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Characteristics of anoxic phosphors removal in sequence batch reactor

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Abstract

The characteristics of anaerobic phosphorus release and anoxic phosphorus uptake were investigated in sequencing batch reactors using denitrifying phosphorus removing bacteria (DPB) sludge. The lab-scale experiments were accomplished under conditions of various nitrite concentrations (5.5, 9.5, and 15 mg/L) and mixed liquor suspended solids (MLSS) (1844, 3231, and 6730 mg/L). The results obtained confirmed that nitrite, MLSS, and pH were key factors, which had a significant impact on anaerobic phosphorus release and anoxic phosphorus uptake in the biological phosphorous removal process. The nitrites were able to successfully act as electron acceptors for phosphorous uptake at a limited concentration between 5.5 and 9.5 mg/L. The denitrification and dephosphorous were inhibited when the nitrite concentration reached 15 mg/L. This observation indicated that the nitrite would not inhibit phosphorus uptake before it exceeded a threshold concentration. It was assumed that an increase of MLSS concentration from 1844 mg/L to 6730 mg/L led to the increase of denitrification and anoxic P-uptake rate. On the contrary, the average P-uptake/N denitrifying reduced from 2.10 to 1.57 mg PO_4^{3-}-P/mg NO_3^--N. Therefore, it could be concluded that increasing MLSS of the DEPHANOX system might shorten the reaction time of phosphorus release and anoxic phosphorus uptake. However, excessive MLSS might reduce the specific denitrifying rate. Meanwhile, a rapid pH increase occurred at the beginning of the anoxic conditions as a result of denitrification and anoxic phosphate uptake. Anaerobic P release rate increased with an increase in pH. Moreover, when pH exceeded a relatively high value of 8.0, the dissolved P concentration decreased in the liquid phase, because of chemical precipitation. This observation suggested that pH should be strictly controlled below 8.0 to avoid chemical precipitation if the biological denitrifying phosphorus removal capability is to be studied accurately.

Key words: biological phosphorus removal; nitrite; MLSS; pH; denitrifying phosphorus removing bacteria (DPB); anaerobic-anoxic processes

Introduction

An anaerobic/anoxic (A_2) process has been proposed for biological phosphorus removal since 1980 (Vlekke *et al*., 1988; Wanner *et al*., 1992; Kern-Jesperen and Henze, 1993; Kuba and van Loosdrecht, 1993, 1996; Meraouki *et al*., 1999). These new processes are based on the activity of denitrifying phosphorus removing bacteria (DPB), which can use nitrate as an electron acceptor, therefore, allowing simultaneous denitrification and phosphorus uptake using the same COD. This is of significance as organic carbon content in most of the municipal wastewater is often limited for phosphorus and nitrogen removal. Employing DPB in the biological nutrient removal processes also makes it possible to reduce aeration and sludge production (Copp and Dold, 1998).

In the biological nitrogen removal system, nitrite appears as an intermediate in the course of nitrification and denitritication. Generally, the accumulation of nitrite is known to cause severe problems in the biological processs. Nowadays, nitrification and denitrification via nitrite has been confirmed experimentally and practically (Abeling and Seyfried, 1992; Yang and Alleman, 1992; Akunna *et al*., 1993). Investigation of the shortened nitrogen removal pathway via nitrite, Turk and Mavinic (1986) revealed: (1) 40% reduction of COD demand during denitrification; (2) 63% higher rate of denitrification; (3) 300% lower biomass yield during anaerobic growth. If nitrites can be used as electron acceptors in the denitrificaiton dephosphorus reaction by DPB, short-cut nitrification and anoxic phosphorus removal can be integrated in the same reactor system. Theoretically, organic matter requirement and oxygen consumption in the biological system will diminish. This is of particularly interesting when biologically removing nitrogen and phosphorus from wastewater with low COD. However, several previous studies have demonstrated that nitrite as an electron acceptor caused inhibitory effects on anoxic phosphorus uptake (Comeau *et al*., 1986; Kerrn-

