Performance and metabolic aspects of a novel enhanced biological phosphorus removal system with intermittent feeding and alternate aeration

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ABSTRACT

A novel enhanced biological phosphorus removal (EBPR) system, which combined the intermittent feeding design with an anaerobic selector, was examined using on-line oxidation reduction potential (ORP), nitrate and ammonium probes. Two experimental periods were investigated: the aerobic and anoxic phases were set at 40 and 20 minutes respectively for period I, and set at 30 and 30 minutes for period II. Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5) and P removal were measured as high as 87%, 96% and 93% respectively, while total Kjeldahl nitrogen (TKN) and NH_4^+ removal averaged 85% and 91%. Two specific denitrification rates (SDNRs), which corresponded to the consumption of the readily biodegradable and slowly biodegradable COD, were determined. SDNR-1 and SDNR-2 during period I were 0.235 and 0.059 g N g^{-1} volatile suspended solids (VSS) d^{-1} respectively, while the respective rates during period II were 0.105 and 0.042 g N g^{-1} VSS d^{-1}. The specific nitrate formation and ammonium oxidizing rates were 0.076 and 0.064 g N g^{-1} VSS d^{-1} for period I and 0.065 and 0.081 g N g^{-1} VSS d^{-1} for period II respectively. The specific P release rates were 2.79 and 4.02 mg P g^{-1} VSS h^{-1} during period I and II, while the respective anoxic/aerobic uptake rates were 0.42 and 0.55 mg P g^{-1} VSS h^{-1}. This is the first report on an EBPR scheme using the intermittent feeding strategy.

Key words | denitrifying phosphorus accumulating organisms (denitrifying PAOs – DPAOs), enhanced biological phosphorus removal (EBPR), nitrification and denitrification rates, phosphorus release rates, phosphorus uptake rates

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INTRODUCTION

The most widespread method implemented for urban wastewater treatment is the conventional activated sludge (CAS) process. This process enables organic carbon (C) removal – part of which (almost 50%) is oxidized to CO_2 and the rest incorporated to new biomass – ammonia oxidation to nitrate and partial (20–30%) phosphorus removal. As long as extended nitrogen (via denitrification) and biological phosphorus removal is of concern, the CAS process should be retrofitted by incorporating anaerobic and anoxic treatment stages in parallel to the main aeration tank. Application of enhanced biological phosphorus removal (EBPR) postulates continuous sludge recirculation between anaerobic and aerobic (or anoxic) conditions. Under anaerobic conditions organic substrate is taken up by phosphorus-accumulating organisms (PAOs) and stored intracellularly with simultaneous phosphate release. In the subsequent aerobic phase, the internally stored organic substrate is oxidized and the energy produced is used for cell growth and excess phosphate uptake (Oehmen *et al.* 2007; Gebremariam *et al.* 2011). Phosphorus is removed from the system via wastage of P-rich excess sludge originating from aerobic conditions. Because the PAOs' anaerobic metabolism is disturbed by the presence of NO_3^- (Kuba *et al.* 1994), adequate N removal (via denitrification) is necessary for achieving effective EBPR. As a consequence, anoxic zones should be incorporated into the system design as well.

Activated sludge treatment plants being operated for nutrient removal should be supplied with sufficient organic substrate in order to achieve both denitrification (by

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denitrifying heterotrophic organisms) and phosphorus removal (by PAOs). However, domestic sewage is generally regarded as a low organic strength wastewater, thereby encouraging competition between the respective bacterial species (Kuba *et al.* 1996; Hamada *et al.* 2006). Availability of organic carbon is often a limiting factor for effective nutrient removal in activated sludge systems treating municipal wastewater. It has been suggested that the influent chemical oxygen demand (COD)/N ratio should not drop below 7, in order to achieve satisfactory denitrification (Kujawa & Klapwijk 1999; Komorowska-Kaufman *et al.* 2006), while the ratio should be kept above 11.1, when P removal is of concern (Shin *et al.* 2001).

