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ORIGINAL PAPER

A sequencing batch reactor system for high-level biological nitrogen and phosphorus removal from abattoir wastewater

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Abstract A sequencing batch reactor (SBR) system is demonstrated to biologically remove nitrogen, phosphorus and chemical oxygen demand (COD) to very low levels from abattoir wastewater. Each 6 h cycle contained three anoxic/anaerobic and aerobic sub-cycles with wastewater fed at the beginning of each anoxic/anaerobic period. The step-feed strategy was applied to avoid high-level build-up of nitrate or nitrite during nitrification, and therefore to facilitate the creation of anaerobic conditions required for biological phosphorus removal. A high degree removal of total phosphorus (>98%), total nitrogen (>97%) and total COD (>95%) was consistently and reliably achieved after a 3-month start-up period. The concentrations of total phosphate and inorganic nitrogen in the effluent were consistently lower than 0.2 mg P l^{-1} and 8 mg N l^{-1}, respectively. Fluorescence in situ hybridization revealed that the sludge was enriched in *Accumulibacter* spp. (20–40%), a known polyphosphate accumulating organism, whereas the known glycogen accumulating organisms were almost absent. The SBR received two streams of abattoir wastewater, namely the effluent from a full-scale anaerobic pond (75%) and the effluent from a lab-scale high-rate pre-fermentor (25%), both receiving raw abattoir wastewater as feed. The pond effluent contained approximately 250 mg N l^{-1} total nitrogen and 40 mg P l^{-1} of total phosphorus, but relatively low levels of soluble COD (around 500 mg l^{-1}). The high-rate lab-scale pre-fermentor, operated at 37°C and with a sludge retention time of 1 day, proved to be a cheap and effective method for providing supplementary volatile fatty acids allowing for high-degree of biological nutrient removal from abattoir wastewater.

Keywords Biological nutrient removal · EBPR · Pre-fermentation · SBR · Slaughterhouse · Step-feed · PAO

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Introduction

The meat processing industry requires large quantities of water, much of which is discharged as wastewater containing high levels of chemical oxygen demand (COD) and nutrients such as nitrogen

(N) and phosphorus (P). Over the past two decades, biological COD and N removal from abattoir wastewater has received much greater attention than has the biological P removal. Reliable biological COD and nitrogen removal systems have been successfully developed and applied for abattoir wastewater treatment using continuous activated sludge systems (Beccari et al. 1984; Frose and Kayser 1985; Willers et al. 1993). However, P removal continues to be achieved primarily through chemical precipitation, despite biological P removal being a much cheaper and more environmentally sustainable option.

The main challenges for biological phosphorus removal from abattoir wastewater are:

- The wastewater contains a high level of ammonia and organic nitrogen. The complete nitrification of these nitrogen components produces a high level of nitrate, which has proved to be an obstacle to the development of a stable and reliable Bio-P removal process (Pitman et al. 1983; Comeau et al. 1986; Furumai et al. 1999). Phosphorus removal requires alternating anaerobic and aerobic/anoxic conditions. The high level of nitrate (due to the high influent nitrogen concentrations) makes the creation of true anaerobic conditions in the system difficult;
- Abattoir wastewater contains substantial amounts of fat, oil and grease (FOG), which would deteriorate the sludge settleability when directly fed to activate sludge systems. Primary treatment is typically required before abattoir wastewater is treated in biological nutrient removal systems. In Australia, the raw abattoir wastewater is typically pre-treated in anaerobic ponds with a hydraulic retention time ranging between 7 and 14 days. While reducing the FOG content, this anaerobic treatment process also removes a large fraction of the COD from the wastewater, resulting in COD limitations (particularly volatile fatty acids—VFAs) for N and P removal (Keller et al. 1997).

In this paper, we demonstrate the use of a sequencing batch reactor (SBR) system for biological nitrogen and phosphorus removal from abattoir wastewater. In recent years, the use of SBRs for the biological treatment of wastewater has been widely extended from lab-scale studies to full-scale wastewater treatment plants (Tilche et al. 1999; Artan et al. 2001; Keller et al. 2001; Puig et al. 2004). SBRs offer a great deal of operational flexibility as it allows for easy adjustment of aerobic, anoxic and anaerobic periods through temporal control of aeration and filling (Wilderer et al. 2001).

To address the first challenge described above, a step-feed scheme, characterised by several aerobic and anoxic phases in a SBR cycle with the wastewater fed to the reactor during the anoxic phases is employed (Anderottola et al. 2000; Lin and Jing 2001; Puig et al. 2004). This operational strategy allows timely removal of nitrate so that, when an adequate amount of COD is available, nitrate build-up is avoided (Puig et al. 2004).