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Jespersen *et al*., 1994). Kuba and van Loosdrecht (1993), even reported that nitrite (approximately 5–10 mg NO_2^--N/L) strongly inhibited the phosphorus uptake activities in an anaerobic/anoxic sequencing batch reactor (A_2SBR). Recently, a series of batch tests were carried out using phosphate accumulating organisms (PAO) sludge and the results indicated that the presence of nitrite inhibited both aerobic and anoxic phosphate uptake (Saito *et al*., 2004). On the other hand, Meinhold *et al*. (1999) discovered that nitrite at low concentration level (4–5 mg NO_2^--N/L) is not detrimental to anoxic phosphorus uptake and can still serve as an electron acceptor for anoxic phosphorus uptake. During another experiment involving an anaerobic/aerobic/anoxic/aerobic SBR ($(AO)_2$SBR), it was found that nitrite up to 10 mg/L was not an inhibitor to phosphorus removal processes, therefore, nitrite was deemed to be an alternative electron acceptor to oxygen or nitrate (Lee *et al*., 2001). A review of the above literature revealed that controversial conclusions of the nitrite influence on the biological anoxic phosphors removal were proposed. Thus, the exact metabolic behavior of DPB with respect to anoxic phosphorus uptake using nitrites as electron acceptors remains to be adequately evaluated, if it is aimed at establishing a stable performance of denitrifying dephosphorus removal processes.

Performance of denitrifying phosphorus removal is also affected, among other factors, by pH and the concentration of mixed liquor suspended solids (MLSS). Impact of pH on the biological phosphorus removal process (BPR) has been studied using PAOs sludge during recent years (Comeau *et al*., 1986; Filipe *et al*., 2001; Schuler *et al*., 2002). It has been observed that increasing pH enhanced the anaerobic P release rate of enhancing biological phosphorus removal (EBPR) biomass. The same pH influence was also obtained for the DPB sludge cultivated in an A_2SBR (Comeau *et al*., 1986; Smolders *et al*., 1994). Although, because of the chemical precipitates, the P release rate has been shown to decrease when the pH increases to 8 (Kuba *et al*., 1997). Unfortunately, no study has been done to estimate the effect of pH on anoxic phosphorus uptake using DPB sludge, and also there are few publications reporting the influence of MLSS on denitrifying phosphorus removal.

According to the literature, most of the studies on biological phosphorus removal are focused on the aerobic phosphorus uptake metabolism up to now, whereas, there are relatively few publications investigating the anoxic phosphorus removal characteristics. It is well known that the denitrifying dephosphorus processes are truly very significant to treat the sewage or industry wastewater with low C/N. In this study, several types of batch tests were conducted using the DEPHANOX sludge, enriched under anaerobic-anoxic conditions. The purpose of this research, therefore, was to validate and study the effect of nitrite, MLSS, and pH, on phosphate release and anoxic phosphate uptake performances, and to explore the optimal operational parameters threshold for the stable performance of denitrifying phosphorus removal processes.

1 Materials and methods

1.1 Activated sludge

All experiments employed, activated sludge obtained from a lab-scale DEPHANOX process with a real sewage wastewater feed (Fig.1) (Kuba *et al*., 1996). The process has proved to be very efficient because it drives the utilization of organic substrate either for phosphorus or for nitrogen removal. It has been demonstrated that the DEPHANOX system can achieve for good nutrient efficiency when it treats synthetic wastewater (Kuba *et al*., 1996), piggery wastewater (Sorm *et al*., 1996), and municipal wastewater (Hu *et al*., 2000).

In this study, the DEPHANOX system was operated continuously over 300 d (Wang *et al*., 2004). The SRT of DPB sludge maintained at 14 d and a sludge concentration was around 4500 mg/L. In addition, NO_3^--N was scarcely detected in the final effluent and the effluent TP concentration was usually below 0.5 mg/L during the whole running period.