In order to achieve efficient denitrification, maximum use of the available carbon for nitrate reduction is highly desirable. Under this view, the incorporation of an intermittent feeding (IF) process with discontinuous wastewater introduction and periodic aeration in a single activated sludge reactor has been suggested (Kantartzi *et al.* 2010). Characteristic of this process is the cycle operation, with sequential anoxic/aerobic periods, where the wastewater is fed in pulses at the beginning of the anoxic period of each cycle. As a result, all the available organic matter is used for the efficient denitrification of the nitrate originating from the previous aerobic (nitrifying) part of the operational cycle. Little or no carbon is oxidized under aerobic conditions, thereby resulting in significant energy savings for aeration.

The present study aims to investigate the performance of a novel EBPR system that combines the IF design with an anaerobic selector installed prior to the main reactor, in order to achieve biological P removal in conjunction with nitrification/denitrification.

METHODS

Pilot-scale setup

A pilot-scale activated sludge system consisting of an anaerobic tank (selector), a continuous stirred tank reactor (main bioreactor) and a sedimentation tank was used in this study (Figure 1). The main bioreactor was operated under alternate anoxic/aerobic conditions by discontinuous application of aeration through an aeration element mounted at the reactor bottom, over an operational cycle of 1 hour. The influent wastewater was continuously pumped to the anaerobic reactor and the mixed liquor from the latter was fed to the main bioreactor periodically, added rapidly (for 3–4 minutes), once at the beginning of each cycle under anoxic conditions (aeration off) (Kantartzi *et al.* 2010). A sludge recirculation stream was directed from the sedimentation tank to the anaerobic selector to sustain adequate suspended solids concentration. During the anoxic phase of the operational cycle, the bioreactor content was kept in suspension by means of a low-speed (40–50 rpm) overhead stirrer, whereas a high-speed (100–120 rpm) mixer fixed at the bottom of the reactor was operated during the aerobic phase, in order to efficiently disperse the air bubbles and enhance oxygen dissolution. The basic design characteristics of the bioreactors used are shown in Table 1. The

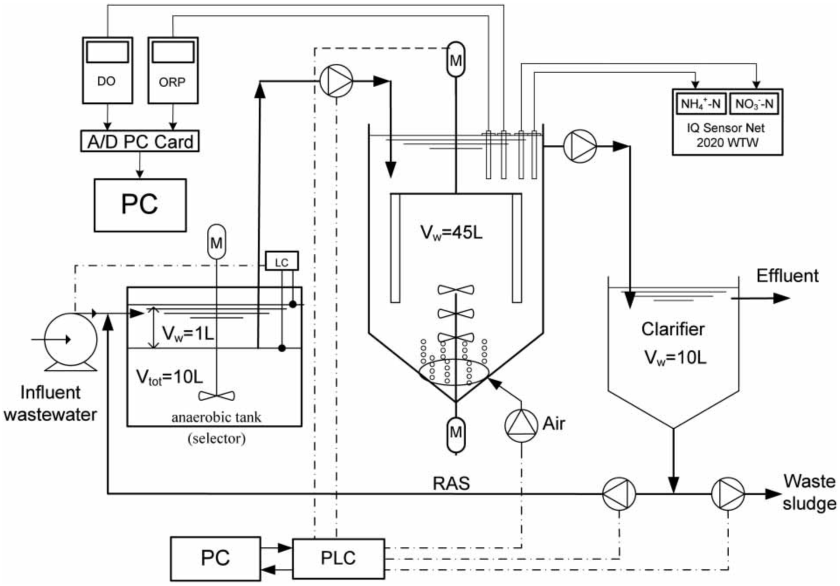

Figure 1 | Flow chart of the pilot-scale EBPR system used in the study.

