A two-sludge system for simultaneous biological C, N and P removal via the nitrite pathway

M. Marcelino, D. Wallaert, A. Guisasola and J. A. Baeza

ABSTRACT

Nitrogen removal via the nitrite pathway results in significant savings in both aeration costs and COD requirements for denitrification when compared to the conventional biological nitrogen removal process. Implementation of the nitrite pathway for simultaneous C/N/P removal in a single sludge system has a major drawback: the aeration phase disfavours denitrifying phosphorus removal. A possible configuration to overcome this issue is the utilisation of a two-sludge system where autotrophic and heterotrophic populations are physically separated. This paper experimentally demonstrates the feasibility of a nitrite-based two-sludge system with sequencing batch reactors (SBR) for the treatment of urban wastewater: a heterotrophic SBR with denitrifying PAOs for P removal and an aerobic SBR for N removal. Partial nitrification was attained in the autotrophic SBR so that shortcut biological nitrogen removal was achieved by using the anoxic dephosphatation activity of DPAOs. Finally, the effect of operating this system without pH control was studied using different influent pH values (pH = 6.8, 7.5 and 8.2) and, despite some efficiency lost due to the pH fluctuations, the system was able to remove most of the C, N and P present in the wastewater.

Key words | nitrite DPAO, nitrite pathway, pH control, SBR, two-sludge

M. Marcelino
D. Wallaert
A. Guisasola (corresponding author)
J. A. Baeza
Departament d'Enginyeria Química,
Universitat Autònoma de Barcelona,
08193, Bellaterra, Catalonia,
Spain
E-mail: *albert.guisasola@uab.cat*

INTRODUCTION

Simultaneous biological removal of phosphorus and nitrogen should be an objective of many WWTPs. In these systems, different biomass fractions (nitrifiers, denitrifiers and Polyphosphate-Accumulating Organisms (PAOs)) must coexist. A fraction of PAOs, called denitrifying PAOs (DPAOs), are able to utilise nitrate/nitrite for phosphorus removal. The benefits of denitrifying dephosphatation have been widely detailed in the literature (Vlekke *et al.* 1988): simultaneous P and N removal is achieved with less sludge production and significant COD and aeration cost savings. Thus, a single sludge with nitrifiers, DPAOs and ordinary heterotrophic organisms (OHOs) for the removal of the excess organic matter could be a fine solution for conventional wastewaters. Nowadays, the utilisation of the nitrite pathway or shortcut biological nitrogen removal is gaining a lot of interest, particularly as a preferred option for the treatment of low COD/N wastewaters. The nitrite pathway, i.e., partial oxidation of ammonia to nitrite and posterior denitrification from nitrite using organic matter as the electron donor, also results in savings in both aeration costs and COD requirements for denitrification (Turk & Mavinic 1986).

Different strategies have been experimentally used to achieve the nitrite pathway in systems with sludge retention:

(i) Control of *aerobic phase length* with which aeration is terminated as soon as ammonia is completely oxidised (Peng *et al.* 2004; Lemaire *et al.* 2008, among others)
(ii) Operation at *low DO levels* (Munch *et al.* 1996; Ruiz *et al.* 2003 or Ma *et al.* 2009). The growth rate of AOB and NOB is influenced by oxygen limitations. Many researchers have experimentally demonstrated that NOB has lower oxygen affinity in comparison to AOB (e.g. Guisasola *et al.* 2005). Consequently, an optimum range of DO can be found for a certain SRT where AOB can grow but NOB growth is not sustained.

The integration of the nitrite pathway in a single-sludge SBR for the simultaneous removal of C, N and P from abattoir wastewater has recently been described in the literature (Lemaire *et al.* 2008). The major handicap of single sludge

doi: 10.2166/wst.2011.398

systems is that the sludge goes through all the stages (i.e. anaerobic, aerobic and anoxic) and the long aerobic hydraulic time required for a successful (partial) nitrification results in adverse conditions for anoxic phosphorus uptake: intracellular polymers can be aerobically used by PAOs resulting in less COD available for the subsequent denitrification. Lemaire *et al.* (2008) successfully overcame this problem by distributing the reactor feed in three different filling periods. A different solution could be the two-sludge system proposed by Kuba *et al.* (1996). This system was based on the physical separation between DPAOs and nitrifiers, resulting in an anaerobic-anoxic heterotrophic SBR (HET-SBR) and a nitrifying autotrophic aerobic SBR (AUT-SBR).