To address the second challenge, the proposed system is equipped with a high-rate pre-fermentor, which is used to provide additional VFAs when the anaerobic pond effluent does not contain a sufficient amount for the biological phosphorus and nitrogen removal.

Details of the design, operation and performance of the proposed system are presented. Further potential optimisations are also highlighted.

Material and methods

Reactor set-up and operation

A lab-scale SBR with a working volume of 7 l was used in this study. The SBR was seeded with non-EBPR (enhanced biological phosphorus removal) sludge from a full-scale SBR treating abattoir wastewater in Queensland, Australia. About 1 l of EBPR sludge (MLSS around 4 g l^{-1}) enriched in a lab reactor (Lemaire et al. 2006) was added on day 60 to initiate the EBPR process in the reactor as there seemed to be no EBPR organisms present in the initial seed sludge. According to quantification with fluorescence in situ hybridisation (FISH, see below for further explanation), the lab reactor EBPR sludge contained 70% *Candidatus Accumulibacter Phosphatis* (called *Accumulibacter* spp. hereafter), a known polyphosphate accumulating organism (PAO). The SBR was operated with a cycle time of 6 h in a temperature-controlled room (18–22°C). This cycle time is commonly used in SBRs treating abattoir wastewater in Australia, and has previously been demonstrated to enable satisfactory COD and N removal from abattoir wastewater (Keller et al. 1997). In each cycle, 1 l of abattoir wastewater

Table 1 Operating conditions of lab-scale SBR (6 h cycle)

SBR sequences	HRT = 42 h	
	Duration (min)	DO ($mgO_2\ l^{-1}$)
Fill no-mix 1	5	~0
No-aerated mix 1 (anoxic or anaerobic[a])	30	~0
Aerated mix 1 (no aeration in the last 5 min)	55	1.5–2
Fill mix 2	3	~0
No-aerated mix 2 (anoxic or anaerobic[a])	70	~0
Aerated mix 2 (no aeration in the last 5 min)	35	1.5–2
Fill mix 3	2	~0
No-aerated mix 3 (anoxic or anaerobic[a])	60	~0
Aerated mix 3 (sludge wasted at the end)	20	1.5–2
Settle	70	~0
Decant	10	~0

[a] When nitrate and nitrite depleted

(more details given below) was pumped into the reactor over the three filling periods with a volume distribution of 0.5, 0.3 and 0.2 l, respectively. Each filling period was followed by non-aerated (either anoxic or anaerobic depending on when the oxidised nitrogen was completely consumed) and aerated periods (Table 1). During aerated periods, air was provided intermittently using an on/off control system to keep the dissolved oxygen (DO) level between 1.5 and 2 $mgO_2\ l^{-1}$. After the settling period, 1 l supernatant was removed from the reactor resulting in a hydraulic retention time (HRT) of 42 h. A volume of 115 ml of mixed liquor was wasted every cycle resulting in a sludge retention time (SRT) of 15 d. The pH in the system was recorded, ranging between 7.1 and 7.9, but not controlled. The ORP signal was also recorded to give indications of the nitrate levels in the reactor during the anoxic periods. The reactor was mixed with an overhead mixer except during the settling, decanting and first filling periods.

Wastewater

The wastewater used in this study was from a local abattoir in Queensland, Australia. At this site, the raw effluent passes through four parallel anaerobic ponds before being treated in a SBR for biological nitrogen and COD removal. The anaerobic ponds serve to reduce FOG and COD, and also to produce easily biodegradable COD, particularly VFAs, to facilitate the down-stream biological nitrogen removal. The four anaerobic ponds produced VFAs at different concentrations due to different organic loading rates. Pond A, which was the only easily accessible pond for wastewater collection in the study, was under-loaded leading to much lower COD and VFAs concentrations in comparison to other ponds (see Table 2). Therefore, extra VFAs were added to pond A effluent to simulate the higher VFA levels present in other ponds, as will be detailed in Table 3.