Table 1 | Basic design characteristics of the pilot-scale plant

Parameter	Period I	Period II
Operational period, d	0–77	78–110
Volume, L		
Anaerobic	5	5
Main bioreactor	45	45
Settling tank	10	10
Influent flow rate, L/d	48	48
Return sludge flow, L/d	24	24
Aerobic/anoxic phase, min	40/20	30/30
Sludge retention time, d	10	10
Temperature, °C	20 ± 2	20 ± 2

operation of the technical equipment (peristaltic pumps, aeration pump, stirrers) was controlled by a programmable logic controller.

Wastewater characteristics

Wastewater from the local university campus (Xanthi, Northern Greece) was used as influent to the pilot plant. Sludge from the municipal wastewater treatment plant of Xanthi was used as the inoculum. Mean values for the main sewage characteristics are given below (with standard deviation in parenthesis): total COD 480 (±74) mg L^{-1}, soluble COD 203 (±55) mg L^{-1}, biochemical oxygen demand (BOD_5) 325 (±55) mg L^{-1}, total suspended solids (TSS) 207 (±77) mg L^{-1}, volatile suspended solids (VSS) 174 (±73) mg L^{-1}, NH_4^+ 55 (±12) mg N L^{-1}, total Kjeldahl nitrogen (TKN) 74 (±10) mg L^{-1}, PO_4^{3-} 7.9 (±3.2) mg P L^{-1}, TP 9.8 (±3.3) mg L^{-1}, pH 7.3 (±0.2) and electric conductivity 1,360 (±215) μS cm^{-1}.

Experimental procedure

The experiment was divided into two different periods, depending on the relative anoxic/aerobic phase duration of the reactor's operational cycle. During the first period, the aerobic and anoxic phases were maintained at 40 and 20 minutes respectively, whereas for the second period, a 30 and 30 minute phase distribution was chosen. During the whole experimental period, the influent flow rate remained constant at 48 L/d, resulting in a mean hydraulic residence time (HRT) of 25 hours (excluding settling HRT). The operational parameters during the entire experimental period are summarized in Table 2.

Table 2 | Operational parameters of the pilot-scale plant

Parameter	Mean ± STDEV
$L^{a}_{org,v}$ (g BOD L^{-1} d^{-1})	0.35 ± 0.07
F/M (g BOD g^{-1} $MLVSS^{g}$ d^{-1})	0.15 ± 0.06
$L^{b}_{N,V}$ (g TKN L^{-1} d^{-1})	0.08 ± 0.01
$L^{c}_{N,VSS}$ (g TKN g^{-1} MLVSS d^{-1})	0.03 ± 0.01
$L^{d}_{P,V}$ (g P L^{-1} d^{-1})	0.01 ± 0.01
$L^{e}_{P,VSS}$ (g P g^{-1} MLVSS d^{-1})	0.004 ± 0.002
$MLSS^{h}$ (g L^{-1})	3.22 ± 1.04
MLVSS (g L^{-1})	2.68 ± 0.85
$MLSS^{f}_{RAS}$ (g L^{-1})	8.60 ± 3.11
$MLVSS_{RAS}$ (g L^{-1})	6.89 ± 2.38
SVI (mL g^{-1})	166 ± 34

[a] Volumetric organic loading rate.
[b] Volumetric nitrogen loading rate.
[c] Specific nitrogen loading rate.
[d] Volumetric phosphorus loading rate.
[e] Specific phosphorus loading rate.
[f] Return activated sludge.
[g] Mixed liquor volatile suspended solids.
[h] Mixed liquor suspended solids.

Analytical methods

Samples were collected from the plant influent and the effluent of each tank, and analysis was conducted according to the protocols described in *Standard Methods* (Clesceri *et al.* 1998). Before sampling, the system was operated for a start-up period of 20 days (≈2·sludge retention time, SRT) in order to ensure adequate biomass acclimatization. For the acclimatization period, operational conditions were the same with those of period I. Total and soluble COD were analyzed by the closed reflux titrimetric method. BOD_5 concentration was determined according to the 5 day BOD test. TKN was assessed by using the macro-Kjeldahl distillation method, whereas ammonia nitrogen was analyzed according to the titrimetric method on samples that had been taken through preliminary distillation. Nitrite was determined by using the 4500-B colorimetric method. Phosphate concentration was determined by the stannous chloride method and total phosphorus (TP) analysis was conducted according to de Haas *et al.* (2000) after the samples' persulfate digestion. All soluble parameters were determined in samples passed through a 0.45 μm membrane filter.