The objective of this paper is to show the feasibility of a two-sludge system similar to that proposed by Kuba *et al.* (1996), but with shortcut biological nitrogen removal. With this aim, the operation of the AUT-SBR was modified so that the activity of nitrite oxidising biomass (NOB) was almost suppressed without leading to the washout of ammonia oxidising biomass (AOB). On the other hand, the HET-SBR was operated to favour the growth of nitrite-based DPAOs (nitrite-DPAOs) so that a significant amount of the phosphorus was taken up under nitrite-reducing conditions. This is not a straightforward issue, since the feasibility of a long-term nitrite-based EPBR system is nowadays a research subject. Finally, the effect of operating this system without pH control was studied using influents with different pH value (Vargas *et al.* 2011). It should be noted that a similar two-sludge system including nitrite pathway has recently been proposed in Zhou *et al.* (2008), which involved the use of granular sludge in the anaerobic/anoxic reactor and biofilms in the aerobic reactor. The system was demonstrated to achieve high-level removal of nitrogen and phosphorus from abattoir wastewater.

MATERIALS AND METHODS

Two-sludge system

Figure 1 shows the two-sludge system proposed in this paper, using two 25 L SBRs and an interchange vessel (25 L). Two different configurations have been used in this study (6-hour and 8-hour). Figure 1 shows the latter. In short, one reactor (HET-SBR) operates under alternating anaerobic/anoxic conditions whereas a second aerobic reactor (AUT-SBR) is in charge of nitrification. The interchange vessel prevents the mixing of both supernatants. In the HET-SBR, organic matter is taken up linked to phosphorus release during the anaerobic phase, whereas ammonium concentration remains almost constant. Then, after a settling period, the supernatants from both SBRs (streams B and D) are exchanged using the interchange vessel. Stream D contains the nitrification products, which will be used as an electron acceptor for anoxic phosphorus uptake. The final effluent of the process (stream E) should contain low amounts of COD, P and N. Figure 1 also shows that an aerobic period may be included after the anoxic phase. This aerobic period is required when the influent contains a shortage of ammonium (high COD/N and low N/P ratios) which would result in an incomplete phosphorus removal. This aerobic phase does not affect DPAO activity since the system is aerated after the anoxic DPAO activity is already finished.

The feed was prepared and stored in a stirred and cooled tank (9 °C) to avoid microbial contamination. It was heated

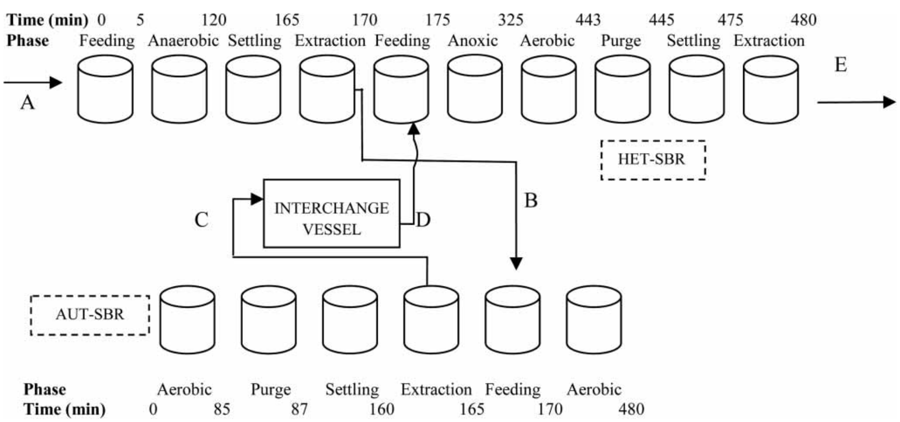

Figure 1 | Operational scheme for HET-SBR and AUT-SBR with a cycle length of 8 hours.

