO_4^{3-}-P will be released, as shown by Fig. 2. Without nitrate in the influent, PO_4^{3-}-P concentration reaches 21.86 mg·L^{-1} at the end of anoxic period, but it drops to 7.89 mg·L^{-1} at the influent nitrate concentration of 20 mg·L^{-1}. Consequently, PO_4^{3-}-P removal is worse and effluent PO_4^{3-}-P concentration is increased from 0.08 to 2.05 mg·L^{-1}.

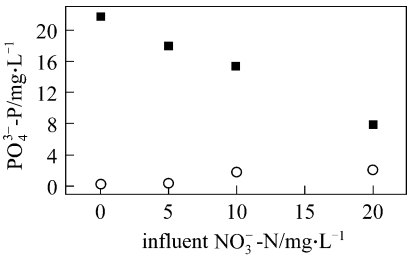

Figure 2 Effect of nitrate concentration on phosphorus removal
■ end of anaerobic period; ○ end of aerobic period

3.2 Effects of nitrite on acetate uptake competition between denitrification and PO_4^{3-}-P release

The nitrate reduction rate decreases to a low level when nitrate is denitrified almost completely. When the ratio of influent COD to nitrite concentration is large, as shown by Figs. 3 (a) and 3 (b), PO_4^{3-}-P release rate increases to a certain value and maintains at that level for the rest of anoxic period as happened in SBR5 and SBR6. The phosphorus removal is excellent (Fig. 4). However, when the ratio is small, much organic substrate is used by denitrifying bacteria as electron donor, deteriorating the PO_4^{3-}-P release process, since there is no maximum PO_4^{3-}-P release rate in the feeding period [Fig 3 (c)]. When the influent nitrite concentration is as high as 30 mg·L^{-1}, PO_4^{3-}-P release is inhibited so severely that its rate changes little and maintains at a low level during the entire feeding period.

Interestingly, the nitrite reduction rate increased a little when nitrate was denitrified completely (data not shown), which was associated with the increase of PO_4^{3-}-P release rate. This phenomenon occurred in SBR5, SBR6, SBR7 and SBR8 (Fig. 3). The reason may be that when nitrate is denitrified completely, bio-P bacteria have more opportunity to use acetate to synthesis poly-β-hydroxybutyrate (PHB). More polyphosphate will be hydrolyzed to provide enough ATP for this process. While nitrite is present in the medium, it has more opportunity to be reduced by denitrifying bacteria through taking more influent acetate as electron donor. If the ratio of influent COD to nitrite concentration is as large as those in SBR5, SBR6 and SBR7, nitrite reduction rate will drop to a low level when nitrite is denitrified completely. It can maintain at a high level when the ratio is as small as that in SBR8.

The phosphorus removal is not affected by the presence of nitrite in SBR5 and SBR6, but it is deteriorated in SBR7, as happened in SBR4. Although the effluent PO_4^{3-}-P concentration is almost the same, the amount of PO_4^{3-}-P released in the anoxic period is very different. According to Eqs. (1) and (2), 40% COD is saved in denitrification process when NO_2^--N is used as electron acceptor instead of NO_3^--N. More acetate is used to synthesize PHB in SBR7, releasing more PO_4^{3-}-P in feeding period. It should be pointed out that although denitrifying 20 mg·L^{-1} NO_3^--N or 30 mg·L^{-1} NO_2^--N to N_2 consumes almost the same amount of organic substrate, the phosphorus removal nearly stops in SBR8 while it still proceeds in SBR4. The reason maybe that nitrite has toxicity effect on