Besides off-line analysis on grab samples, on-line monitoring of oxidation reduction potential (ORP) and nitrate was also conducted in the main bioreactor. The ORP level was monitored by a SenTix ORP electrode connected to a pH

340i analyzer (WTW), while NO_3^- and NH_4^+ concentrations were assessed by using appropriate ion-selective electrodes (IQ SensorNet NitraLyt® and AmmoLyt®, WTW).

Statistical analysis

The Pearson correlation test ($p < 0.05$) and Student's *t*-test were performed in order to evaluate relationships between variables and compare treatment means.

RESULTS AND DISCUSSION

The pilot plant efficiency regarding total COD, BOD_5, TKN, NH_4^+, total nitrogen (TN) and TSS removal is depicted in Figure 2. The influent and effluent concentrations of each single parameter as well as the respective plant removal efficiency is shown for the whole experimental period (period I and period II).

Organic matter removal

The system efficiency concerning total COD removal was good, with an average removal ratio equal to 87% (±5.6) and an effluent concentration of 64 (±24) mg L^{-1}. Similarly, the system BOD_5 removal efficiency reached 96% (±1.6) and the effluent concentration was 14 (±3.6) mg L^{-1}. Interestingly, total COD removal efficiency was found to be significantly higher ($p < 0.05$) during period II compared to period I, with the respective mean values being 89.6% and 83.3%.

