RESEARCH REPOSITORY

Wong, P.Y., Ginige, M.P., Kaksonen, A.H., Cord-Ruwisch, R., Sutton, D.C. and Cheng, K.Y. (2015) Simultaneous phosphorus uptake and denitrification by EBPR-r biofilm under aerobic conditions: effect of dissolved oxygen. Water Science & Technology, 72 (7). pp. 1147-1154.

http://researchrepository.murdoch.edu.au/id/eprint/28618/

Simultaneous phosphorus uptake and denitrification by EBPR-r biofilm under aerobic condition: effect of dissolved oxygen

Pan Yu Wong, Maneesha P. Ginige, Anna H. Kaksonen, Ralf Cord-Ruwisch, David C. Sutton and Ka Yu Cheng

ABSTRACT

A biofilm process, termed enhanced biological phosphorus removal and recovery (EBPR-r), was recently developed as a post-denitrification approach to facilitate phosphorus (P) recovery from wastewater. Although simultaneous P uptake and denitrification was achieved despite substantial intrusion of dissolved oxygen (DO > 6 mg/L), to what extent DO affects the process was unclear. Hence, in this study a series of batch experiments was conducted to assess the activity of the biofilm under various DO concentrations. The biofilm was first allowed to store acetate (as internal storage) under anaerobic condition, and then was subjected to various conditions for P uptake (DO: $0 - 8$ mg/L; nitrate: 10 mg-N/L; phosphate: 8 mg-P/L). The results suggest that even at a saturating DO concentration (8 mg/L), the biofilm could up take P and denitrify efficiently (0.70 mmol e$^-$/gTS*h). However, such aerobic denitrification activity was reduced when the biofilm structure was physically disturbed, suggesting that this phenomenon was a consequence of the presence of oxygen gradient across the biofilm. We conclude that when a biofilm system is used, EBPR-r can be effectively operated as a post-denitrification process, even when oxygen intrusion occurs.

Key words | electron acceptors, polyphosphate accumulating organism, post-denitrification

Pan Yu Wong
Maneesha P. Ginige
Anna H. Kaksonen
Ka Yu Cheng (corresponding author)
CSIRO, Land and Water Flagship,
Floreat, Western Australia 6014,
Australia
E-mail: kayu.cheng@csiro.au

Pan Yu Wong
Anna H. Kaksonen
David C. Sutton
School of Pathology and Laboratory Medicine,
University of Western Australia,
Nedlands, Western Australia 6009,
Australia

Ralf Cord-Ruwisch
Ka Yu Cheng
School of Engineering and Information Technology,
Murdoch University,
Perth, Western Australia 6150,
Australia

INTRODUCTION

Low effluent concentrations for total phosphorus (TP) and total nitrogen (TN) are increasingly being imposed on wastewater treatment plants worldwide. Europe and North America in particular have enforced discharge limits of 0.1 mg/L for TP and 3 mg/L for TN (Boltz *et al.* 2012). While the strict TP limits are largely achieved through chemical precipitation, biological post-denitrification is applied to meet the TN discharge limit. With much of the readily biodegradable organic carbon in the influent being oxidised during upstream aerobic/anoxic oxidative processes, post-denitrification is heavily reliant on the addition of carbon sources (e.g. methanol) (Wei *et al.* 2014). The cost of adding external carbon is a significant burden to the wastewater industry, and one way to offset this cost is through resources recovery from wastewater.