before entering the system in a heat exchanger vessel. The composition of the feed was: $MgCl_2 \cdot 7\ H_2O$ (68 mg/L), NaCl (60 mg/L) and $CaCl_2 \cdot 2\ H_2O$ (30 mg/L). The micronutrient composition was: yeast extract (1 mg/L), $FeSO_4 \cdot 7H_2O$ (4 mg/L), $CuSO_4 \cdot 5H_2O$ (2 mg/L), $MnSO_4 \cdot H_2O$ (3 mg/L), $ZnSO_4 \cdot 7H_2O$ (4 mg/L) and H_3BO_3 (0.02 mg/L). Organic matter, nitrogen and phosphorus were added as CH_3CH_2COONa, NH_4Cl and KH_2PO_4 respectively, mimicking the composition of a municipal wastewater. Their concentration depended on the operation period, being 300 mg COD/L, 40 mg N/L and 10 mg P/L for a typical composition.

The interchange volume between reactors is a key parameter for the operation of two-sludge systems. In this research, 80% of volume interchange was chosen, which resulted in a plant capacity of 80 L/d assuming 4 cycles per day. Both SBRs were monitored with a DO probe (Desin DO2-WW), a pH probe (Desin EPH-M11), a redox probe (EPR-M11) and a temperature probe (Pt-100). The signal converters (Desin TM-3659) transmit the signal to a data acquisition card (Advantech PCI-1711) connected to a PC, with LabWindows CVI 9.0 software for monitoring and control of the process. The data acquisition card has several digital outputs for actuation over the pumps, stirrers and aeration valves. Temperature was not controlled but the lab temperature was around 20 °C during the research period. DO was controlled during the aerobic phase between 1.5 and 2.0 mg DO/L with an on/off controller. pH was controlled at 7.5 during periods I and II by automatic acid dosage in the HET-SBR (HCL 1 M) and base dosage in the AUT-SBR (Na_2CO_3, 1 M). Finally, an average biomass concentration of 1,500 mg VSS/L was maintained throughout this study.

The plant was seeded with sludge withdrawn from Manresa WWTP (Catalonia, Spain) where nitrification–denitrification was performed and a small amount of PAO were detected using Fluorescence *In Situ* Hybridization techniques (results not shown). The HET-SBR was bioaugmented in the start up period with an enriched nitrite-DPAO sludge from another experimental set-up. This nitrite-DPAO sludge failed to use nitrate even when exposed to it in a long-term experiment (Guisasola *et al.* 2009).

Achievement of partial nitrification

The aerobic phase length control strategy was used to achieve partial nitrification. This strategy eliminates the period of the aerobic phase when oxygen and nitrite coexist after ammonium oxidation and thus, NOB growth is significantly disfavoured. The ammonia depletion point can easily be detected using DO or pH signals. However, in this research, an on-line OUR measurement was implemented for a proper detection of the ammonia depletion point. OUR was calculated as the first derivative of the DO drop when aeration was stopped by the on/off controller. The fundamentals of this OUR-based control strategy are depicted in Figure 2. Given the fact that nitritation involves a much higher oxygen consumption rate than nitratation, OUR decreases after ammonium depletion. The on-line detection of this decrease indicates the point in time when ammonium is depleted. The proposed strategy consists of finishing the aerobic phase (i.e. deactivating the on/off controller, closing the aeration valve and switching off the stirrer) when OUR decreases from a certain setpoint (OUR_{MINSP}). OUR_{MINSP} was periodically changed to be 50% of the maximum OUR value.