1.2 Batch experiments

The tests were conducted as batch experiments in 4-L SBRs made of glass and fitted with mixers (Fig.2). The mixers were stirred continuously to keep the activated sludge in suspension except in the settling period. During the reaction, the dissolved oxygen (DO), pH, and oxidation-reduction potential (ORP) were detected on-line

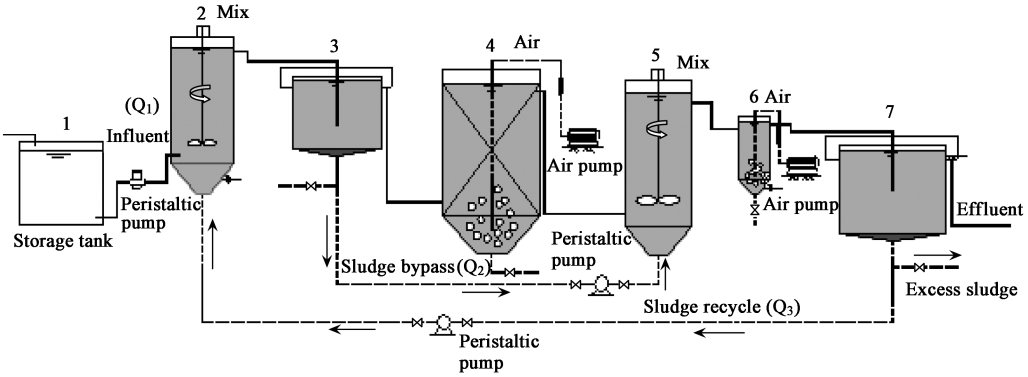

Fig. 1 Schematic diagram of the DEPHANOX process. (1) storage tank; (2) anaerobic tank; (3) internal settler; (4) fixed biofilm nitrification; (5) anoxic tank; (6) post-aeration tank; (7) final settler.

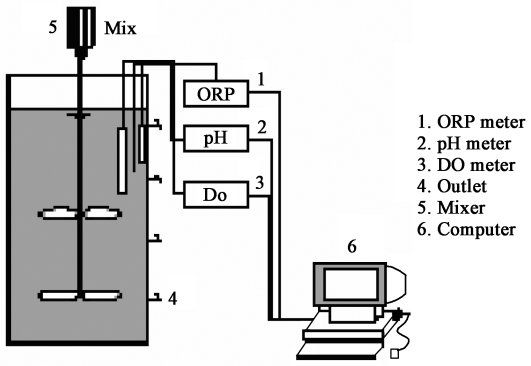

Fig. 2 Experimental SBR system with the control equipment.

automatically. Samples were collected from the reactor at regular intervals and were immediately centrifuged at 3000 r/min for 1 min.

1.3 Experimental approach

In each test series, several SBRs were operated in parallel. The sludge taken from settlers of the DEPHANOX process was usually diluted by means of tap water, before it was transferred into the reactors. After settling for 30 min, the liquid phase was decanted and the sludge was resuspended in tap water. SBRs were operated on the synthetic wastewater prepared with tap water. COD, phosphorus, nitrite, and nitrate concentration were adjusted by adding NaAc, KH_2PO_4, $NaNO_2$, and KNO_3 respectively. The pH was manually controlled at 7.0±0.1 by addition of HCl (0.5 mol/L) or NaOH (1 mol/L), to avoid chemical phosphate precipitation. The batch experiments were repeated at different SBR operational conditions, four times, and the average results were reported.

1.3.1 Experiment No. 1: effect of nitrite concentration on the phosphorus uptake

The activated sludge (1400 ml) used for this test was withdrawn from the internal settler. After being washed twice in tap water (to ensure that no external carbon source existed), the sludge was distributed to three SBRs. Synthetic wastewater was pumped into the SBRs during the first 5 min, to supply the initial PO_4^{3-}-P concentration of 10 mg/L. Simultaneously, reactors received different amounts of nitrite (equivalent to 5.5, 9.5 and 15 mg NO_2^--N/L). The MLSS in all runs were maintained at approximately 2850 mg/L. The react retention time was 4 h. The ambient temperature in the experiments was around 24°C, and the measured sludge temperature was around 25°C.

1.3.2 Experiment No. 2: effect of MLSS on phosphorus release and anoxic uptake

The sludge used in this experiment was transferred from the final settler and distributed to three reactors with different volume (V_A = 140 ml; V_B = 250 ml, and V_C = 515 ml). Synthetic wastewater was pumped into each reactor at the start of the anaerobic phase to give 100 mg COD/L and 9.0 mg PO_4^{3-}-P/L initially. The measured MLSS of the three reactors were 1844, 3231, and 6730 mg/L, respectively. After anaerobic reaction for 3 h, an anoxic period started by adding potassium nitrate (NO_3^- concentration of 30 mg/L). Then anoxic react was carried on for 4 h, temperature 22±1°C.