Raw wastewater and anaerobic pond effluent from the abattoir were collected on a weekly basis and

Table 2 Characteristics of the different types of wastewater used in this study

Parameter	Pre-fermented raw wastewater	Anaerobic pond A effluent[b]	Anaerobic pond B effluent
TCOD ($mg\ l^{-1}$)	7,460–9,300	430–720	740–950
SCOD ($mg\ l^{-1}$)	2,360–2,840	205–245	440–531
VFA[a] ($mg\ COD\ l^{-1}$)	703–869	24–32	272–358
TN ($mg\ l^{-1}$)	271–317	218–262	240–262
NH_4–N ($mg\ l^{-1}$)	139–160	207–224	220–226
TP ($mg\ l^{-1}$)	44–53	33–37	37–40
PO_4–P ($mg\ l^{-1}$)	38–43	32–34	33–36

The intervals represent the mid-95% range

[a] Acetate and propionate only

[b] Pond effluent used in this study; additional acetate and propionate was added (see Table 3) to simulate Pond B effluent, which was non-accessible for wastewater collection on site

Table 3 Characteristics of the SBR influent during its 9-month operation

Influent parameters	Day 0–30	Day 30–80	After day 80
Ratio VFA:TP	3.7	12.2	15.1
Ratio TCOD:TN	5.5	8.7	12
% Pre-fermented raw wastewater in influent	15	15	25
VFAs added to Pond A to simulate other ponds	No	Yes[a]	Yes[a]

[a] 250 mg COD l^{-1} acetate, and 100 mg COD l^{-1} propionate

stored at 4°C. The raw wastewater was subjected to 1 day pre-fermentation before being pumped into the SBR. The pre-fermentation was performed in a 50-l tank continuously mixed with a submersible pump. No inoculum was introduced in the pre-fermentor, and hence the microbial population present in the raw abattoir wastewater was used to carry out the fermentation. The temperature inside the tank was kept at 37°C by a heating probe and would not require any special heating system in a full-scale installation due to the temperature of the abattoir raw wastewater (typically around 40°C). The aim of this pre-fermentation step was to increase the level of easily biodegradable COD, in particular VFAs, which is critical for bio-P removal. The characteristics of the pre-fermented raw wastewater and the anaerobic pond effluent are compared in Table 2.

The abattoir closed down for a month over Christmas due to annual maintenance. During this period, the SBR cycle operation was modified in order to preserve the reactor biomass as no wastewater was available. The sludge was aerated and mixed for 15 min in each 6 h cycle and was allowed to settle for the rest of the cycle. The performance during this starvation and recovery period is the focus of another paper (Yilmaz et al. 2007).

Analyses

The ammonia ($NH_3 + NH_4^+$), nitrate (NO_3^-), nitrite (NO_2^-) and total phosphate (PO_4–P) were analysed using a Lachat QuikChem8000 Flow Injection Analyser (Lachat Instrument, Milwaukee). Total and soluble chemical oxygen demand (TCOD and SCOD, respectively), total Kjeldahl nitrogen (TKN), total phosphorus (TP), mixed liquor suspended solid (MLSS) and volatile MLSS (MLVSS) were analysed according to the standard methods (APHA 1995). VFAs were measured by Perkin–Elmer gas chromatography with column DB-FFAP 15 m × 0.53 mm × 1.0 µm (length × ID × film) at 140°C, while the injector and FID detector were operated at 220 and 250°C, respectively.

Fluorescence in situ hybridisation (FISH) was performed as specified in Amann (1995). Oligonucleotide probes used in this study were the combination of EUB338 i-iii (EUBmix) for the detection of all bacteria (Daims et al. 1999), the combination of PAO462, PAO651 and PAO846 (PAOmix) for *Accumulibacter* spp. (Crocetti et al. 2000), Actino-658 and Actino-221 for proposed *Actinobacteria* PAO (Kong et al. 2005), the probe combination (GAOmix) of GAOQ989 (Crocetti et al. 2002) and GB_G2 (Kong et al. 2002) for *Competibacter* spp., the probe combination (DF1mix) of TFO_DF218 and TFO_DF618 for Cluster 1 *Defluviicoccus vanus*-related spp. (Wong et al. 2004) and the probe combination (DF2mix) of DF988, DF1020 and helper probes H966 and H1038 for Cluster 2 *Defluviicoccus vanus*-related spp. (Meyer et al. 2006). FISH quantification was performed as described in Crocetti et al. (2002).

Model based simulations

To demonstrate the benefits of the multi-step feeding strategy in avoiding high-level build-up of nitrate, and hence enhancing P removal in the SBR, a model was devised based on the IWA ASM2d (Henze et al. 1999). A complete description of the model is provided in Lemaire et al. (2008). In the simulation studies described below, default parameter values recommended in Henze et al. (1999) were used. The wastewater composition used in the simulations is summarised in Table 4.

