Influence of ORP variation, carbon source and nitrate concentration on denitrifying phosphorus removal by DPB sludge from dephanox process


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Abstract The effect of added carbon source and nitrate concentration on the denitrifying phosphorus removal by denitrifying phosphorus removal bacteria sludge was systematically studied using batch experiments, at the same time the variation of ORP was investigated. Results showed that the denitrifying and phosphorus uptake rate in the anoxic phase increased with the high initial anaerobic carbon source addition. However, once the initial COD concentration reached a certain level, which was in excess of the PHB saturation of Poly-p bacteria, residual COD carried over to the anoxic phase inhibited the subsequent denitrifying phosphorus uptake. This was equal to supplementing the external carbon source to the anoxic phase, furthermore the higher the external carbon source concentration the more powerful the inhibition caused. High nitrate concentration in the anoxic phase increased the initial denitrifying phosphorus rate. Once the nitrate was exhausted, phosphate uptake changed to phosphate release. Moreover, the time of this turning point occurred later with the higher nitrate addition. On the other hand, through on-line monitoring the variation of the ORP with different initial COD concentration, it was found that ORP could be used as a control parameter for phosphorus release, but it is impossible to utilize ORP for controlling the denitrification and anoxic phosphorus uptake operations.

Keywords Anaerobic-anoxic processes; biological phosphorus removal; carbon source; denitrifying phosphorus removal bacteria; nitrate; ORP

Introduction

An anaerobic-anoxic (A₂) process has been proposed for biological phosphorus removal (Vlekke et al., 1988; Wanner et al., 1992; Kerr-Jespersen and Henze, 1993; Kuba et al., 1993, 1996; Meraouki et al., 1999). These new processes are based on the activity of denitrifying phosphorus removal bacteria (DPB) which are capable of using nitrate as an electron acceptor in simultaneous removal of phosphorus and nitrogen from wastewater. The main advantage of applying DPB is the possible saving of COD and energy and less sludge production (Kuba et al., 1997).

The effect of the various carbon and nitrate levels on P release and uptake behaviours in batch tests using PAOs sludge from conventional lab-scale (enhancing biological phosphorus removal) (EBPR) systems has been studied (Malnou et al., 1984; Hascoet and Florentz, 1985; Gerber et al., 1986). Malnou et al. (1984) reported that nitrate is an inhibiting factor to the phosphorus release process in the “anaerobic” phase where COD is available, and no (net) phosphorus release occurred until denitrification was complete. Hascoet and Florentz
(1985) showed that when nitrate is present together with substrate, release of phosphate and uptake of phosphate (with denitrification) occur simultaneously. As to the denitrifying phosphorus removal process, carbon and nitrate are two absolutely necessary and sensitive substances (Kuba et al., 1993; Bortone et al., 1994). Whether the quantities of these two are controlled correctly or not determines the phosphorus and nitrogen removal efficiency. However, few studies have been conducted systemically on the influence of carbon and nitrate concentration on phosphorus removal using DPB sludge.

On the other hand, oxidation-reduction potential (ORP) has been demonstrated to be practical and useful for process control for activated-sludge processes (Charpentier et al., 1998), digestion (Al-Ghusain and Hao, 1995), and other oxidation-reduction processes (Chang et al., 1996). Specifically, a correlation was found between phosphate release and ORP (Shapiro et al., 1965; Koch and Oldham, 1985). At the same time, since nitrates have been introduced in the phosphorus uptake phase as electron acceptors, it had been suggested that ORP measurement could be used for process control in A2SBR because its profile corresponds to the profile of electron acceptors (Kuba et al., 1993). Unfortunately, no experiments have actually been carried out to study the variation of ORP of the anaerobic-anoxic denitrifying phosphorus removal periods and to further verify the feasibility of ORP as a process control parameter.