Among many resources in wastewater, phosphorus (P) is of interest because it is a non-renewable resource, and its scarcity for agricultural purposes could potentially threaten global food security (Cordell *et al.* 2011). Wong *et al.* (2013)

proposed and validated a post-denitrification process, termed enhanced biological phosphorus removal and recovery (EBPR-r), that facilitates nitrogen (N) removal but also enables P recovery from wastewater. The EBPR-r process involves two steps, in which a biofilm consisted of phosphate accumulating organisms (PAOs) is alternately exposed to a wastewater stream and a separate recovery stream. As the PAOs can use nitrate (NO$_3^-$) as a final electron acceptor for P uptake, the first step of the process facilitates both denitrification and P removal from the wastewater. In the absence of soluble carbon in wastewater, the internal carbon storage polymers (i.e. such as poly-β-hydroxy-alkanoates; PHA) act as electron donors and an energy source to facilitate this step. The subsequent anaerobic step, the biologically captured P is released into a smaller recovery stream. External carbon (i.e. acetate) is added to facilitate P release, and the biofilm simultaneously replenishes its carbon reserves by intracellular storage of carbon supplied to the recovery

Uncorrected Proof

2   P. Y. Wong et al. | Phosphorus uptake and denitrification under aerobic condition   Water Science & Technology | in press | 2015

stream. The volume difference maintained between the wastewater and the recovery stream (e.g. ratio of 4:1) enables recovery of P as a concentrated solution.

It has been suggested that dissolved oxygen (DO) concentrations >0.2 mg/L inhibit denitrification (Gerardi 2003). In conventional post-denitrification processes, anoxic conditions prevail largely because of the rapid consumption of oxygen (O_2) by heterotrophic bacteria during carbon oxidation. Facilitating an anoxic environment in a similar manner is not feasible in the EBPR-r process, as external carbon is supplied only to the recovery stream. Except for carbon stored intracellularly, no soluble carbon is available in the wastewater stream. Under these conditions, Wong et al. (2013) observed elevated DO concentrations (DO > 6 mg/L) during N and P removal, but surprisingly the high DO concentrations did not appear to inhibit denitrification and P removal.

Storage-driven denitrification is commonly observed in the simultaneous nitrification, denitrification and phosphorus removal processes (SNDPR) (Zeng et al. 2003; Lemaire et al. 2008). In which, nitrifiers make use of the O_2 to facilitate partial nitrification under low DO concentration (<1 mg/L), and hence are largely responsible for creating the anoxic conditions for denitrification. Findings based on the SNDPR process are not directly useful for understanding denitrification in the EBPR-r process, which has been observed to take place at much higher levels of DO (>6 mg/L) with little contribution of nitrifiers (Wong et al. 2013). If large-scale EBPR-r is to be implemented, a clear understanding of the impact of DO on post-denitrification and P removal is critical.

Hence, the aim of this study was to explore the impact of DO on simultaneous storage-driven denitrification and P removal by an EBPR-r biofilm. The specific objectives included assessment of the importance of the biofilm structure, and the levels of DO that can be tolerated by the bacteria without impeding denitrification. First, batch experiments were conducted to quantify P uptake, NO_3^- removal and O_2 consumption kinetics by an intact biofilm exposed to various concentrations of DO (0–8 mg/L) and NO_3^- (0–50 mg-N/L). Secondly, the EBPR-r biofilm was physically disturbed to investigate the effect of biofilm structure on the P and N removal efficiencies.

MATERIALS AND METHODS

Reactor configuration and synthetic wastewater

A laboratory scale sequencing batch biofilm reactor (master reactor) was operated continuously in EPBR-r configuration

for a 2-year period, as described previously (Wong et al. 2013). A schematic diagram of the reactor process is shown in Figure S1 in the supporting document. In brief, 1,000 biofilm carriers (Kaldnes® K1 polyethene) were equally distributed among eight adjoining stainless steel mesh compartments. Over a 6-h cycle the biofilm carriers were alternately exposed for 4 h to a wastewater stream (7.2 L) for P uptake, and for 2 h to a separate recovery stream (1.8 L, 25% of the volume of the wastewater stream) for anaerobic P release.