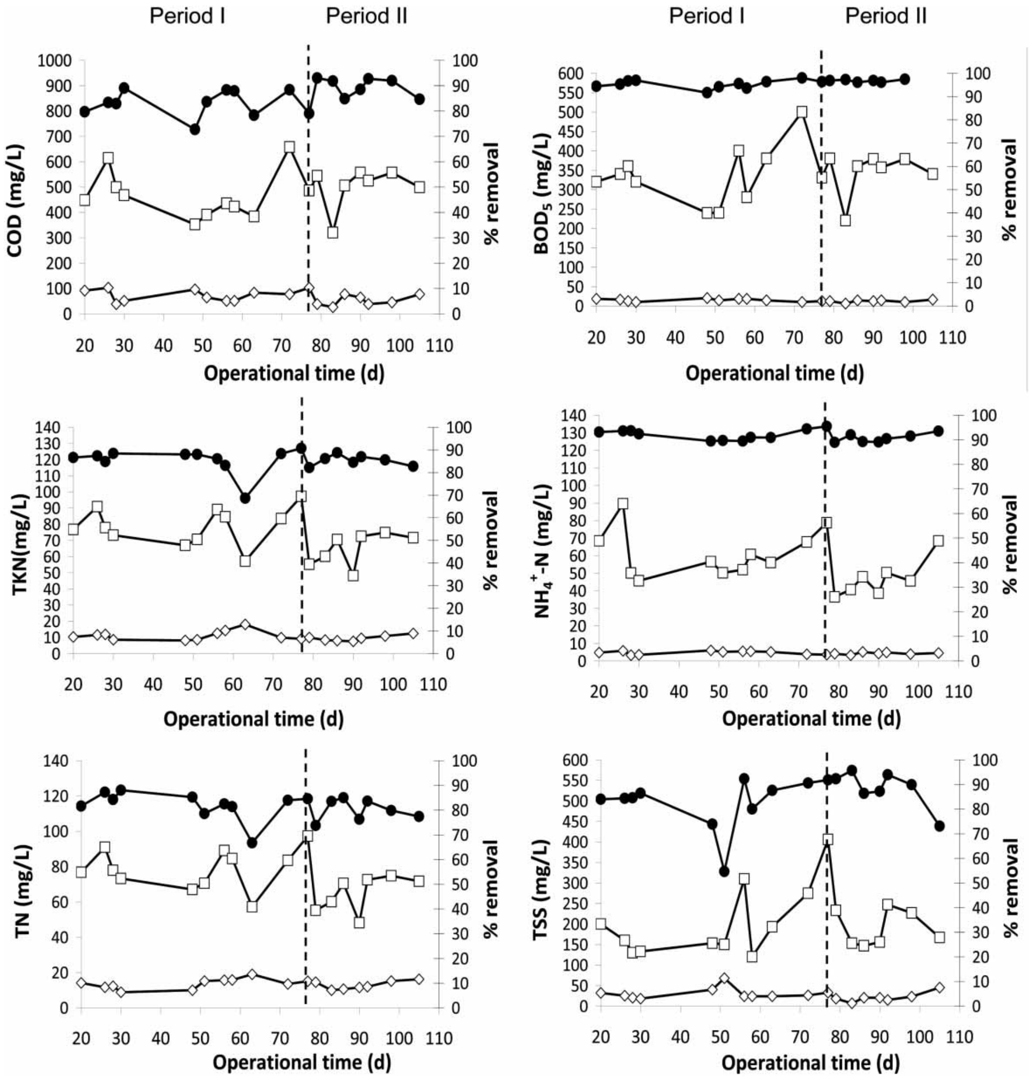

Figure 2 | Variation of influent (□) and effluent (◇) concentrations, and the respective removal percentages (•) of basic pollution parameters throughout the experimental period.

Nitrogen removal

The system operation resulted in high ammonia nitrogen removal, with the corresponding removal percentage and mean effluent concentration being 92% (±2.1) and 4.5 (±0.9) mg N L^{-1} respectively. No statistically significant difference was identified for the ammonia removal between periods I and II, indicating that the aeration period of 30 minutes was high enough to achieve satisfactory nitrification. Moreover, TKN removal was identified as high as 86% (±4.8) during the whole experimental period, with a mean effluent concentration of 10.5 (±2.6) mg L^{-1}.

The nitrification and denitrification rates as well as the ammonia oxidizing rates were calculated based on the on-line NO_3^--N and NH_4^+-N data obtained during periods I and II; see Figure 3(a). Two specific denitrification rates (SDNRs) were observed as influenced by the kind of the organic matter available to denitrifiers, i.e. the readily and slowly biodegradable organic carbon. SDNR-1 was observed during the first minutes of anoxic phase, whereas SDNR-2 was obtained as soon as nitrate became minimal (below ORP knee). In both experimental periods, the system demonstrated a significant denitrification capacity, since SDNRs were sufficient to achieve an NO_3^--N concentration of 2.8 (±2.0) mg L^{-1} in the effluent. NO_2^--N was measured off-line and identified in negligible amounts (less than 0.5 mg L^{-1}). During period I, SDNR-1 and SDNR-2 were 0.235 (±0.069) and 0.059 (±0.020) g N g^{-1} VSS d^{-1} respectively, while the rates during period II were 0.105 (±0.035) and 0.042 (±0.005) g N g^{-1} VSS d^{-1} respectively; see Figure 3(a). As expected, consumption of the readily biodegradable COD led to higher denitrification rates than those determined during the utilization of slowly biodegradable COD. SDNRs during period I were greater than those obtained during period II. This phenomenon can be attributed to the proliferation of denitrifying PAOs (DPAOs) during the latter period due to the prolonged anoxic phase (see also the 'Phosphorus removal' section), which exhibit a complex behavior with the simultaneous presence of an electron acceptor (i.e. nitrate) and an electron donor (i.e. organic matter) during the first minutes of the anoxic phase (Wachtmeister *et al.* 1997; Kapagiannidis *et al.* 2013).

The specific nitrate formation rate (SNFR) and the specific ammonium oxidizing rate (SAOR) were estimated as 0.076 (±0.021) and 0.064 (±0.023) g N g^{-1} VSS d^{-1} for period I, and 0.065 (±0.015) and 0.081 (±0.010) g N g^{-1} VSS d^{-1} for period II respectively. No statistically significant differences were observed for SNFR and SAOR at any period examined.