1.3.3 Experiment No. 3: effect of pH

This experiment can be divided into two parts. (1) pH effect on phosphorus release. The sludge withdrawn from the final settler was immediately distributed to six reactors. Synthetic wastewater was pumped into each reactor to give 100 mg COD/L initially. At the beginning of the anaerobic phase, the pH was adjusted from 7.0 to a set point (6.0, 6.5, 7.0, 7.5, 8.0, and 8.5). The anaerobic react time lasted for 1.5 h. (2) pH effect on anoxic phosphorus uptake. The activated sludge withdrawn from the internal settler was divided into two reactors (reactors A and B). Initial PO_4^{3-}-P and NO_3^--N of the two runs were 20 mg/L and 42 mg/L respectively. The initial value of pH was set at 7.0. During the whole period of the reaction, the pH of run B was controlled at 7 ± 0.1 by the addition of HCl, but the pH was not controlled in run A, although its variations were recorded. The temperature of the two reactors was kept at 22±1°C.

1.4 Analytical methods

DO and temperature were measured continuously using a WTW oxygen probe. Continuous monitoring of pH and oxidation reduction potential (ORP) were carried out using two WTW inolab pH level 2 meters with an ORP electrode and a pH probe (WTW). COD_{Cr}, PO_4^{3-}-P, NO_3^--N, NO_2^--N, and MLSS were measured according to standard methods (APHA, 1995).

2 Results and discussion

2.1 Effect of nitrite concentration

The detected nitrite concentration in the nitrification tank of DEPHANOX was very low (NO_2^--N⩽1 mg/L), which implied that the activated sludge employed for the batch experiments had never been acclimated under anoxic conditions with nitrite as an electron acceptor. Experiment No. 1, shown in Fig.3, exhibits the typical dynamic profiles of PO_4^{3-}-P and NO_2^--N concentration. The nitrite and phosphorus reduction rates of these three runs can be observed with the help of Table 1.

Fig.3 reveals that phosphate uptake performance of runs A and B appeared not to be negatively influenced by nitrite at concentrations of 5.5 mg/L and 9.5 mg/L. Phosphate concentration in both these reactors decreased with the

Table 1 Relationship between initial phosphorus uptake rate and nitrite reduction with P/N ratio

Batch No.1	Initial P/N (mmol/mmol)	Phosphorus uptake rate (0–30 min) (mgP/(g MLSS·h))	Denitrification rate (0–30 min) (mgN/(g MLSS·h))
Run A	0.90	2.26	3.86
Run B	0.48	0.86	2.56
Run C	0.31	–	2.23

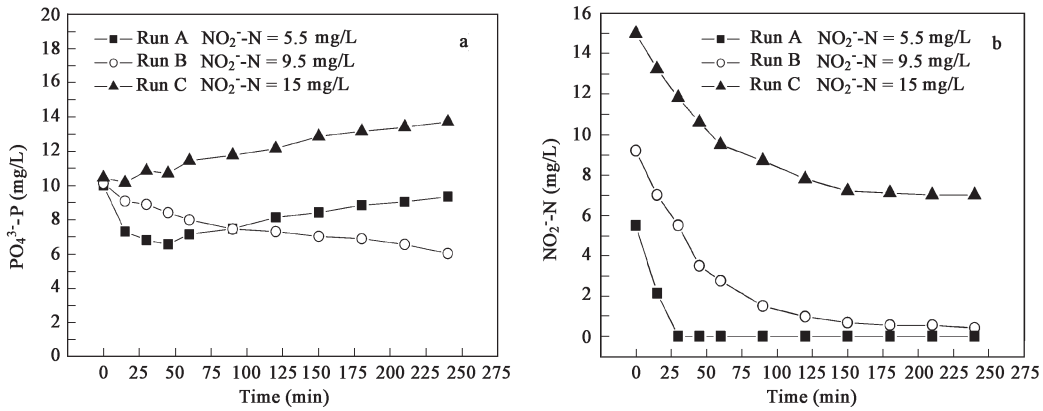

Fig. 3 Effect of NO_2^--N concentration on anoxic phosphate uptake.

reduction of nitrite. Moreover, the nitrite consuming rate in the initial 30 min in run A was much higher than in run B. Contrarily, P-uptake behavior of run C was completed inhibited.