Two scenarios, one with three feeding periods as used in the experimental study and one with a single feeding period, were evaluated. In the case of a single feed, the SBR cycle consisted of one non-aerated period and one aerated period, followed by a settling and a decanting period. A volume of 1 l of influent was fed into the SBR at the start of the non-aerated period. The lengths of the non-aerated and aerated periods used in the single-feed case were equal to the total lengths of the three non-aerated periods and

Table 4 Wastewater composition used in simulation

Parameter	Value	Unit
S_A	500	mg COD l^{-1}
S_F	600	mg COD l^{-1}
S_{N_2}	15	mg N l^{-1}
S_{NH4}	206	mg N l^{-1}
S_P	37	mg P l^{-1}
S_{HCO}	73	mmol l^{-1}
S_I	120	mg COD l^{-1}
X_I	100	mg COD l^{-1}
X_S	200	mg COD l^{-1}
X_H	10	mg COD l^{-1}
X_{ash}	10	mgSS l^{-1}

three aerated periods, respectively, used in the three-feed case. All other operational parameters were the same, and as per previous description.

Results

Nutrient removal performance of the SBR

Figure 1 presents the influent COD, N and P, and effluent N and P concentrations, along with the MLSS concentration in the reactor and its volatile fraction, during the 9-month operation of the SBR. Also presented in Fig. 1 is the fraction of *Accumulibacter* spp., the only PAO found in the system. The putative *Actinobacteria* PAOs were not found in the sludge. Glycogen accumulating organisms (GAOs), namely *Competibacter* spp. and the putative *Defluviicoccus vanus*-related spp. (Cluster 1 and 2), were negligible (<1% of the total microbial population at all time). Complete nitrification was achieved in the SBR after less than 1 week of operation as shown by the absence of ammonia in the effluent (Fig. 1c). However, denitrification was incomplete and NO_x–N (nitrate + nitrite) accumulated in the reactor reaching 60 mg l^{-1} in the effluent during the first 30 days of operation (Fig. 1c). To improve the denitrification, extra VFAs (i.e. acetate and propionate) were added to pond A effluent on day 30 to simulate the VFA concentrations in the non-accessible ponds (typically 250 mg COD l^{-1} acetate and 100 mg COD l^{-1} propionate). As a result, the effluent NO_x–N level dropped to 15 mg l^{-1}. Phosphorus removal was negligible during the first 60 days (Fig. 1c) likely due to the presence of nitrate during most of the cycle and the lack of strict anaerobic conditions in the system, which is detrimental to PAO metabolism. The fact that non-EBPR sludge was used to seed the reactor could have also contributed to the slow development of PAOs.

After the introduction of 1 l lab-scale EBPR sludge enriched in *Accumulibacter* spp. on day 60, P removal improved dramatically, and consistent high-level of P removal was achieved and maintained thereafter. The process data clearly suggests that the enriched *Accumulibacter* spp. culture managed to survive and develop in a very different environmental setting. This is confirmed by the FISH quantitation results (Fig. 1c) and the decrease of the organic fraction in the biomass due to intracellular poly-P storage by *Accumulibacter* spp. (Fig. 1d).

However, Fig. 1c also shows that while P removal was improving, NO_x–N started to accumulate again in the system. It was believed that a shortage of easily biodegradable COD in the reactor triggered this NO_x–N accumulation as PAOs and denitrifiers were now competing for the carbon sources. Prior to the addition of PAOs, all the easily biodegradable COD contained in the feed was consumed by denitrifiers during the non-aerated periods, resulting in a low NO_x–N concentration at the end of a cycle. It enabled the creation of anaerobic conditions during the first non-aerated period and the freshly introduced PAOs were able to take up a significant fraction of the influent COD in this period, as clearly demonstrated by cycle studies (data not shown). This reduced the availability of organic carbon for ordinary denitrifiers leading to the accumulation of NO_x–N. This condition, if not amended, would eventually lead to the washout of PAOs as high-level of NO_x–N eliminates anaerobic conditions required by PAOs. In order to further increase the amount of VFA available for P and N removal, the amount of pre-fermented raw wastewater in the influent was increased from 15 to 25% on day 80 resulting in a higher VFA:TP ratio and TCOD:TN ratio in the influent (Table 3). Denitrification improved immediately and from day 100 onwards, less than 10 mg N l^{-1} remained in the effluent. There was one interruption to the reactor operation between day 125 and 160, when the abattoir closed down and no wastewater could be supplied to the SBR (Fig. 1). The reactor biomass concentration decreased by 30% during this long