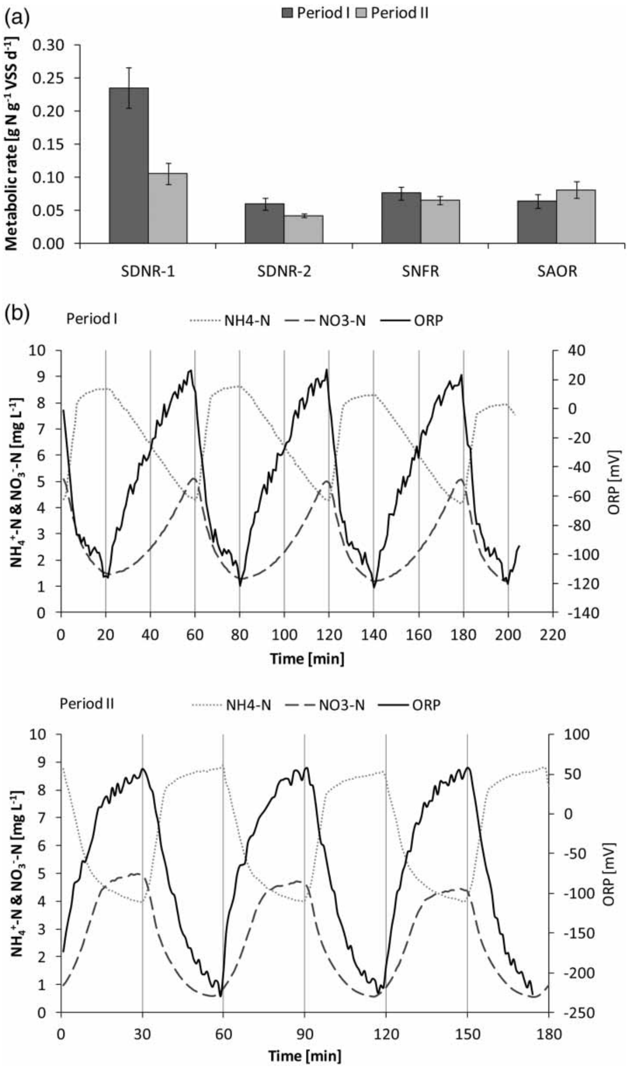

Figure 3 | (a) Determination of N metabolic rates in the intermittently aerated and fed EBPR system ($n = 15$) and (b) typical variations of ORP and the corresponding NH_4^+-N and NO_3^--N concentrations in the IF reactor during successive operational cycles of experimental periods I and II. Abbreviations: SDNR, specific denitrification rate; SNFR, specific nitrate formation rate; SAOR, specific ammonium oxidizing rate.

Figure 3(b) shows the typical variations of the ORP and the corresponding NO_3^--N concentration during three successive operational cycles for a typical day of periods I and II. In any case, NO_3^- was eliminated by the end of the anoxic period, as also indicated by the low negative value of ORP (less than −100 mV). As soon as aeration was turned on, ORP increased, reaching a maximum of 30 and 50 mV for period I and period II respectively. At the same time point, NO_3^- concentration became maximal. As shown in Figure 3(b), complete denitrification was achieved within a short period of time during period I (less than 20 minutes), while a period of 30 minutes was needed for the elimination of NO_3^- during period II. The strong correlation between ORP and NO_3^- concentration values suggests the use of on-line sensors as efficient operational control parameters for activated sludge systems designed for N removal (Caulet *et al.* 1998). Such on-line control could ensure maximum N removal efficiency, operational stability and optimum operation of aeration pumps, so significant energy requirements could be saved.