RESULTS AND DISCUSSION

Period I: Start-up

The start-up period aimed at achieving partial nitrification in the AUT-SBR as well as enriching the HET-SBR with nitrite-DPAO. The long-term objective in the HET-SBR was to have most of the phosphorus taken up under nitrite-reducing conditions. It should be noted that complete anoxic phosphorus uptake does not only depend on the amount of nitrite-DPAO present, but also on the influent COD/N/P ratios. Depending on the COD/N ratio, the amount of nitrite produced in the AUT-SBR may not be high enough to take up all the P released under anaerobic

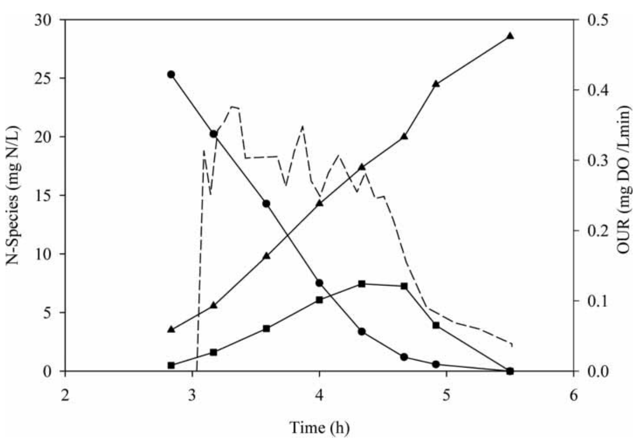

Figure 2 | OUR (dashed) and N-species profiles for a cycle in AUT-SBR with complete nitrification. Ammonium (●), nitrate (▲) and nitrite (■).

conditions in the HET-SBR and thus, an extra post-anoxic aerobic phase should be added in the HET-SBR to avoid incomplete phosphorus removal.

Figure 3 shows a conventional 6-hour cycle during the start-up period. As can be observed in the AUT-SBR, partial nitrification was not yet fully achieved (N-NO_2/N-NH_4 is only around 0.7). With respect to the HET-SBR, the results showed a faster adaptation to nitrite when compared to nitrate: i) nitrite-based anoxic P uptake was faster with nitrite and ii) nitrate was utilised only when nitrite was not present because of the HET-SBR bioaugmentation with an enriched nitrite-DPAO sludge. The potential utilisation of nitrite and nitrate for dephosphatation is a current focus of research (Flowers *et al.* 2009). In our case, nitrate was present at the start of the anoxic phase as long as nitratation was not completely suppressed. The nitrate utilisation linked to phosphorus uptake observed in this period could be caused either by a slow growth of nitrate-DPAO or by the presence of flanking species. These flanking species would reduce nitrate to nitrite while only nitrite-DPAO would denitrify from nitrite onwards. When partial nitrification was achieved nitrite was the sole electron acceptor under anoxic conditions.

Period II: Steady-state operation

Figure 4 shows the steady-state operation of the two-sludge plant. Partial nitrification was achieved with nitrate

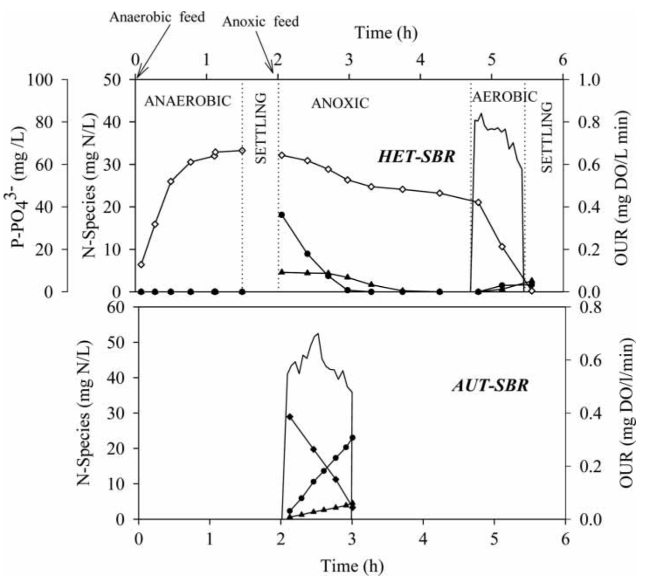

Figure 3 | Profiles of OUR (solid), phosphorus and N-species for a cycle in HET-SBR and AUT-SBR in the start-up period. Ammonium (◆), nitrate (▲), nitrite (•) and phosphorus (◇).