Both the wastewater and recovery streams contained a standard growth medium consisting of (per L of DI water): 39 mg $MgSO_4$, 20 mg $CaCl_2 \cdot 2H_2O$, 11 mg NH_4Cl (3 mg/L NH_4^+-N), 200 mg $NaHCO_3$ and 3 mL of a trace element solution (Wong et al. 2013). The wastewater stream also contained 8 mg-P/L phosphate (supplemented as 1 M phosphate buffer), 10 mg-N/L nitrate (as sodium nitrate) and 11.6 mg/L N-Allylthiourea, the latter added to prevent nitrification during the P-uptake phase (Ginestet et al. 1998). To restore intracellular PHA reserves during the anaerobic P release, 520 mg/L sodium acetate was added to the recovery stream, which corresponded to 400 mg/L chemical oxygen demand. Concentrated stock solutions (15×) of the media comprising each of the streams were prepared, and the pH was adjusted to 7.0 ± 0.2 using 2 M HCl. Defined volumes of the stock solution and deionised water were simultaneously pumped into the reactor at the beginning of each phase to achieve the desired concentrations.

Kinetic experiments using intact biofilm

To elucidate the use of O_2 and NO_3^- by the EBPR-r biofilm when both electron acceptors were present, two sets of experiments were performed in duplication (Figure 1). (1) The activity of the biofilm was investigated using an initial
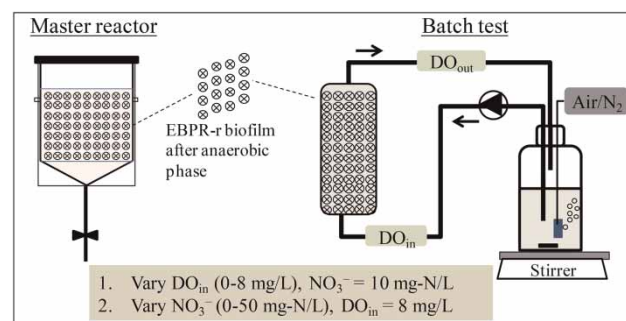

Figure 1 | A schematic diagram of the batch experiment setup designed to assess the ability of the enriched biofilm to denitrify, and to remove P from wastewater using stored PHA.

Uncorrected Proof

3   P. Y. Wong *et al.* | Phosphorus uptake and denitrification under aerobic condition   **Water Science & Technology** | in press | 2015

NO_3^- concentration of 10 mg-N/L, but the bulk DO concentration was varied (0, 2, 4, 6 and 8 mg/L). (2) Constant influent bulk DO concentration of 8 mg/L was maintained, but the initial NO_3^- concentration was varied (0, 5, 10, 20, 30 and 50 mg-N/L).

In each experiment, biofilm carriers (~330) were removed from two compartments of the master reactor at the end of the anaerobic P-release phase, at which time the biomass had stored PHAs (Bond *et al.* 1999). The carriers were immediately transferred into a column reactor (440 mL working volume; diameter 45 cm, height 300 cm; Figure 1), where biological P uptake was triggered by recirculating (7.85 L/h; Masterflex®, USA) a P-containing wastewater stream (2.4 L, 8 mg-P/L) for 4 h. Two luminescent DO probes (PDO₂; Barben Analyser Technology, USA) were installed in the recirculation line, one before (DO_{in}) and one after (DO_{out}) the column reactor. The influent DO_{in} was controlled at 0–8 mg/L by sparging air or nitrogen into the aeration vessel (2.0 L), while NO_3^- was added (as 4 M $NaNO_3$) into the wastewater stream to give an initial concentration of 0–50 mg-N/L. The monitoring and control of DO were performed using a programmable logical controller and software (LabVIEW, National Instruments, USA).