Phosphorus removal

The profile of PO_4^{3-} concentration at the various stages of the pilot plant is shown in Figure 4(a). Phosphorus concentration was found to follow the typical pattern of EBPR systems, namely P was released under anaerobic conditions and taken up during the following anoxic/aerobic conditions. Interestingly, anaerobic P release was found to be significantly higher during period II ($p < 0.05$) as compared to period I. In fact, P release was 23.3 ($\pm$6.1) mg L^{-1} in the anaerobic reactor during period I, compared to 36.7 ($\pm$10.8) mg L^{-1} PO_4^{3-}-P released during period II. As denitrification was found to be high enough in both periods, the difference in P release during period I is likely to be attributed to PAO poly-hydroxyalkanoates (PHAs) content minimization due to the extended aeration phase applied (40 minutes). Similarly, in a study by Brdjanovic *et al.* (1998), implementation of excessive aeration in an EBPR system resulted in gradual PHA depletion by PAO cells with adverse effects on the EBPR efficiency.

Regarding Figure 4(b), P removal efficiency during period II ($93.7 \pm 5.89\%$) was higher than in period I ($82.4 \pm 10.7\%$). Moreover, a direct relationship between the P released in the anaerobic tank and the P taken up in the intermittent reactor in both periods was identified; see Figure 4(c) (Pearson r coefficients of 0.99 and 0.95 for periods I and II respectively, $p < 0.01$), indicating that greater P release in the anaerobic period can lead to greater P uptake during the anoxic/aerobic period in an intermittently aerated and fed bioreactor (Zhao *et al.* 1998). In comparison to period I, the higher P removal efficiency during period II can be attributed to the greater anoxic period applied (30 minutes versus 20 minutes), since a longer anoxic phase can lead to the proliferation of DPAOs that can be enriched under extended anoxic periods (Peng *et al.* 2006; Wang *et al.* 2009) as also verified by the greater P release and uptake rates during period II (Table 3).

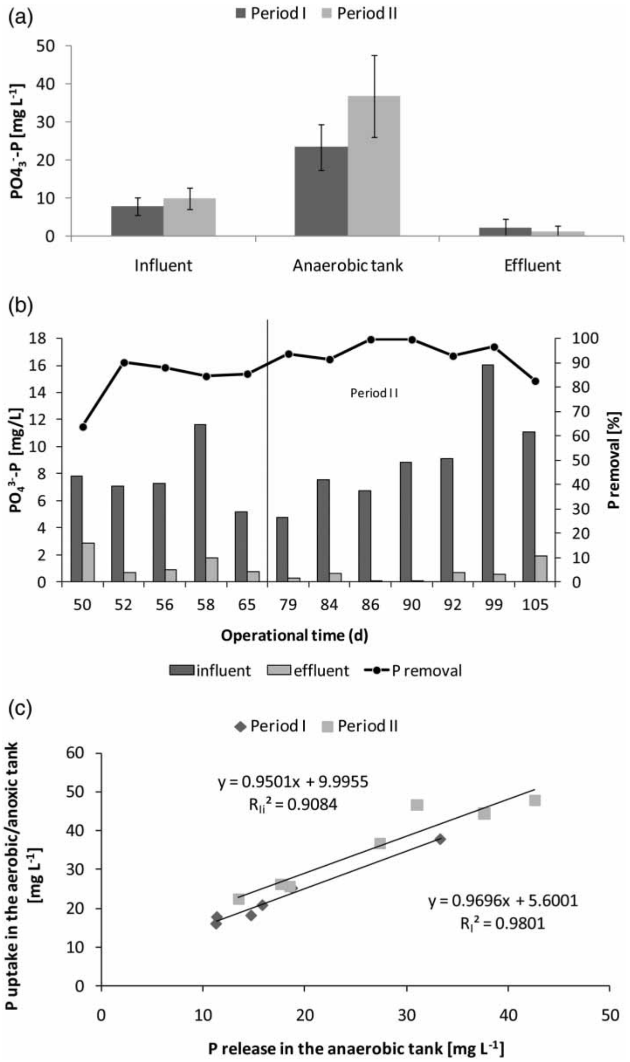

Figure 4 | (a) PO_4^{3-}-P concentration profile at the various stages of the intermittently aerated and fed plant, (b) influent and effluent PO_4^{3-}-P concentration profiles and P removal efficiencies throughout the whole experimental period and (c) relationship between P release in the anaerobic tank and P uptake in the intermittently aerated and fed bioreactor throughout the experimental period.