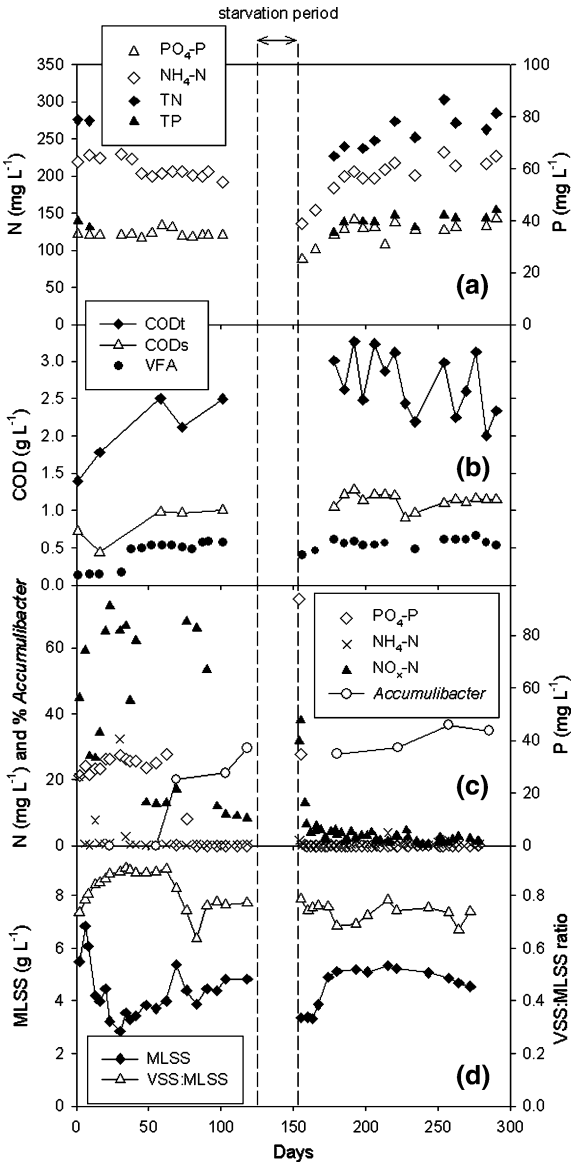

Fig. 1 Characteristics of the influent (**a**) and **b** effluent nutrient levels and the *Accumulibacter* spp. population (**c**) and MLSS and the VSS:MLSS ratio in the reactor (**d**). No wastewater was fed during the 33 days of starvation period

starvation period but the nitrifying, denitrifying and phosphorus removal capabilities of the biomass were adequately maintained through the use of an alternating anoxic/anaerobic and aerobic starvation strategy, as demonstrated by the quick recovery of the reactor performance when wastewater feed was resumed (within 4 days). A detailed report of this starvation period can be found in Yilmaz et al. (2007).

According to the effluent and MLSS data (Fig. 1c, d), the SBR reached a steady state around day 170. Table 5 details the SBR effluent quality between day 170 and 275. For comparison, the COD and nutrient levels in the influent are also presented. The SBR process consistently achieved 95, 97 and 98% of TCOD, TN and TP removal, respectively. The remaining COD in the effluent could be regarded as non-biodegradable and represented about 5% of the total COD initially present in the influent. It was observed that the sludge volume index (SVI) was relatively high throughout the study period, between 180 and 250 ml $gMLSS^{-1}$. This could have partially been caused by the remaining high fat/oil/grease content of the pre-fermented raw wastewater, as suggested by Johns (1995). However, the suspended solids concentration in the effluent was lower than 25 mg l^{-1} at all times (data not shown).

Figure 2 shows the nitrogen and phosphorus transformations in a typical SBR cycle study during the steady state period. At the end of each aerobic period, NH_4–N was fully oxidised, and the low level of NO_x–N accumulated was then removed in the following anoxic period. The small amount of NO_x–N carried over to the next cycle (less than 0.5 mg l^{-1}) was quickly denitrified at the beginning of the first anaerobic period. Anaerobic P release by PAOs mostly occurred in the first non-aerated period due to the absence of NO_x–N in the SBR bulk liquid. PO_4–P was then fully taken up during the subsequent aerobic periods.

Model-based evaluation of the impact of multi-step feeding strategy

Figure 3 shows the simulated effluent PO_4–P and NO_x–N concentrations as well as the PAO populations in the two scenarios evaluated. With the multi-step feeding strategy, excellent P and N removal was achieved (Fig. 3a), which agrees well with the experimental results presented in Fig. 1. In comparison, when only one feeding period was simulated (Fig. 3b), the effluent PO_4–P stabilised at an elevated level of approximately 4 mg P l^{-1} while the effluent NO_x–N level stayed above 20 mg N l^{-1} (in comparison to 2.5 mg N l^{-1} with multi-step feeding scenario). The multi-step feed operation resulted in a PAO population size that was 30% higher than that in the single-feed case due to more favourable conditions for PAOs created by the better NO_x–N