For run A, nitrite was denitrified rapidly accompanied by anoxic phosphorus uptake, and then 5 mg NO_2^--N/L of the nitrite was completely exhausted within the initial anoxic 30 min. Simultaneously, the PO_4^{3-}-P concentration reached the minimum value (7.15 mg/L) at 30 min and was subsequently followed by a second "endogenous" phosphorus release. In run B, phosphate uptake and denitrification occurred simultaneously, immediately after the nitrite solution was added (about 9.5 mg/L). However, the phosphate uptake rate and nitrite reduction rate were lower compared to the rates of reactor A (Table 1). The results implied that the phosphate uptake behavior of the DPB was negatively affected, but not completely inhibited when the DPB was exposed to comparatively higher nitrite concentration (i.e., at 9.5 mg/L). This relatively good tolerance of the DPB's anoxic capability against nitrite probably came from the anoxic metabolism of the nitrite, giving lower concentration near the cell (Saito *et al*., 2004).

In the case of run C, the anoxic phosphorus uptake was not observed, although nitrite concentration decreased slowly. It was probably because the severe inhibition caused by the nitrite occurred when the DPB was abruptly exposed to a higher nitrite concentration, which had exceeded a certain threshold concentration. Furthermore, it was noted from Fig.3a, that this kind of severe inhibition lasted as long as the nitrite remained in the system. Weon *et al*. (2002) deemed that the mechanism of nitrite inhibition had been associated with its effect on bacterial membranes and energy generation. However, the exact mechanism of nitrite toxicity remains to be clarified. In contrast, the profile of nitrite concentration decreased gradually with the constant denitrifying rate. The reduction of the nitrite might be attributed to the denitrification by the denitrifying bacteria, but not denitrifying phosphorus removing bacteria (DPB). Saito *et al*. (2004) proposed that nitrite did not have a negative impact on the enzyme system related to denitrification, but rather on the enzyme system related to phosphate uptake and potential polyphosphate formation. This could be the reason for the fact that nitrites reduced but phosphates did not.

Experiment results of run A revealed that the nitrite concentration reached nearly zero at 30 min. Subsequently, strict anaerobic conditions were induced, which resulted in a phosphorus release. For run C, the phosphate release at a low rate was also observed. This phosphate release, further referred to as a "secondary release", is presumably associated with the storage of organic substrates (such as, PHB), but endogenous effects might also contribute to this P-release, though to a lesser extent.

The above result is in good agreement with the findings of Meinhold *et al*. (1999), and Lee *et al*. (2001), that nitrite was not an inhibitor to denitrifying phosphorus removal, if only nitrite concentration was controlled under the threshold value.

As mentioned in the introduction of this article, the threshold value of nitrite obtained from different experiments may be significantly different (Kuba and van Loosdrecht, 1993; Saito *et al*., 2004; Meinhold *et al*., 1999; Lee *et al*., 2001). Actually, the threshold value mainly depends on the sludge condition, such as, the extent of sludge adaptation to nitrite. If DPB sludge is incubated using nitrites as electron acceptor, it can be predicted that an increase in nitrite-denitrification rate will be included in the denitrifying phosphorus removal process. Accordingly, the obstacle that the low COD of municipal wastewater limits for P and N removal can be better avoided.

2.2 Effect of MLSS concentration

As an activated sludge process, the performance of denitrifying phosphorus removal processes is also influenced by the MLSS concentration. In this study, the performance of anoxic phosphorus uptake, with respect to the difference of MLSS, was evaluated under a constant NO_3^--N concentration of 33 mg/L. The variation of COD, nitrate, and phosphate concentration at different MLSS is highlighted in Fig.4.

