Journal of Environmental Sciences Vol.16, No.4, pp.548—552, 2004

ISSN 1001—0742
CN11—2629/X

Article ID: 1001-0742(2004)04-0548-05 **CLC number**: X131.2; X703 **Document code**: A

Effect of carbon source and nitrate concentration on denitrifying phosphorus removal by DPB sludge

WANG Ya-yi[1], PENG Yong-zhen[2], Wang Shu-ying[2], PAN Mian-li[3]

(1. College of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150008, China. E-mail: yayiwang@163.com; 2. College of Environment and Energy Engineering, Beijing University of Technology, Beijing 100022, China; 3. Hangzhou Huadong Gene-Technology Institute, Hangzhou 310000, China)

Abstract: Effect of added carbon source and nitrate concentration on the denitrifying phosphorus removal by DPB 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 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 to the PHB saturation of poly-P bacteria, residual COD carried over to anoxic phase inhibited the subsequent denitrifying phosphorus uptake. Simultaneously, phosphate uptake continued until all nitrate was removed, following a slow endogenous release of phosphate. High nitrate concentration in anoxic phase increased the initial denitrifying phosphorus rate. Once the nitrate was exhausted, phosphate uptake changed to 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 ORP could be used as a control parameter for phosphorus release, but it is impossible to utilize ORP for controlling the denitrificaion and anoxic phosphorus uptake operations.

Keywords: biological phosphorus removal; carbon source; nitrate; ORP; denitrifying phosphorus removal bacteria (DPB); anaerobic-anoxic processes

Introduction

An anaerobic-anoxic(A_2) process has been proposed for biological phosphorus removal since the last 1980's(Vlekke, 1988; Wanner, 1992; Kerrn-Jesperen, 1993; Kuba, 1993; 1996; Meraouki, 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 simultaneously removal phosphorus and nitrogen from wastewater. The main advantage of applying DPB is the possible saving of COD and energy(aeration) and less sludge production(Kuba, 1997).

Effect of the various carbon and nitrate levels on P release and uptake behaviors in batch tests using PAOs sludge from conventional lab-scale enhanced biological phosphorus removal(EBPR) system has been studied previously(Malnou, 1984; Hascoet, 1985; Gerber, 1986; 1987). Malnou *et al*.(Malnou, 1984) reported nitrate is an inhibiting factor to phosphorus release process in the "anaerobic" phase where COD is available, and no(net) phosphorus release occurred until denitificaion was complete. While, Hascoet and Florenta(Hascoet, 1985) showed when nitrate is present together with substrate, release of phosphate and uptake of phosphate(with denitrification) occur simultaneously. As to denitrifying phosphorus removal process, carbon and nitrate are two absolutely necessary and sensitive substances(Kuba, 1993; Boritone, 1994). Whether the quantities of these two are controlled correctly or not determines the phosphorus and nitrogen removal efficiency finally. 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(ORP) has been demonstrated to be practical and useful for process control for activated-sludge processes(Charpentier, 1998), digestion(Al-Ghusain, 1995), and other oxidation-reduction processes(Chang, 1996). Specifically, a correlation was found between phosphate release and ORP(Shapiro, 1965; Koch, 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 A_2 SBR because its profile corresponds to the profile of electron acceptors(Kuba, 1993). Unfortunately, none experiments actually carried out to study the variation of ORP of anaerobic-anoxic denitrifying phosphorus removal periods and further to 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

Foundation item: The National Natural Science Foundation of China(No.20377003) and the Key Project of Beijing Education Committee(No.K_Z200310005003)

ORP as the control parameter during the denitrifying phosphorus removal.

1 Materials and methods

1.1 Activated sludge

The activated sludge used for these tests comes from a lab-scale dephanox process designed according to the denitrifying phosphorus principle(Kuba, 1996). The system fed with domestic sewage was operated over a period of 300 d. The SRT of DPB sludge in the dephanox process is 14 d and maintains a sludge concentration of around 4500 mg/L. During the batch experiments being carried out, NO_3^--N was scarcely detected in the final effluent of the dephanox process and TP concentration of the effluent was below 0.5 mg/L (Fig.1).

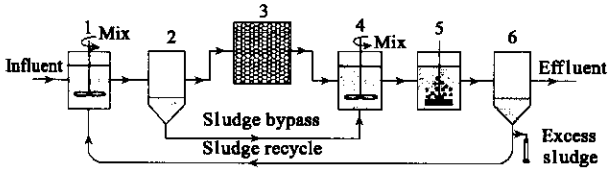


Fig.1 Configuration of dephanox process

1. anaerobic tank; 2. internal settler; 3. fixed-biofilm nitrification; 4. anoxic tank; 5. post-aeration tank; 6. final settler

1.2 Reactor system

The tests were conducted as batch experiments in 4 L SBRs made of glass and fitted with mixers. The mixers were stirred continuously to keep the activated sludge in suspension expect in the settling period. The temperature of the reactor was kept at 20—23℃. During the reaction DO, pH and ORP detected on-line. Samples were collected from the reactor at regular intervals and were immediately centrifuged at 3000 r/min for 1 min.