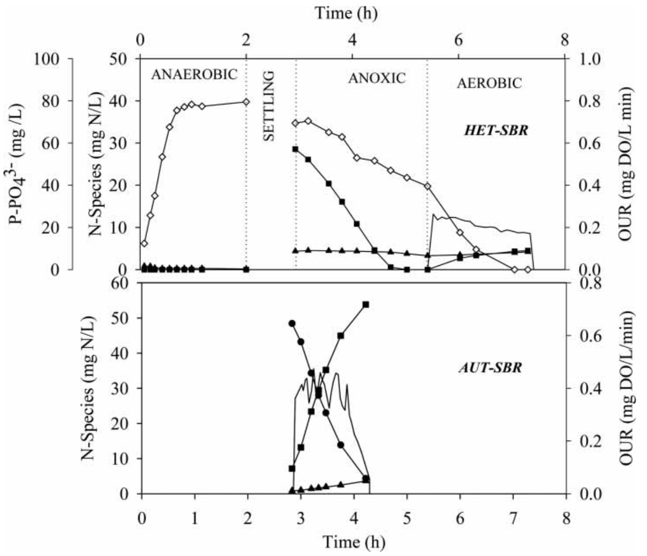

Figure 4 | Profiles of OUR (solid), phosphorus and N-species for a cycle in HET-SBR and AUT-SBR in the steady-state period. Ammonium (•), nitrate (▲), nitrite (■) and phosphorus (◇).

formation in the AUT-SBR being negligible. The maximum OUR value was 33% lower than previous OUR values (see Figure 3) because the NOB were likely washed out of the system. With respect to the HET-SBR, all the nitrite formed was successfully used for anoxic dephosphatation. Nitrate utilisation was not observed in this cycle, indicating that the hypothesis of nitrate-reducing flanking species could be discarded. As nitrite was present in most of the anoxic cycle, nitrate utilisation for P uptake was virtually not observed. As shown in Figure 3, nitrate was utilised only when nitrite was depleted. A significant phosphorus release (80 mg P-PO_4^{3-}/L) was observed under anaerobic conditions. Almost half of this phosphorus was anoxically taken up using nitrite as electron acceptor. Finally, complete phosphorus removal was achieved under aerobic conditions. Moreover, a known advantage of this system was the low sludge production due to the utilisation of nitrite DPAO to denitrify part of the influent organic matter. The system operated under stable conditions for more than four months.

Period III: No controlled pH

It was demonstrated in period II that a two-sludge system for simultaneous biological COD, N and P removal via nitrite was feasible. However, this system was operated under controlled pH conditions (setpoint pH = 7.5), which is not realistic from a real application point of view. For this reason, the system efficiency was tested without pH control in a range of different fixed influent pH values (6.8, 7.5, 8.2).

Each of the influent pHs was tested over more than two weeks. Figures 5 and 6 show an example cycle with an influent wastewater with pH fixed at 8.2 and without pH control in the system. Several interesting observations could be drawn:

(a) With respect to the HET-SBR reactor, the variation of the influent pH in the proposed range was not really sensitive for the operation of the plant. The main reason is that under anaerobic conditions, the pH tends to converge to a value near 7.2. Marcelino *et al.* (2009) demonstrated experimentally and through a simulation-based study that this convergence had a chemical explanation: the buffer capacity of phosphorus released as $H_2PO_4^-$, since the pKa value for the second deprotonation equilibrium is 7.2 at 25 °C. Figure 5 shows the experimental results of a conventional cycle from the HET-SBR without pH control. As can be observed, pH decreases when Stream D (see Figure 1) from the AUT-SBR enters the HET-SBR. As will be discussed below, the pH of this stream is to some extent acidic (around 6). The anoxic P uptake causes a pH increase up to 8, due to the denitrification of nitrite to nitrogen gas. This pH variation may result in different rates of nitrite and P utilisation. The results obtained with the three different pH influents were very similar, as pH stabilised around 7.2 at the end of the anaerobic phase.