The objective of this study was to examine the effect of added carbon source and nitrate concentration on denitrifying phosphorus removal using DPB sludge from a lab-scale Dephanox process in SBRs. Furthermore, the variation of ORP was investigated in order to study the feasibility of the ORP as the control parameter during the denitrifying phosphorus removal.

**Materials and methods**

**Activated sludge**

The activated sludge used for these tests came from a lab-scale Dephanox process designed according to the denitrifying phosphorus principle (Kuba et al., 1996). The system was fed with domestic sewage and operated over a period of 300 days. The SRT of DPB sludge in the Dephanox process is 14 days and maintains a sludge concentration of around 4,500 mg/L. During the batch experiments NO₃⁻-N was scarcely detected in the final effluent of the Dephanox process and the TP concentration of the effluent was below 0.5 mg/L.

**Reactor system**

The tests were conducted as batch experiments in 4 L SBRs made of glass and fitted with mixers, see Figure 2. The mixers were stirred continuously to keep the activated sludge in suspension except in the settling period. The temperature of the reactor was kept at 20–23°C. During the reaction DO, pH and ORP were detected on-line. Samples were
collected from the reactor at regular intervals and were immediately centrifuged at 3,000 rpm for 1 min.

Experimental procedure

In each test series, three 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. COD, phosphorus and nitrate concentration were adjusted by adding NaAc, KH$_2$PO$_4$ and KNO$_3$, respectively. pH was strictly controlled at 7 ± 0.05 by the addition of HCl or NaOH to prevent chemical precipitates forming.

Experiment No 1. Effect of anaerobic carbon additions and variation of ORP. The sludge was transferred from the final settler and then distributed to three reactors. Here it is noted that the PHB (Poly-β-Hydroxybutyrate) in the DPB cells has been consumed completely after aeration for 0.5 h in the post-aeration tank. At the beginning of the anaerobic phase, synthetic wastewater was pumped into the three SBRs during the first 5 min to give initial acetate concentration of 100, 200 and 300 mg COD/L, respectively, and phosphate concentration of 10 mg/L. Three hours later, nitrate was added to each reactor (corresponding concentration of 60 mg NO$_3$–/L), in this phase, simultaneous dephosphatation and denitrification occurred by DPB. The concentration profiles of nutrients were tracked for 4 h.

Experiment No 2. Effect of anoxic carbon additions. The activated sludge was withdrawn from the final settler. Synthetic wastewater was pumped into each reactor at the start of the anaerobic phase to give 150 mg COD/L and 5 mg PO$_4^{3–}$/L initially. After anaerobic reaction for 3 h, an anoxic period was initiated by adding potassium nitrate (NO$_3$– concentration of 65 mg/L). At the same time the three reactors received different amounts of acetate, i.e. 0 mg COD/L, 60 mg COD/L and 120 mg COD/L, respectively. Then anoxic reaction was carried on for 4 h.

Experiment No 3. Effect of nitrate concentration on the phosphorus uptake. The sludge used in this test was taken from the internal settler. After being washed twice in the tap water (to ensure no external carbon source existed), the sludge was distributed to three reactors. 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 nitrate (equivalent to 5, 15 and 40 mg NO$_3$–/L). The reaction retention time was 3 h.
Analytical methods

The dissolved oxygen (DO) and temperature were measured continuously using a WTW oxygen probe. Continuous monitoring of pH and ORP was 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 and MLSS were measured according to Standard Methods (APHA, 1995).

Results and discussion

Effect of different anaerobic carbon source additions

During the experiments the MLSS were maintained at 5,000 mg/L, and at the beginning of the anaerobic phase the COD amount in the three reactors was controlled to around 100, 200 and 300 mg/L respectively. Experiment 1 shown in Figure 3 exhibits the typical ORP profiles along with the COD, PO$_4^{3-}$ and NO$_3^-$ dynamic profiles.