Table 5 Influent and effluent characteristics between day 170 and 275

Parameter (mg l^{-1})	Influent ($N = 13$)		Effluent ($N = 32$)		Removal of TCOD, TN and TP (%)
	Mid-95% range	Mean	Mid-95% range	Mean	
TCOD	2,600–3,120	2,870	129–151	140	95
SCOD	1,150–1,320	1,240	114–127	121	
TKN	236–277	256	5.3–7.7	6.5	97
NH_4–N	196–215	206	0.2–0.8	0.5	
NO_x–N	Not detected		1.9–2.8	2.3	
TP	38–41	40	0.7–1.4	1.0	98
PO_4–P	35–38	37	0.04–0.09	0.06	

N represents the number of samples analysed between day 170 and 275

removal. The simulations results clearly demonstrated the benefits of the multi-step feeding strategy in terms of N and P removal.

Performance of the pre-fermentor

The impact of the 1-day pre-fermentation performed on raw wastewater is depicted in Fig. 4. The overall VFA concentration more than doubled as a direct result of this pre-fermentation. Acetate and propionate were the most abundant VFAs in the raw abattoir wastewater before and after pre-fermentation with propionate having a slightly higher production rate than acetate. Also shown in Fig. 4 is the impact of pre-fermentation on the NH_4–N and PO_4–P concentrations. While PO_4–P concentration stayed constant, NH_4–N concentration doubled due to partial mineralisation of the organic nitrogen which represents around 75% of the raw wastewater total nitrogen. The 1 week storage of the pre-fermented raw wastewater in the cold room at a temperature of 4°C affected VFAs levels more than nutrient levels with a 20% reduction of acetate and propionate concentration.

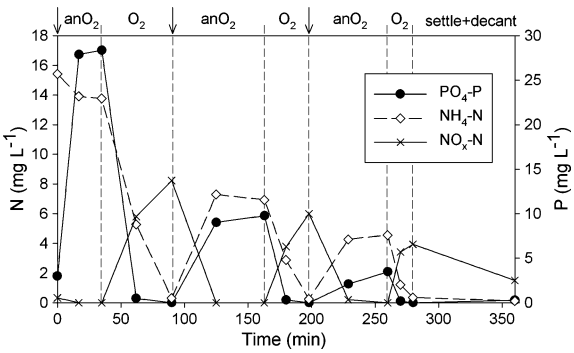

Fig. 2 Nitrogen and phosphorus profiles during a SBR cycle study performed on day 243. *Vertical arrows* indicate the three feeding periods

Discussion

Multi-feed strategy to promote biological P removal

Biological phosphorus removal from wastewaters containing a high level of nitrogen, such as abattoir wastewater, is challenging. Large accumulation of nitrate or nitrite must be avoided in order to secure anaerobic conditions required by PAOs. Several studies using the SBR technology to simultaneously remove COD, N and P from piggery wastewater have been reported (Tilche et al. 1999; Obaja et al. 2003, 2005). However, the characteristics of piggery wastewater differ greatly from those of abattoir wastewater. The large amount of inorganic salts, minerals and metal ions present in the piggery wastewater promote chemical P removal by precipitation, as evidenced by the scarce P release observed during the anaerobic stage of the process (Bortone et al. 1994). Subramaniam et al. (1994) and Keller et al. (1997) attempted to achieve simultaneous COD, N and P removal biologically from abattoir wastewater using SBR systems. However, P removal was quite unstable due to intermittent recycling of high levels of NO_x–N to the anaerobic period.

The use of a multi-feed strategy in this study aimed to limit the level of NO_x–N recycled to the

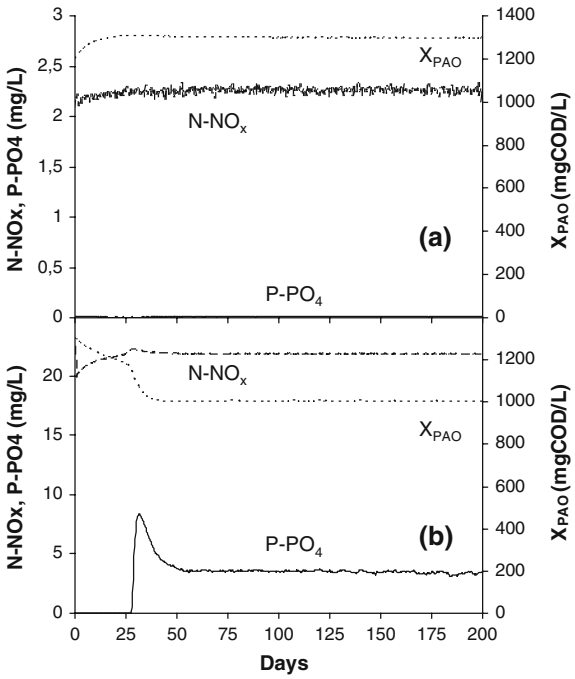

Fig. 3 The simulated concentration of PO_4–P and NO_x–N in the reactor effluent and the PAO population sizes (**a**) with the 3-step feeding strategy and (**b**) with a single feeding step