(b) With respect to the AUT-SBR value, the suppression of the pH control resulted in detrimental effects on partial nitrification due to the acidification caused by ammonium oxidation to nitrite. Figure 6 shows an example of one cycle in the AUT-SBR. The initial pH in the aerobic reactor was always close to 7.2 as mentioned above and, then, pH decreased as nitritation advanced. This decrease caused a reduction of the nitritation rate and ammonium was present in almost all the aerobic phase. The most likely cause of this reduction is the increase of free nitrous acid concentration, a known AOB inhibitor, due to the low pH. Hence, the OUR control strategy could not be applied and NOB started to appear due to the coexistence of nitrite and oxygen. However, the ratio $N\text{-}NO_2/N\text{-}NH_4$ was around 0.8, which means that most of the ammonium was partially nitrified. This detrimental effect could only be mitigated with a highly buffered influent to avoid high pH variations. Therefore, the available alkalinity in the real influent plays a major role in these systems.

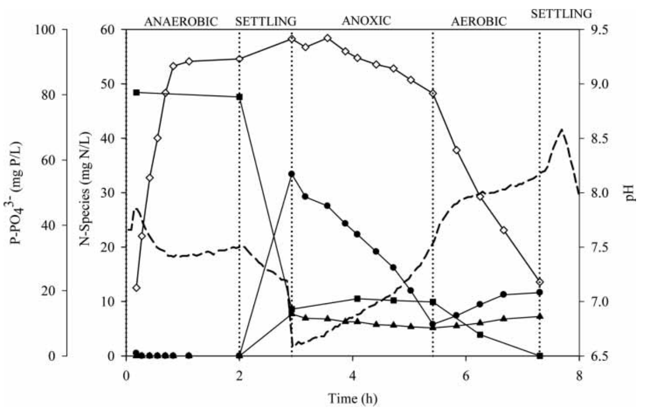

Figure 5 | Profiles of pH (dashed), phosphorus and N-species for a cycle in HET-SBR with the pH control deactivated. Ammonium (■), nitrate (▲), nitrite (•) and phosphorus (◇).

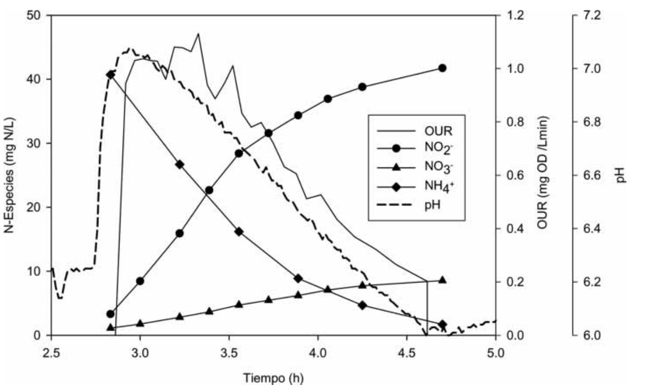

Figure 6 | OUR (solid), pH (dashed) and N-species profiles for a cycle in AUT-SBR with the pH control deactivated. Ammonium (◆), nitrate (▲), nitrite (•).

CONCLUSIONS

This research has demonstrated the feasibility of a two-sludge system for simultaneous biological carbon, nitrogen and phosphorus removal using shortcut biological nitrogen removal and nitrite-based dephosphatation. This system overcomes the potential drawbacks of a one-sludge system for the treatment of low COD/N wastewaters. The system was operated without pH control for three different influents (pH = 6.8, 7.5 and 8.2) and, despite some efficiency lost due to the pH fluctuations, the system was able to remove most of the C, N and P present in the wastewater. The P released in the initial anaerobic phase acted as a buffer and the pH tended to converge to 7.2 independently of the influent pH.