When EBPR processes e.g. A/O and A$_2$O systems are considerable, it is possible to obtain higher phosphorus removal efficiency with more carbon source added in the anaerobic stage, but this is not the case with anaerobic/anoxic phosphorus removal. As Figure 3 shows, the test with initial additions of 300-acetate COD mg/L released more phosphorus compared to the others. It was hypothesized that high initial COD concentration would lead to more intracellular PHB stored by the DPB, subsequently availing to a significant anoxic phosphorus uptake rate and denitrification rate. After nitrate addition, phosphorus uptake was observed in all the tests. However, it is interesting to notice that in the high initial COD addition test (COD = 300 mg/L, Figure 3c), the released phosphorus could not be fully taken up within the anoxic period. Contrarily, as to the runs of the additions of 100 and 200 mg/L, phosphorus uptake was observed in all the tests.

Figure 3 The relationships between the variation in the concentration of COD, NO$_3^-$-N, PO$_4^{3-}$-P and ORP with different initial COD concentration.
200 mg COD/L, acetate was completely removed in the anaerobic phase (COD < 20 mg/L) and phosphorus concentrations were both near to zero at a certain time in the anoxic period.

On the basis of the data shown in Figure 3, with increased initial COD concentration of the tests, the mean specific rates of denitrification and phosphorus uptake in the initial 30 min after nitrate added were: 5.9, 12.33 and 16.35 mgNO$_3^-$-N/g MLSS·h (the specific rate of denitrification); 14.86, 15.79 and 13.63 mgPO$_4^{3-}$-P/g MLSS·h (the specific rate of phosphorus uptake). Obviously, the P uptake rate and the associated denitrification rate, increased with increased amounts of acetate added (COD increased from 100 mg/L to 200 mg/L). But when the initial COD increased up to 300 mg/L, anoxic phosphorus uptake rate decreased instead. It is speculated that anaerobic COD residue for the anoxic phase might be the major cause for this drop. The relatively high concentration of acetate was probably in excess of the PHB saturation of Poly-p bacteria and residual acetate in the anoxic stage thus hinders phosphorus uptake at the very beginning because of the competition for nitrate by denitrification, which in turn led to a limitation of nitrate for P uptake.

In addition, as illustrated in Figures 3b and 3c, the phosphate uptake ceased due to the exhausting of NO$_3^-$-N from the mixed liquor, and subsequent ("endogenous") release of phosphorus occurred, i.e. there was a slow rise in the phosphorus curve after the nitrate concentration was close to zero. The phenomena of phosphorus release without electron acceptors (nitrate) and donors (HAc) might result from energy production for maintenance due to Poly-p degradation.

**Typical ORP profiles of different anaerobic carbon source additions**

As Figure 3 shows, ORP was found to vary with the anaerobic–anoxic cycles as a result of sequential phosphorus release and denitrification in the A$_2$SBR. It started with the negative value and dropped continuously during the anaerobic period with increased phosphate concentration. The rate of ORP decrease displayed a perfect relativity with the phosphorus release rate and COD consumption rate. Especially during the initial 60 min, ORP decrease sharply accompanied with the quick variation of COD and phosphate concentrations. Comparing the curves of the ORP of the three systems (with the increased initial COD) it can be found the greater the influent biodegradable COD concentration, the lower the ORP measurement at the end of anaerobic period, e.g. the ORP went down to values of –210, –265 and –312 mV, respectively, at the end of the anaerobic reaction. Through on-line monitoring the change of the ORP decrease rate, whether the carbon source was enough or not could be known and the carbon addition could be controlled to accelerate the phosphorus release.

A significant increase in the ORP was observed after filling with nitrate, and a jump point was markedly shown in the profile (points A, B and C shown in Figure 3). Such an increase would be due to the presence of oxidized nitrogenous compounds. Thereafter the ORP dropped significantly because of denitrification ability. During the first 180 min of anoxic operation, ORP dropped quickly, but between 180–420 min the ORP decrease rate apparently became slow because of the reduced concentration of nitrate. When the nitrate concentration dropped below the detection limit, phosphorus release was initiated, along with a simultaneous slow drop in ORP (Figures 3b and 3c).