The capability of phosphorus release and HAc uptake under anaerobic conditions increased with increasing MLSS concentration (Figs.4a and 4b). This may be because of an increase in the energy required for cell maintenance. Fig.4b also reveals that it was run C in which the phosphorus concentration approached a stable value first (a plateau occurred).

After the addition of nitrate, the phosphorus uptake

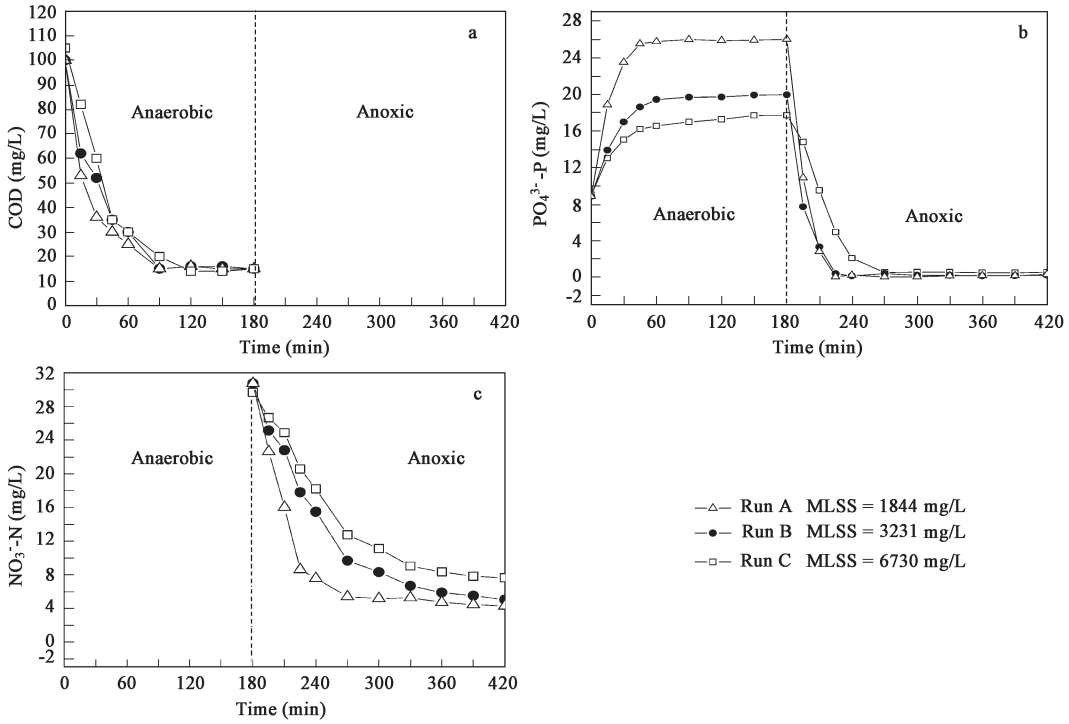

Fig. 4 Effect of MLSS on phosphorus release and anoxic phosphate uptake.

was immediately observed in all tests. Varying the MLSS from 1844 mg/L to 3231 mg/L, improved the phosphorus removal efficiency. Nevertheless, it is interesting to find that increasing MLSS up to 6730 mg/L resulted in a small change in the specific denitrification and phosphorus uptake rate (Fig.5). It has been clearly shown in Fig.4 that the denitrification and P-uptake rate accelerated with a corresponding increase in MLSS. Although, based on the values of Fig.5, it is noted that the amount of phosphate taken up per nitrogen denitrified, over a period of the initial 30 min, was reduced from 2.10 to 1.57 mg PO_4^{3-}-P/mg NO_3^--N, with increasing MLSS. This observation suggests that the high level of MLSS concentration might lead to a lower specific rate of P-uptake and denitrification. The diminution of both rates at high MLSS may be caused by the competition between different trophic groups found in the sludge.

It was also observed that, with limited nitrate amount addition, the batch test with high MLSS could easily result in the second phosphorus release than the low one (personal experience of the authors, data not shown). Generally, this second phosphorus release was unfavorable for optimum phosphorus removal control. In this study, therefore, it was suggested that MLSS be controlled between the range of 3000–4000 mg/L, which seemed to be the optimal condition for the denitrifying phosphorus removal process.