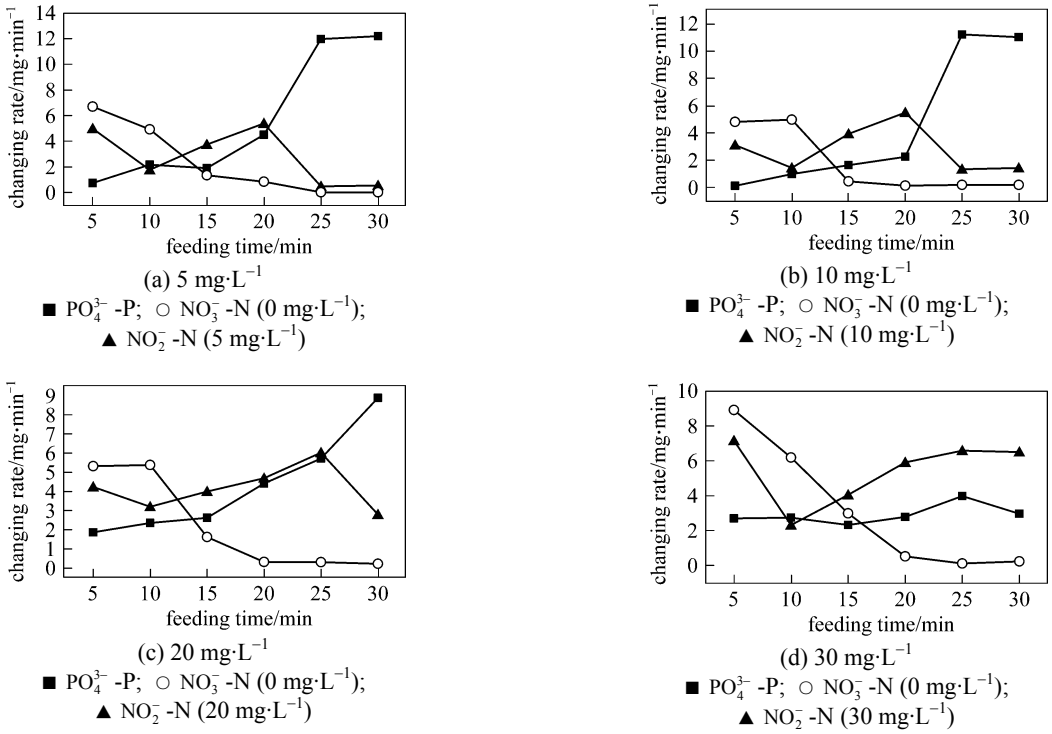

Figure 3 Polyphosphate release and nitrogenous compound deoxidize rate at different influent concentration of NO_2^--N

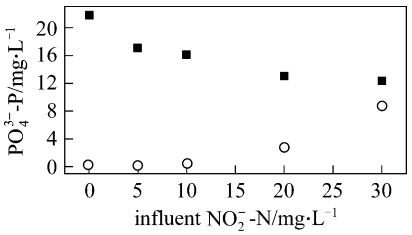

Figure 4 Effect of nitrite concentration on phosphorus removal
■ end of anaerobic period; ○ end of aerobic period

bio-P bacteria, seriously influencing their metabolic process, which is in accordance with the results in literature [17].

It was found that PO_4^{3-}-P release rate maintained at a low level in anoxic period when NO_x^--N concentration was large (date not shown) in all experimental stages. The rate would not increase obviously until NO_x^--N was denitrified to a concentration lower than 0.5 mg·L^{-1}. Akin and Ugurlu considered that phosphorus release could take place under anoxic condition whether nitrate existed or not as long as organic substrate was present [18]. Peng *et al*. [19] pointed out when NO_3^--N concentration was higher than 0.5 mg·L^{-1}, PO_4^{3-}-P release would stop, while in our experiment it proceeded. The researchers [18, 19] believed that PO_4^{3-}-P release process would be influenced seriously when NO_x^--N concentration was higher than 0.5 mg·L^{-1}. The threshold is so little that even 0.5 mg·L^{-1} NO_3^--N or NO_2^--N will restrain PAOs to use acetate obviously. PAOs are sensitive to the presence of NO_x^--N, and this maybe one of the reasons why EBPR systems are usually unstable.

3.3 Variations of parameters during feeding phase

Denitrification process using acetate as organic carbon source can be represented as follows.

$$5CH_3COOH + 8NO_3^- \longrightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \quad (5)$$

In the above heterotrophic denitrification reaction, one equivalent of alkalinity is produced when one equivalent of NO_3^--N is reduced, which equals to 3.57 g of alkalinity (as $CaCO_3$) production with 1 g of NO_3^--N reduced. Denitrification process contained two steps. The first step is from NO_3^--N to NO_2^--N, and the second step is from NO_2^--N to N_2. Alkalinity was mainly produced in the second step. According to Eq. (3) acidity is produced in PO_4^{3-}-P release process, and its amount depends on the reaction conditions. pH value increases in denitrification process and decreases in PO_4^{3-}-P release process. According to this principle, pH variation during the feeding period gives some informations about the biochemical reactions for denitrification and PO_4^{3-}-P release. Because denitrifying bacteria are prone to acetate than PAOs, pH should first increase during the feeding period, then decrease if PO_4^{3-}-P release process becomes dominate, as shown by Figs. 5 and 6.

pH variation curves during the feeding period can be divided into three parts approximately: slow increase,

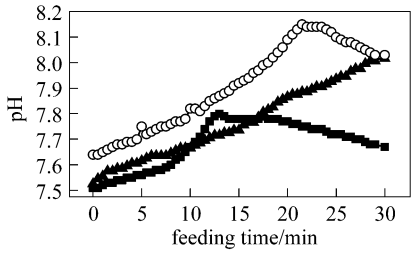

Figure 5 pH variation in feeding period at different influent NO_3^--N concentrations

■ NO_3^--N (5 mg·L^{-1}); ○ NO_3^--N (10 mg·L^{-1}); ▲ NO_3^--N (20 mg·L^{-1})

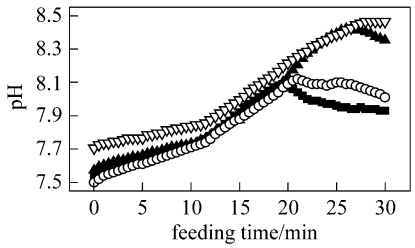

Figure 6 pH variation in feeding period at different influent NO_2^--N concentrations