The major handicap observed when working without pH control was that the nitration rate was severely affected by the pH decrease under aerobic conditions. This decrease caused difficulties in applying the aerobic phase length control strategy for obtaining partial nitrification in this reactor. Therefore, the application of the proposed system will depend on the available alkalinity in the real influent.

ACKNOWLEDGEMENTS

This work was supported by the Ministerio of Ciencia y Tecnología (CTQ2007-61756/PPQ). The authors are members of the GENOCOV group (Grup de Recerca Consolidat de la Generalitat de Catalunya, 2009 SGR 815). Marcos Marcelino is grateful for the grant received from the Spanish government (FPU AP 2004-7085).

REFERENCES

Flowers, J., He, S., Yilmaz, S., Noguera, D. & McMahon, K. D. 2009 Denitrification capabilities of two biological phosphorus removal sludges dominated by different 'Candidatus Accumulibacter' clades. *Environomental Microbiology Reports 1* (6), 583–588.

Guisasola, A., Jubany, I., Baeza, J. A., Carrera, J. & Lafuente, J. 2005 Respirometric estimation of the oxygen affinity constants for biological ammonium and nitrite oxidation. *Journal of Chemical Technology Biotechnology* **80** (4), 388–39.

Guisasola, A., Qurie, M., Vargas, M., Casas, C. & Baeza, J. A. 2009 Failure of an enriched nitrite-DPAO population to use nitrate as an electron acceptor. *Process Biochemistry* **44** (7), 689–695.

Kuba, T., van Loosdrecht, M. C. M. & Heijnen, J. J. 1996 Phosphorus and nitrogen removal with minimal COD requirement by integration of denitrifying dephosphatation and nitrification in a two-sludge system. *Water Research* **30** (7), 1702–1710.

Lemaire, R., Marcelino, M. & Yuan, Z. 2008 Achieving the nitrite pathway using aeration phase length control and step-feed in an SBR removing nutrients from abattoir wastewater. *Biotechnology and Bioengineering* **100** (6), 1228–1236.

Ma, Y., Peng, Y., Wang, Y., Yuan, Z. & Wang, X. 2009 Achieving nitrogen removal via nitrite in a pilot-scale continuous predenitrification plant. *Water Research* **43** (3), 563–572.

Marcelino, M., Guisasola, A. & Baeza, J. A. 2009 Experimental assessment and modelling of the proton production linked to phosphorus release and uptake in EBPR systems. *Water Research* **43** (9), 2431–2440.

Munch, E. V., Lant, P. & Keller, J. 1996 Simultaneous nitrification and denitrification in bench-scale sequencing batch reactors. *Water Research* **30** (2), 277–284.

Peng, Y., Chen, Y., Wang, S., Peng, C., Liu, M., Song, X. & Cui, Y. 2004 Nitrite accumulation by aeration controlled insequencing batch reactors treating domestic wastewater. *Water Science and Technology* **50** (10), 35–43.

Ruiz, G., Jeison, D. & Chamy, R. 2003 Nitrification with high nitrite accumulation for the treatment of wastewater with high ammonia concentration. *Water Research* **37**, 1371–1377.

Turk, O. & Mavinic, D. 1986 Preliminary assessment of a shortcut in nitrogen removal from wastewater. *Canadian Journal of Civil Engineering* **13**, 600–605.

Vargas, M., Guisasola, A., Artigues, A., Casas,, C. & Baeza, J. A. 2011 Comparison of a nitrite-based anaerobic–anoxic EBPR system with propionate or acetate as electron donors. *Process Biochemistry* **46** (3), 714–720.

Vlekke, G. J. F. M., Comeau, Y. & Oldham, W. K. 1988 Biological phosphorus removal from wastewater using oxygen or nitrate in sequencing batch reactors. *Environmental Technology Letters* **9**, 791–796.

Zhou, Y., Pijuan, M. & Yuan, Z. 2008 Development of a 2-sludge, 3-stage system for nitrogen and phosphorous removal from nutrient-rich wastewater using granular sludge and biofilms. *Water Research* **42** (12), 3207–3217.

First received 27 April 2010; accepted in revised form 19 October 2010