2.3 Effect of pH

2.3.1 Effect of pH on phosphorus release

The pH of a combined biological nutrient removal system requires that it be carefully monitored, as the various processes, such as, nitrification, denitrification, P-release, and P-uptake, have specific pH ranges within which they can be optimized. It has been found that variation of pH in the denitrifying phosphorus removal process, which introduces denitrification to the phosphorus

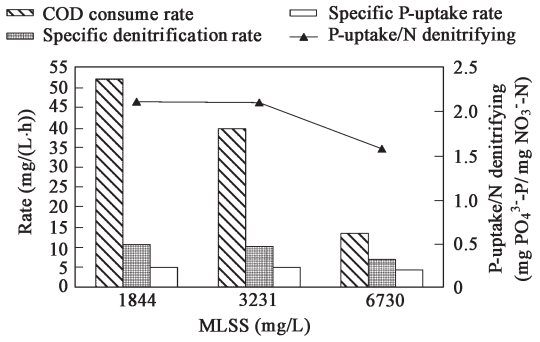

Fig. 5 COD, nitrate consumption rate and phosphorus uptake rate versus biomass concentration. Initial 0–30 min of anaerobic and anoxic reaction.

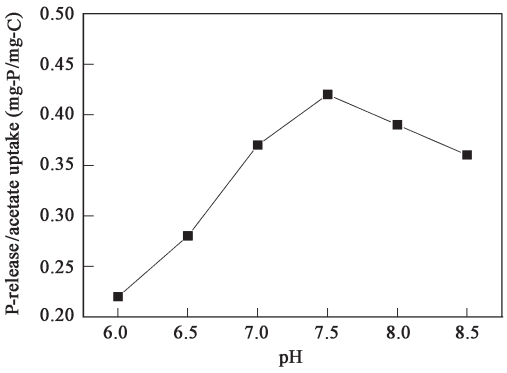

Fig. 6 Correlation between the anaerobic P-release/acetate uptake ratio and pH.

uptake period is much more complicated than the conventional EBPR process. Fig.6 illustrates the correlation between the anaerobic P-release/acetate uptake ratio and pH. For the investigated pH range of 6.0–8.5, anaerobic P-release/acetate uptake was found to increase from 0.22 to 0.42 mg-P/mg-C, with increasing the pH. The result was consistent with the previous investigations, in which it was presumed that interpretation of pH influence on the energy need for acetate uptake was on account of the increasing electrical potential differences across the cell membrane with increasing pH (Lee *et al*., 2001; Sorm *et al*., 1996). Also, Kaback (1976) and Baronofsky *et al*. (1984) illuminated the detailed mechanism of the influence of pH, which contributed to the anaerobic P-release/acetate increasing with pH. In this study, the P-release/acetate uptake apparently decreased from 0.42 to 0.39 mg-P/mg-C, abruptly, when pH was increased up to 8.0. This was mainly caused by the "natural" chemical precipitation phosphate compounds. At the end of the experiment, the pH of run A was adjusted to 7.0 by the addition of HCl. It was interesting to find that the phosphate concentration increased and did not maintain at 0 mg/L. This experimental phenomenon was similar to that of Kuba *et al*. (1997), who studied the pH influence by DPB sludge from an A_2SBR system.

The above experiments proved that phosphorus precipitation does occur with pH⩾8.0. As pH increases significantly during the denitrifying phosphorus removal phase, the following experiment was conducted to explore whether the high pH (e.g. pH⩾8.0) would disturb the biological anoxic phosphorus uptake.

2.3.2 Effect of pH on anoxic phosphorus uptake

The impact of pH on anoxic phosphorus uptake is shown in Figs.7 and 8. Fig.7 reveals that there is no remarkable difference between runs A and B in terms of variation of nitrate concentration. The dynamic of phosphate concentration, however, should be discussed in detail. Fig.8 indicates that there is a rapid pH increase at the beginning of the anoxic period in run A, which may be caused by the stripping of H^+ produced during denitrification. In terms of the phosphorus uptake react of the two reactors, the calculated specific phosphate uptake rate is almost the same as it was at the beginning of anoxic stage.