■ NO_2^--N (5 mg·L^{-1}); ○ NO_2^--N (10 mg·L^{-1}); ▲ NO_2^--N (20 mg·L^{-1}); ▽ NO_2^--N (30 mg·L^{-1})

rapid increase and rapid decrease. Each part is related to different reaction process. The slow increase is associated with denitrification process of NO_3^--N and NO_2^--N, the rapid increase is associated with NO_2^--N denitrification and rapid decrease is associated with PO_4^{3-}-P release. It is found that when NO_3^--N is denitrified completely, the slope of pH increase is usually larger. The reason is that most alkalinity is produced when NO_2^--N is denitrified to N_2, and its denitrifying rate is higher than that when NO_3^--N is denitrified to NO_2^--N. Thus the less NO_3^--N or NO_2^--N in the influent, the earlier the pH peak value will appear. Interestingly, the slopes of slow increase of pH are almost the same in all SBR stages, probably because denitrifying from NO_3^--N to NO_2^--N is the rate-limiting step in denitrification process. Denitrification process follows 0 order reaction [20], so the factor to determine its rate is the amount of enzyme participating the reaction that are almost the same in all SBR stages [21].

3.4 Effects of NO_x^--N on using ways of organic substrate

Organic substrate uptake in the feeding period can be used in two ways. One part of them is converted into storage polymer such as polyhydroxybutyrate (PHB) when the external substrate is acetate, and the other part is used for direct microbial growth. Ash *et al*. [22] found that the more NO_3^--N in the influent made more acetate uptake convert to simultaneous direct growth. In our study, operation patterns were the same for all experimental stages. At the end of aerobic period and before the mixing was stopped, 100 ml excess sludge was withdrawn to maintain a relatively stable MLSS. However, MLSS still showed a slight fluctuation (as shown in Tables 2 and 3) in the experimental stages. It was observed that the average MLSS increased slightly when more NO_3^--N was present in the influent, demonstrating that NO_3^--N can stimulate microorganisms directly to use organic substrate for their growth. In addition, it was found that the average MLSS would increase with influent NO_2^--N concentration (except in SBR 8). The increasing extent was larger than those when NO_3^--N was present in the influent, because NO_2^--N inhibites polymer storage more significantly than NO_3^--N. In other words, the main reason for PHB storage inhibition is NO_2^--N accumulation in the reactor which always happens when the ratio of influent COD to nitrogenous compound concentration is not high enough to denitrify all NO_3^--N available [23]. If influent nitrogenous compound is changed from NO_3^--N to NO_2^--N, NO_2^--N accumulation extent will become larger. When influent NO_2^--N increases to a certain extent, it may disturb microbial biochemical reactions, so the average MLSS decreases when influent NO_2^--N is high, such as 30 mg·L^{-1}.

Table 2 Average MLSS at different influent NO_3^--N concentration

NO_3^--N concentration/mg·L^{-1}	MLSS/mg·L^{-1}
0	1666
5	1689
10	1691
20	1712

Table 3 Average MLSS at different influent NO_2^--N concentration

NO_2^--N concentration/mg·L^{-1}	MLSS/mg·L^{-1}
5	1737
10	1847
20	1916
30	1866

Experimental results in this study indicate that the competition for carbon source between denitrification and PO_4^{3-}-P release strongly depends on the ratio of influent COD to NO_x^--N concentration. When the ratio is small, PO_4^{3-}-P release does not proceed sufficiently. Meanwhile, NO_2^--N is prone to accumulation, deteriorating the PO_4^{3-}-P removal performance. However, even when NO_x^--N concentration is large, PO_4^{3-}-P release proceeds at a low rate, though the mechanism is still unknown. In this experiment, sodium acetate is used as electron donor. For other carbon source such as glucose and sodium propionate,

results may be different.

4 CONCLUSIONS

NO_3^--N reduction rate is almost independent of the ratio of influent COD to NO_3^--N concentration under anoxic condition, and it is only determined by the amount of enzyme participating the denitrification process. More NO_x^--N in the influent reduces the amount of PO_4^{3-}-P released during feeding period. PO_4^{3-}-P release rate maintains at a low level when NO_x^--N concentration in solution is higher than 0.5 $mg \cdot L^{-1}$, but it increases obviously when NO_x^--N is denitrified to a concentration lower than 0.5 $mg \cdot L^{-1}$. However, PO_4^{3-}-P release proceeds simultaneously with the denitrification even when influent NO_x^--N concentration is large. In addition, NO_2^--N accumulation deteriorates microbial activity seriously. The pH variation during the feeding period gives some information about the biochemical reaction process of denitrification and PO_4^{3-}-P release. When more NO_3^--N is present in the influent, more acetate uptake in feeding period is used for direct microorganism growth.

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