Table 3 | Specific P release and uptake rates and COD/TP and COD/TKN ratios in both operational periods

Parameter	**Period I**	**Period II**
COD/TP	44.8 ± 19.0	67.0 ± 36.9
COD/TKN	6.0 ± 0.9	7.9 ± 1.9
Specific anaerobic phosphate release rate ($HRT_{AN}^{a} = 2.5$ h) (mg P g^{-1} VSS h^{-1})	2.79 ± 0.55	4.02 ± 1.03
Specific phosphate uptake rate ($HRT_{IF}^{b} = 22.5$ h) (mg P g^{-1} VSS h^{-1})	0.42 ± 0.06	0.55 ± 0.19

[a] Hydraulic retention time in the anaerobic tank.
[b] Hydraulic retention time in the intermittent feeding tank.

The specific anaerobic P release rates in the intermittently aerated and fed EBPR system studied were 2.79 (±0.55) and 4.02 (±1.03) mg P g^{-1} VSS h^{-1} during phases I and II, while the respective specific anoxic/aerobic P uptake rates were 0.42 ± 0.06 and 0.55 (±0.19) mg P g^{-1} VSS h^{-1}. Wang *et al.* (2009) identified P release and uptake rates of the same order by using a step-fed anaerobic/anoxic/oxic (A^2/O) system.

Apart from the use of sequencing batch reactors (SBRs) in EBPR (Li *et al.* 2008; Podedworna & Zubrowska-Sudoł 2012), the adoption of other intermittent feeding and alternate aeration systems is limited. Zheng & Long (2008) developed a combined fixed-film EBPR system treating municipal wastewater, which consisted of an anaerobic biofilter, two intermittent aerated biofilters and an aerobic biofilter. Moreover, an anaerobic-intermittent aerobic bioreactor was adopted by Lee *et al.* (2007) to perform EBPR. An intermittently aerated cylindrical oxidation ditch system was used by Lim *et al.* (2006) for enhanced biological phosphorus removal. Asadi *et al.* (2012) also investigated simultaneous C, N and P removal from an industrial estate's wastewater using an upflow aerobic/anoxic sludge bed reactor. However, this is the first report on an EBPR system where the pulse feeding strategy was used, in contrast to the configurations mentioned above, which were operated under continuous flow.

CONCLUSIONS

A pilot-scale, activated sludge plant was designed and operated for combined carbon, nitrogen and phosphorus removal from domestic sewage. The system operated under the intermittent feeding and alternate aeration concept in a continuously stirred bioreactor with the utilization of an anaerobic selector installed in front of the main reactor and receiving the influent. The experimental results proved the system to be highly efficient regarding carbon, nitrogen and phosphorus removal. Accordingly, mean removal percentages of COD, BOD_5 and TSS were determined as high as 87%, 96% and 86% respectively. TKN and ammonia nitrogen removal averaged 85% and 91%. The implementation of an operational pattern of 30 minutes aeration on and 30 minutes aeration off was proved to be adequate for achieving superior nitrification-denitrification, while on-line monitoring of ORP, ammonium and nitrate concentrations was proved to be an efficient asset for process optimization. Two SDNRs, which corresponded to the consumption of the readily and slowly biodegradable COD, were determined. Consumption of the readily biodegradable COD by the denitrifiers led to higher denitrification rates than those determined during the utilization of slowly biodegradable COD. Moreover, the system demonstrated typical EBPR performance, with P removal percentage values up to 93.7%. The specific P release and uptake rates were up to 4.02 and 0.55 mg P g VSS h^{-1}, depending on the relative duration of the anoxic/aerobic phases.

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