With on-line monitoring of the variation of the ORP, the distinctive signal whether the phosphorus was taken up completely or not could not be indicated clearly. Furthermore, the "nitrate knee" on the ORP curve, described as the point where NO$_3^-$ is significantly removed during the typical denitrification period, did not occur in this study. This might be because the metabolism pattern of denitrification in the anaerobic–anoxic system changed due to the major carbon source for denitrification not being an external carbon source but the internal carbon source (PHB) stored by Poly-p bacteria in the preceding anaerobic...
stage. As a result, the characteristic of the ORP curve is no longer similar to the typical one. A similar result (lack of nitrate knee during denitrification) was also found by Ra et al. (1999) who applied the internal organic carbon source to accomplish the biological nutrient removal in TSSBR (two-stage sequencing batch reactor). The exact reasons need to be found by further study. It seems to be the case that ORP cannot be used to control anoxic phosphorus uptake.

**Effect of carbon source concentration in the anoxic stage**

The disturbance of COD on phosphorus removal is quite complex. Experiment 2 was designed in order to further clarify the carbon source on the phosphorus uptake abilities of DPB sludge. The MLSS in all runs were controlled at 3,000 mg/L and at the beginning of the anoxic stage, acetate corresponding to 0, 60 and 120 mg COD/L were added to the three reactors.

Figure 4 presents the COD, $\text{PO}_4^{3-}$-P and $\text{NO}_3^{-}$-N variations. The characteristics of the phosphorus release curves of three runs looked similar, attributed to the uniform initial experiment conditions. Acetate had been removed completely (COD $\leq$ 20 mg/L) at 150 min and phosphorus concentration reached the plateau in all tests. It is worth noting that even with anoxic acetate present, a net phosphorus uptake still occurred. The nitrate removal rates in the anoxic phase differ substantially for corresponding different anoxic COD addition. In the first 30 min of anoxic operation, with the increased anoxic COD addition the specific phosphorus uptake rates were 14.34, 9.24 and 4.30 mg$\text{PO}_4^{3-}$-P/g MLSS·h; the specific denitrification rates were 10.65, 11.98 and 15.8 mg$\text{NO}_3^{-}$-N/g MLSS·h. Apparently, the tests with anoxic acetate additions exhibited a considerable increase in nitrate removal rate but the decrease of phosphorus removal rate was comparable to that of the reactor without anoxic acetate addition.

![Figure 4](https://iwaponline.com/wst/article-pdf/50/10/153/419352/153.pdf)

**Figure 4** Effect of anoxic COD additions on denitrifying phosphorus removal
Hascoet and Florentz (1985) reported that the bacteria release phosphate under anoxic conditions when COD was present, but the P release rate was reduced compared to anaerobic conditions. Østgaard et al. (1997) even found that with high acetate addition the anoxic P release and PHB accumulation became dominant and masked the phosphorus uptake by DPB. Nevertheless, as Figure 4 shows, in this study the phosphorus release was not found even with the anoxic COD addition of 120 mg/L at 30 min after nitrate was added. It is important to explain that phosphorus release must have happened at the very beginning of the anoxic COD addition, but subsequent COD consumed by nitrate seemed to become dominant and not support phosphorus release any more, i.e. a large amount of acetate-COD removed not for phosphorus release but rather for denitrification by DPB which can use organic substrate directly. Therefore phosphorus uptake did not occur until the COD decreased to a certain level (around 30 mg COD/L). This is the very reason that a small-scale phosphorus uptake was found in two anoxic COD addition tests after anoxic reaction for 30 min.