1.3 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 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 and nitrate concentration were adjusted by adding NaAc, KH_2PO_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 anoxic carbon additions and aviation of ORP. The sludge was transferred from the final settler and then distributed to three reactors. Here it is noted that the PHB (poly-hydroxyoutyrate) in the DPB cells has been consumed completely after aerated for 0.5 h in the post-aeration tank. At the beginning of the anaerobic phase, synthetic waste water was pumped into the three SBRs during the first 5 min to give initial acetate concentration of 100, 200 and 300 COD mg/L respectively, and phosphate concentration of 10 mg/L. Three hours later, nitrate was added into the each reactor(corresponding concentration of 60 mg NO_3^-/L), in this phase, simultaneous dephosphate and denitrifiction occurred by DPB. The concentration profiles of nutrients were tracked for 4 h.

Experiment No 2: Effect of nitrate concentration on the phosphorus release. The sludge was taken from the final settler. At the start of the anaerobic phase, synthetic wastewater was pumped into the SBRs during the first 5 min to give initial COD of 150 mg/L and phosphate concentration of 10 mg/L. At the same time, different dosages of nitrate (corresponding to 5, 30 and 55 mg NO_3^-/L) were added to three reactors. The reaction retention time was 3 h.

Experiment No 3: Effect of nitrate concentration on the phosphorus uptake. The sludge used in this test was taken from internal settler. After washed twice in the tap water(to ensure no external carbon source exist), 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 amount of nitrate(equivalent to 5, 15 and 40 mg NO_3^-/L). The reaction retention time was 3 h.

1.4 Analytical methods

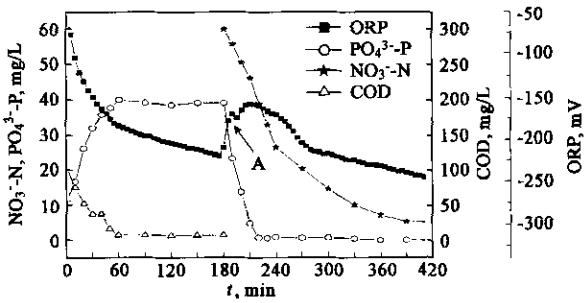
The dissolved oxygen(DO) and temperature were measured continuously using a WTW oxygen probe. Continuous monitoring of pH and ORP were carried out using two WTW inolab pH level 2 meters with an ORP electrode and a pH probe (WTW). COD_{Cr}, PO_4^{3-}-P, NO_3^--N and MLSS were measured according to standard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

2 Results and discussion

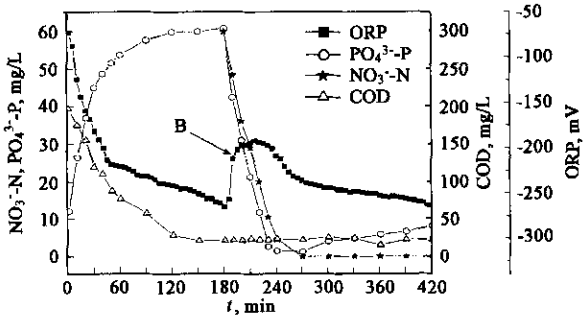
2.1 Effect of different anaerobic carbon source additions

During the experiments the MLSS were maintained at 5000 mg/L, and at the beginning of anaerobic phase the COD amount in three reactors were controlled around 100200 and 300 mg/L respectively. Experiment No.1 shown in Fig.2 exhibits the typical ORP profiles along with the COD, PO_4^{3-} and NO_3^- dynamic profiles.