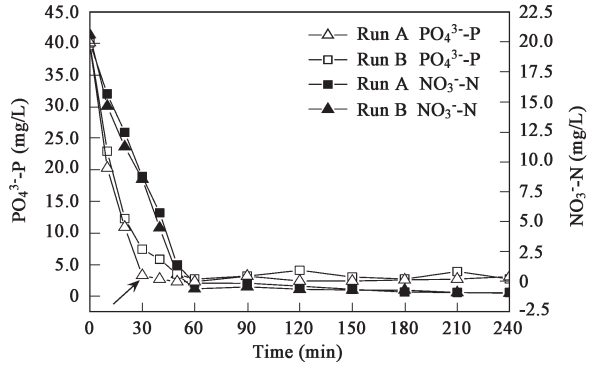

Fig. 7 Dynamics of nitrate and phosphate in run A and B. MLSS A = 2443 mg/L; MLSS B = 2504 mg/L.

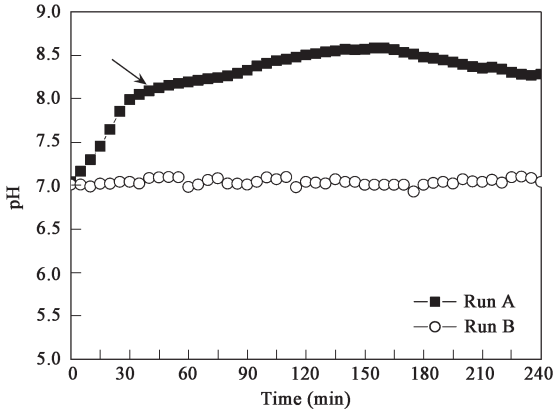

Fig. 8 Variation of pH in run A and B. MLSS A = 2443 mg/L; MLSS B = 2504 mg/L.

Subsequently, the pH in run A increased continuously and reached 8.0 at 30 min. Correspondingly, in the phosphate concentration in run A, there appeared a remarkable decline trend and the phosphate concentration reached 0 mg/L sharply (indicated with an arrow). In case of run B (with pH control), similar phenomena did not take place and the phosphate concentration did not reach 0 mg/L until 60 min. The difference between these two runs was primarily caused by the phosphate chemical precipitation when pH increased to above 8.0.

Parallel tests indicate clearly that pH has a significant impact on the anoxic phosphate uptake. Especially, the rapid pH increase (above 8.0), during the anoxic phosphate uptake period intends to result in the rapid decrease of phosphorus concentration, which is because of chemical precipitation. Undoubtedly, the chemical precipitation should mask the real biological phosphorus uptake capability. According to the above discussion, it seems that the pH should be strictly controlled below 8.0, to avoid chemical precipitation if the biological denitrifying phosphorus removal characteristics are to be studied accurately.

3 Conclusions

This study investigates the effect of nitrite, MLSS, and pH on phosphate release and anoxic phosphate uptake, to determine the optimal operational conditions for the stable performance of denitrifying phosphorus removal processes. Anoxic phosphorus uptake can be achieved successfully when initial nitrite concentrations are 5.5 and 9.5 mg/L. However, increasing nitrite concentration up to 15 mg/L results in the inhibition of anoxic phosphorus uptake. This indicates that nitrite can serve as an alternative electron acceptor for anoxic P-uptake if only nitrite concentration is controlled under the threshold value, which mainly depends on the sludge condition. Further studies reveal that increasing MLSS concentration from 1844 mg/L to 6730 mg/L leads to an increase in the average rate of denitrification and anoxic P-uptake, whereas, the average P uptake/N denitrifying is reduced from 2.10 to 1.57 mgPO_4^{3-}-P/mgNO_3^--N. Therefore, in the case of this test, it is suggested that MLSS be controlled between

3000–4000 mg/L for the optimal operation denitrifying phosphorus removal processes. Furthermore, it has been observed that the pH does affect the anaerobic P-release and anoxic P-uptake significantly. A rapid pH increase (even up to 8), has been observed during the anoxic phosphorus uptake period. Thus, pH should be strictly controlled below 8.0 to avoid chemical precipitation if the biological denitrifying phosphorus removal phenomena are to be studied accurately.

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