In the test with anoxic acetate addition of 0 mg/l, the NO$_3^-$ can be detected (4 mg/L) at the end of cycle and phosphorus release was not found during the whole anoxic period (Figure 4a). But seen from the profile with anoxic COD addition of 120 mg/L (Figure 4c), phosphorus uptake was found to stop when the nitrate concentration reached zero (at 300 min). This was followed by a slow release of phosphorus (“endogenous” phosphorus release) for the remainder of the test. Similar phenomenon was also observed in experiment 1, that endogenous phosphorus release occurred when nitrate and carbon source were completely removed.

As mentioned above, the anoxic acetate addition had the effect of decreasing P-uptake and increasing the denitrification rate. On the other hand, although acetate additions inhibited the phosphorus uptake at the very start of the anoxic operation to some extent, once acetate substrate was depleted, a slow uptake of phosphorus occurred as long as nitrate was present and the rate of denitrification decreased.

Effect of nitrate concentration on anoxic phosphate uptake

Figure 5 displays the typical results of experiment 3. The MLSS were maintained at 4,400 mg/L in the tests. As Figure 5a shows, the rate of the phosphorus uptake was high in the initial 15 min. Calculation of the data involved showed that the mean specific phosphorus uptake rates of the three tests in the initial 15 min were 7.55, 11.5 and 12.63 mgPO$_4^{3-}$-P/g MLSS·h and the corresponding mean specific denitrification were 4.54, 9.54 and 9.91NO$_3^-$-N/g MLSS·h, respectively. This means that the higher concentration of nitrate increased the specific denitrifying and phosphorus uptake initially. The reason for these
results is similar to the explanation by Bortone et al. (1994), that the very large floc size of DPB sludge might limit the nitrate diffusion in the deeper part, therefore denitrification rates increase linearly depending on the initial nitrate concentration.

In the case with a low dose of nitrate (the initial nitrate concentration was 5 mg/L), the PO$_4^{3-}$-P concentration reached the minimal value (11.62 mg/L) when the nitrate concentration became close to zero at 15 min, there was a turning point (point A) in the phosphorus dynamic profile, i.e. the second phosphorus release occurred. For the other two tests, the phosphate concentration did not reach zero until reaction was carried on for 30 min, and at that moment the nitrate consumed completely in the run with initial nitrate 15 mg/L, and endogenous phosphorus release found in the remainder reaction. But to the test of initial nitrate 40 mg/L, there is a zero platform occurred during 30~120 min, however, the denitrification did not cease. After nitrate was completely consumed, slow phosphorus uptake was observed. For the above tests with the same initial substrate concentrations, a switch from phosphorus uptake to phosphorus release under anoxic condition was dependent on the initial nitrate concentration when there was no external carbon. The higher the initial nitrate, the later the switch occurred (points A, B and C as shown in Figure 5b). At the same time, the uptake of phosphorus was reduced compared to the amount of nitrate reduced.

Conclusions

Based on the results obtained in this study, the practical important conclusions can be summarized as follows:

1. The perfect denitrifying phosphorus removal can be accomplished in the anaerobic–anoxic batch process as long as the COD and nitrate were controlled correctly.

2. High initial carbon source addition increases the subsequent denitrification and phosphorus rate at the very beginning. However, once the initial COD concentration reached a certain level, which was in excess of the PHB saturation of Poly-p bacteria, residual COD carried over to anoxic phase inhibited the subsequent denitrifying phosphorus uptake. This was equal to supplementing with an external carbon source to the anoxic phase, furthermore the higher the external carbon source concentration the more powerful the inhibition caused. In addition, whether the nitrate is sufficient or not is the key to anoxic phosphorus uptake.

3. ORP can be used as a control parameter of phosphorus release, but it is impossible to utilize ORP for controlling the denitrification and anoxic phosphorus uptake operations.

4. Nitrate inhibited the pure phosphorus release, but can be applied as an electron by DPB in anoxic phosphorus uptake. High nitrate concentration in the anoxic phase increased the initial denitrifying phosphorus rate. Once the nitrate was exhausted, phosphate uptake changed to phosphate release. Moreover, the time of this turning point occurred later with the higher nitrate addition.

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