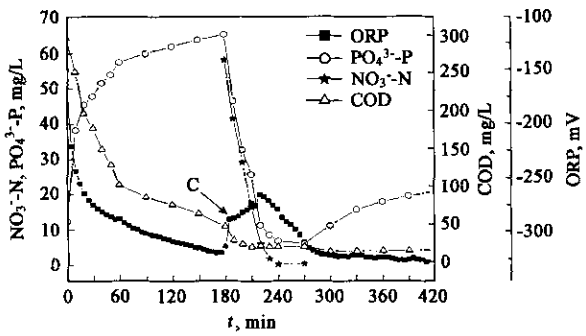
When the EBPR processes e.g. A/O and A_2O systems are considerable, it is possible to obtain higher phosphorus removal efficiency with more carbon source added in the anaerobic stage, but this is not the case with anaerobic/anoxic phosphorus removal. As Fig.2 shows, the test with initial additions of 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



(a) Dynamics of COD, NO_3^--N, PO_4^{3-}-P and ORP, initial COD=100 mg/L



(b) Dynamics of COD, NO_3^--N, PO_4^{3-}-P and ORP, initial COD=200 mg/L



(c) Dynamics of COD, NO_3^--N, PO_4^{3-}-P and ORP, initial COD=300 mg/L

Fig.2 The relationships between the variation in the concentration of COD, NO_3^--N, PO_4^{3-}-P and ORP with different initial COD concentration

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, Fig.2c), 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 mgCOD/L, acetate was completely removed in the anaerobic phase (COD < 20 mg/L) and phosphorus concentrations were both near to zero at certain time in anoxic period.

On the basis of the data shown in Fig.2, with increased initial COD concentration of the tests, the mean specific rate of denitrificaion and phosphorus uptake in the initial 30 min after nitrate added were: 5.9, 12.33 and 16.35 mgNO_3^--N/(gMLSS · h) (the specific rate of denitrificaion); 14.86, 15.79 and 13.63 mgPO_4^{3-}-P/(gMLSS·h) (the specific rate of phosphorus uptake). Obviously, the P uptake rate and the associated denitrification rate, increase for increased amounts of acetate added (COD increased from 100 to 200 mg/L). But when the initial COD increased up to 300 mg/L, \anoxic phosphorus uptake rate decreased instead. It is speculated anaerobic COD residue to the anoxic phase might be the major cause for this drop. The relatively high concentration of acetate probably was in excess to 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 denitrificaion, which in turn led to a limitation of nitrate for P uptake.

In addition, as illustrated in Fig. 2b and 2c, 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 phosphorus curve after the nitrate concentration was closed 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.

2.2 The typical ORP profiles of different anaerobic carbon source additions

As Fig. 2 shows, ORP was found to vary with the anaerobic-anoxic cycles as a result of sequential phosphorus release and denirification 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 consume 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 three systems (Fig.2a, 2b and 2c; 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 the values of −210, −265 and −312 mV respectively at the end of 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 OPR was observed after filling with nitrate, and a jump point was markedly shown in the profile (point A, B and C showed in the Fig.2). Such an increase would be due to the presence of oxidized nitrogenous compounds. Thereafter the ORP dropped significantly because of denitrifyicaion 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 slowly drop in ORP (Fig.2b and 2c).

With on-line monitoring the variation of the ORP, the distinctive signal whether the phosphorus was taken up completely or not could not been indicated clearly. Furthermore, the "nitrate knee" on the ORP curve is described as the point where NO_3^- is significantly removed during the typical denitrification period did not occur in this

study. This might be explained that the metabolism pattern of denitrificaion in the anaerobic-anoxic system changed due to the major carbon source for denitrification being not 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 ORP curve is no long similar to the typical one. Similar result (lake of nitrate knee during denitrificaion) was also found by RA *et al.*, (RA, 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 explained according to the further study. Therefore, it seems to suggest ORP cannot be used to control anoxic phosphorus uptake.

2.3 Effect of nitrate concentration on anaerobic phosphate release

The effect of anaerobic nitrate addition was studied at three levels with experiment No. 2 shown in Fig. 3. The MLSS in the reactors were maintained at 3100 mg/L. An initial release of phosphorus was investigated for all nitrate levels tested and the rate of rapid P release decreased for increasing initial nitrate concentration during the initial rapid phase(0—0.5 h)(i.e. 12.29, 9.45 and 9.32 mgPO_4^{3-}-P/(gMLSS·h)). With increasing initial nitrate concentration, the magnitude of net phosphorus release decreased because part acetate was directly oxidized by nitrate and not converted to PHB. It is worth pointed out that the rate of COD removal were accelerated by denitrify and phosphorus release at the very beginning. For the three tests, the average specific COD removal rates in the first 15 min reached 75.81, 105.11 and 123.59 mgCOD/(gMLSS · h) respectively with increased initial nitrate addition.