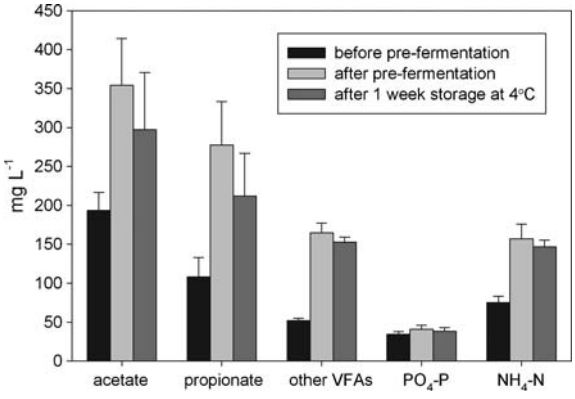

Fig. 4 Concentration of the main VFAs, PO_4–P and NH_4–N in the raw wastewater before and after pre-fermentation, and after 1 week storage at 4°C. Other VFAs include iso-butyric, butyric and iso-valeric acids. (*Error bars* = SD, $n = 11$)

anaerobic period. Figure 2 shows that the strategy was very successful. The NO_x–N level was less than 8 mg l^{-1} throughout the cycle, despite of the high level of NH_4–N and organic nitrogen in the wastewater (over 250 mg l^{-1}, see Table 5). Based on the amount of wastewater fed over the three feeding periods in the SBR cycle (i.e. 0.5, 0.3 and 0.2 l, respectively) and the PO_4–P concentration in the influent (i.e. 35 mg l^{-1}), the amounts of PO_4–P introduced in the SBR bulk liquid during each feeding steps were estimated to be 2.7, 1.5 and 1.0 mg l^{-1}, respectively. By subtracting these amounts from those measured at the end of each anaerobic period (Fig. 2) the true anaerobic P release by PAOs following the three feeding periods was estimated to be 25.3, 4.5 and 1.0 mg P l^{-1}, respectively. The very large discrepancy between the first anaerobic P release and the second and third is mostly due to the very low level of NO_x–N in the bulk liquid prior to the first feeding (<0.5 mg l^{-1}) compared to the second (8 mg l^{-1}) and third (6 mg l^{-1}) feeding (Fig. 2). This indicates that the first non-aerated period was crucially important for P removal as PAOs could freely utilise all the VFA available without having to compete with heterotrophic denitrifiers. Without the multi-feed strategy employed in this study, high level of NO_x–N is likely to have accumulated in the SBR at the end of the cycle and to have been recycled into the first anaerobic period preventing the stable high level of P removal reported. This was further confirmed by the simulation results presented in Fig. 3 where, without the multi-step feeding strategy in place, the PAO population in the SBR decreased and P removal became incomplete due to high levels of NO_x–N in the SBR.

Pre-fermentation of raw wastewater

The performance of a biological nutrient removal system depends greatly on the availability of easily biodegradable carbon sources in the wastewater, particularly VFAs. Considering the fact that it is difficult to control the VFAs content in large anaerobic ponds, a more controllable VFA source is necessary for reliable biological nutrient removal from abattoir wastewater. In this study, a high-rate pre-fermentation step was demonstrated to be a cheap and effective option. Table 3 shows that the VFA:TP ratio increased from 12.2 to 15.1 when the pre-fermented wastewater fraction in the SBR influent increased from 15 to 25% on day 80. This caused an immediate reduction in the nitrate level, with a drastic improvement to the reliability of P removal (Fig. 1b, c). The results show that it is both necessary and practically feasible to include a high-rate pre-fermentor to generate VFAs

that may be supplemented to the nutrient removal SBR when an inadequate amount of VFAs is present in the pond effluent.

However, it should be highlighted that the use of raw wastewater should be minimised. There is evidence suggesting that a high fraction of raw feed deteriorates the sludge settleability (data not shown) likely due to its higher FOG content compared to pond effluent. An over supply of carbon sources through this stream would also increase aeration costs and sludge production in the SBR system. Controlled addition of this stream using an on-line control system would be highly beneficial. However, the control of VFAs supplement to biological phosphorus removal systems in accordance to the actual demand for VFAs (varying with time) is still unresolved (Olsson et al. 2005).