As to the run of initial nitrate concentration of 5 mg/L, the nitrate completely used up within first 15 min, thereby phosphorus release profile was observed to ascend sharply in the second 15 min. Also, the specific phosphorus release rate was accordingly improved from 12.52 to 20.64 mgPO_4^{3-}-P/(gMLSS·h). At 60 min, an endogenous phosphorus release platform appeared when COD was completely removed. For tests with high initial nitrate concentration (nitrate concentration $\geqslant$ 30 mg/L), the nitrate were not used up when the COD concentration had been near to zero at 80 min, and the recorded metabolism completely converted from anaerobic to anoxic. Thereafter, the phosphate release turned into uptake, moreover, the higher the initial nitrate, the later the switch occurred for a given level of nitrate. Above results demonstrated that carbon source present with nitrate will result the phosphorus release and uptake(with denitrification) happen simultaneously. In other words, nitrate causes the negative effect on the pure phosphorus release.

2.4 Effect of nitrate concentration on anoxic phosphate uptake

Fig.4 displays the typical results of experiment No. 3.

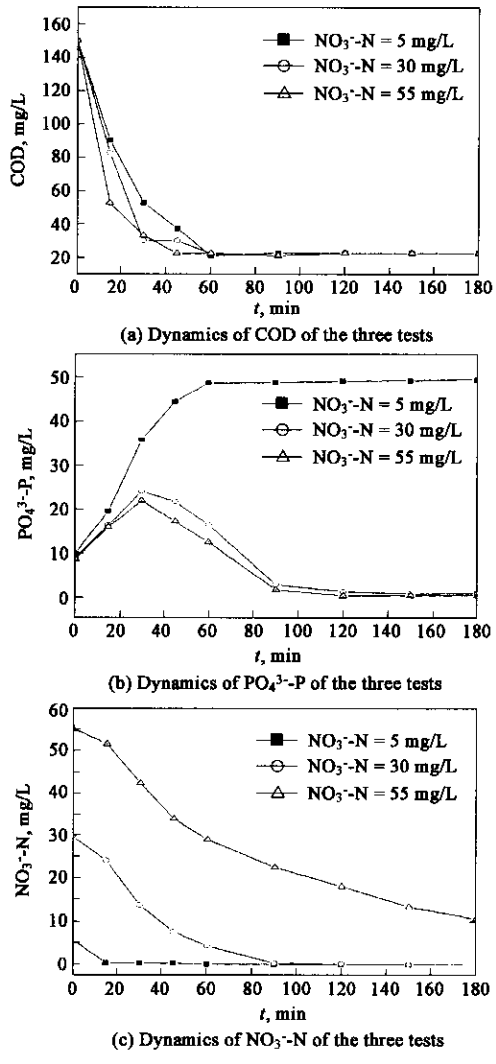
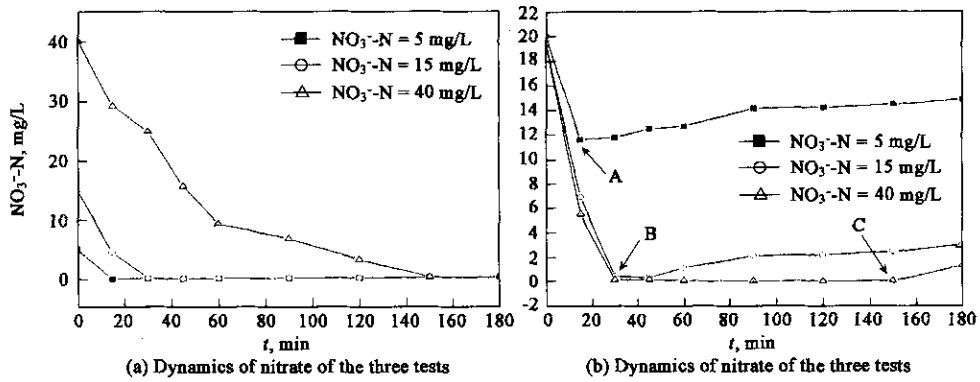


Fig.3 Effect of different NO_3^--N concentration on phosphate release under anaerobic condition

The MLSS were maintained at 4400 mg/L in the tests. As Fig.4a shows, the rate of the phosphorus uptake was high in the initial 15 min. Calculation the involved data showed, the mean specific denitrificaion and phosphorus uptake rate of three tests(5, 15 and 40 mg NO_3^-/L) in the initial 15 min were 7.55, 11.5 and 12.63 mgPO_4^{3-}-P/(gMLSS·h); 4.54, 9.54 and 9.91 NO_3^--N/(gMLSS·h). This means the higher concentration of nitrate increased the specific denitrifying and phosphorus uptake initially. The reason of these results had been explained by Bortone *et al.* (Bortone, 1994) that the very large flock size might limit the nitrate diffusion in the deeper part, therefore denitrification rates increase linearly depending on the initial nitrate concentration.

In case with a low dose of nitrate (an 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 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 at 30



(a) Dynamics of nitrate of the three tests (b) Dynamics of nitrate of the three tests

Fig.4 Effect of different nitrate concentration on anoxic phosphate uptake

min, and at that moment the nitrate consumed 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 phosphors 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(point A, B and C as shown in Fig.4b). At the same time, the uptake of phosphorus was reduced compared to the amount of nitrate reduced.

3 Conclusions

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

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 to the PHB saturation of poly-P bacteria, residual COD carried over to anoxic phase inhibited the subsequent denitrifying phosphorus uptake. In addition, whether the nitrate is sufficient or not is the key to anoxic phosphorus uptake.

Nitrate inhibited the pure phosphorus release, but can be applied as electron by DPB in anoxic phosphorus uptake. High nitrate concentration in 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.

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

References:

Al-Ghusain I A, Hao O J, 1995. Use of pH as control parameter for aerobic/anoxic sludge digestion[J]. J Environ Eng, 121: 225—231.

APHA(American Public Health Association), 1995. Standard methods for the examination of water and wastewater [M]. 19th ed. Water Environment Federation. Washington DC, USA: American Public Health Association.

Bortone G, Malaspina F, Stante L *et al*., 1994. Biological nitrogen and phosphorus removal in an anaerobic/anoxic sequencing batch reactor with separated biofilm nitrification[J]. Wat Sci Tech, 30(6): 303—313.

Chang D N, Lin J G, Chao A C *et al*., 1996. Modified model for on-line control of the chemical oxidation decoloring process[J]. Wat Sci Tech, 34(3/4): 151—157.

Charpentier J, Martin G, Wacheux H *et al*., 1998. ORP regulation and activated suludge: 15 years of experience[J]. Wat Sci Tech, 38(3): 197—208.

Gerber A, Mostert E S, Winter C T *et al*., 1986. The effect of acetate and other short-chain carbon compounds on the kinetics of biological nutrient removal [J]. Wat S A, 12: 7—12.

Gerber A, de Villiers T H, Mostert E S *et al*., 1987. The phenomenon of simultaneous phosphate uptake and release, and its importance in biological nutrient removal[M]. In: Biological phosphate removal from wastewaters (Ramadori R. ed.). Oxford: Pergamon Press.

Hascoet M C, Meunier A, 1985. Influence of nitrate on acetic acid induced biological phosphate removal from wastewater[J]. Wat S A, 11: 1—8.

Kerm J P, Henze M, 1993. Biological phosphorus release and uptake under alternation anaerobic and anoxic conditions in a fixed-film reactor[J]. Wat Res, 27(4): 617—624.

Koch F A, Oldham W K, 1985. Oxidiation-reduction potential—a tool for monitoring, control and optimization for biological nutrient removal systems [J]. Wat Sci Tech, 17: 259—281.

Kuba T, Van Loosdrecht M C M, 1993. Phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor[J]. Wat Sci Tech, 27(5—6): 241—252.

Kuba T, Van Loosdrecht M C M, Heijnen J J, 1996. Phosphorus and nitrogen removal with minimal COD requirement by integration of nitrification in a two-sludge system[J]. Wat Res, 42(1—2): 1702—1710.

Kuba T, Van Loosdrecht M C M, Heijnen J J, 1997. Biological dephosphatation by activated sludge under denitrifying conditions: pH influence and occurrence of denitrifying dephosphatation in a full-scale waste water treatment plant[J]. Wat Sci Tech, 36(12): 75—82.

Malnou D, Meganck M, Faup G M *et al*., 1984. Biological phosphorus removal: study of the main parameters[J]. Wat Sci Tech, 16(10/11): 173—185.

Meraouki J, Filipe C D M, Daigger G T *et al*., 1999. Characterization of the denitrifying fraction of phosphate accumulation of phosphate accumulation organisms in biological phosphate removal[J]. Wat Sci Tech, 39(1): 31—42.

RA C S, LO K V, Shin J S *et al*., 2000. Biological nutrient removal with an internal organic carbon source in piggery waster treatment[J]. Wat Res, 34 (3): 965—973.

Shapiro J, Levin G V, Zea G, 1965. Anoxically induced release of phosphate in wastewater treatment[J]. J Wat Pollut Control Fed, 39:1810—1818.

Vlekke G J K M, Comeau Y, Oldham W K, 1988. Biological phosphorus removal from wastewater with oxygen or nitrate in sequencing batch reactors[J]. Environ Technol Lett, 9: 791—796.

Wanner J, Cech J S, Kos M, 1992. New process design for biological nutrient removal[J]. Wat Sci Tech, 25:445—448.

(Received for review August 11, 2003. Accepted September 22, 2003)