An alternative solution that is being investigated is to reduce the demand for carbon sources by achieving nitrogen removal via nitrite instead of nitrate. This strategy, if successful, would reduce the carbon demand for denitrification by 40% (Turk and Mavinic 1986). This would therefore reduce the amount of additional carbon supply, which in turn will also reduce the overall oxygen requirement. Such an improvement would have significant benefits for the operation of large-scale wastewater treatment facilities. Peng et al. (2004) demonstrated that stable nitrite accumulation during the nitrification process could be obtained through an aeration control system based on pH and DO signals. On-line control systems based on simple pH and DO signals are being developed to achieve this nitrite pathway in our lab-scale SBR.

A further opportunity to reduce the demand for carbon sources is to enhance the denitrification by PAOs. It has been found that *Accumulibacter* spp. are capable of taking up phosphorus under anoxic conditions (Kuba et al. 1993; Meinhold et al. 1999; Zeng et al. 2003a). This is particularly attractive as the same carbon could be used for both denitrification and P removal. However, the exact conditions necessary to promote this type of denitrification are still unclear and further investigations are needed (Zeng et al. 2003a).

The low abundance of GAOs in the sludge

Competibacter spp. have been widely reported to be abundant in both lab-scale EBPR reactors (Mino et al. 1995; Crocetti et al. 2002; Kong et al. 2002; Zeng et al. 2003b) and full-scale EBPR plants (Crocetti et al. 2002; Saunders et al. 2003; Kong et al. 2006). Surprisingly, in this study, *Competibacter* spp. were scarcely present in the reactor representing always less than 1% of the total microbial population. *Defluviicoccus vanus*-related *Alphaproteobacteria* organisms, a new putative GAO recently reported in literature (Wong et al. 2004; Meyer et al. 2006), was also found to be in very low abundance in the reactor.

Several factors have been suggested in the literature that may influence on the competition between PAOs and GAOs. Filipe et al. (2001) and Oehmen et al. (2005) found that pH has a significant impact on the PAO and GAO competition with *Accumulibacter* spp. possessing advantages over *Competibacter* spp. for anaerobic carbon uptake at relatively high pH (>8). The pH in the study fluctuated between 7.1 and 7.9 during a cycle (uncontrolled), which should have unlikely provided any selective advantages to PAOs over GAOs. Saito et al. (2004) reported that the presence of nitrite in the anaerobic or aerobic period inhibited the PAO activity and could therefore enhance the presence of GAOs in the system. The presence of nitrite in the reactor during all three aerobic periods and during the second and third anaerobic periods apparently did not promote the growth of GAOs, as suggested by Saito et al. (2004). Some studies also showed that better EBPR performance was achieved at relatively low temperature (5–15°C) due to a shift in the microbial community from GAOs to PAOs (Whang and Park 2002; Erdal et al. 2003). The temperature used in this study, controlled between 18 and 22°C, is very similar to many reactor studies where GAOs appeared to be a problem, and is therefore not believed to be a significant contributor to the low abundance of GAOs. A more likely reason for the limited growth of GAOs in this reactor could be the large fraction of propionate present in the influent (propionate to acetate COD ratio was 0.8). Pijuan et al. (2004) and Oehmen et al. (2006) revealed that propionate as a carbon source may provide selective advantage to PAOs. The pre-fermentor used in this study largely contributed to the increase of the propionate fraction. If this hypothesis is true, the operation of the pre-fermentor should be optimised to not only maximise the total amount of VFAs produced but also to control the VFAs composition and particularly the acetate to propionate ratio.

Conclusions

A sequencing batch reactor system was demonstrated to effectively remove nitrogen, phosphorus and COD from abattoir wastewater. This provides a more cost-effective and environmentally friendly alternative to chemical phosphorus removal, which is a common practice at present. The following conclusions are drawn:

- It is possible to achieve a high degree (>98%) of biological phosphorus removal from abattoir wastewater in the presence of high levels of nitrogen (200–300 mg N l^{-1}).
- The multi-step feeding strategy prevents high-level accumulation of nitrate or nitrite, and hence facilitates the creation of anaerobic conditions. The strategy is strongly recommended for practical use in the biological treatment of abattoir wastewater.
- It is important to incorporate a high-rate pre-fermentor as an integrated component of the nutrient removal system. This stream, which contains a high-level of VFAs, is effective in providing supplementary carbon sources for both phosphorus and nitrogen removal.

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