Liquid samples were collected from the reactor every 15–45 min and immediately filtered using a 0.22 μm pore size syringe filters (Acrodisc® PF; Pal Corporation, UK). The concentrations of soluble NO_2^--N, NO_3^--N, and PO_4^{3-}-P in the filtrates were determined using ion chromatography (ICS-03000, DIONEX). Changes in the concentrations of PO_4^{3-}-P, NO_x-N (NO_3^--N + NO_2^--N), and NO_2^--N were plotted against time, and the specific P uptake rate (PUR) and the NO_x removal rate (expressed as mg/L*h) were recorded as the slope of the steepest part of the curves. These rates were normalised using the respective total biomass (TS) concentrations, and expressed in mmol/gTS*h. TS was obtained by subtracting the weight of 50 biofilm-free carriers from the dry weight (dried at 60 °C) of 50 EBPR-r carriers supporting biofilm (Wong *et al.* 2013). To compare the reduction (electron-accepting) kinetics of NO_3^- and O_2 using the storage reducing power (i.e. PHAs), both the oxygen uptake rate (OUR) and the NO_x removal rate were transformed into a common unit, termed the electron-accepting rate (mmol e⁻/gTS*h). The percentage of electrons used for O_2 and NO_3^- reduction was calculated by assuming that all the electrons from the storage were captured by either O_2 or NO_3^-. The details of the calculations are given in the supporting information.

Kinetic experiments using dislodged biomass

To confirm if the observed denitrification in the presence of O_2 was due to the presence of an oxygen gradient across the biofilm, kinetic experiments were conducted using biomass dislodged from the carriers. To obtain the biomass, biofilm carriers (~330) were removed from the master reactor at the end of a P-uptake phase (low in PHA storage) and placed into growth medium in a 500 mL flask. Attached biofilm was physically removed by shaking the carriers in standard medium for 2 min. To break down the size of the flocs, the suspended biofilm was repeatedly drawn up and expelled through a needle (gauge 19–1/2) using a 50 mL syringe. An acetate-containing recovery stream (200 mL) was added to the dislodged biomass for PHA replenishment for 2 h. Thereafter, the biomass was concentrated by centrifugation and washed twice with standard medium (without N, P and C) under anaerobic condition to remove any excess acetate. The PHA-rich biomass was then resuspended in 220 mL standard medium under anaerobic conditions for use in batch experiments.

Four batch reactors (250 mL Schott bottles) were operated in parallel to compare the denitrification ability of the biomass in the presence of 10 mg-N/L NO_3^- for 4 h: (1) oxygenated, with 8 mg-P/L phosphate; (2) oxygenated, without phosphate; (3) anoxic, with 8 mg-P/L phosphate; and (4) anoxic, without phosphate. To initiate the experiment, 50 mL of suspended biomass was added to 200 mL of synthetic wastewater. The oxygenated and anoxic condition was achieved by continuously sparging air and nitrogen into the liquid, respectively. Mixing was achieved using a multi-position magnetic stirrer (400 rpm; RT10, IKA). To confirm the observation of denitrification under oxygenated conditions, on a different day the batch tests for the aforementioned conditions (1) and (2) were repeated.

Liquid samples were withdrawn and filtered for analysis of soluble NO_2^--N, NO_3^--N, and PO_4^{3-}-P. The mixed liquor suspended solids (MLSS) value for the suspended biomass was measured according to the standard method (American Public Health Association American Water Works Association & Water Environment Federation (U.S.) 1995). The PUR, NO_x removal rate and the NO_2^- accumulation rate were normalised with the solids concentration, and expressed as mmol/gMLSS*h. The size distribution of the suspended biomass flocs, measured using a laser particle sizer (Malvern Master Sizer), was determined by an external laboratory (CSIRO, Division of Mineral Particle Analysis Service, Waterford, Australia).

Uncorrected Proof

4   P. Y. Wong *et al.* | Phosphorus uptake and denitrification under aerobic condition   Water Science & Technology | in press | 2015

RESULTS AND DISCUSSION

Storage-driven denitrification and P uptake at very high DO concentrations

In a previous study we observed that an EBPR-r biofilm could up take phosphate and remove NO_3^- in the presence of saturating DO (Wong *et al.* 2013). However, as both NO_3^- and O_2 were provided to the biofilm, we were unable to distinguish the independent effects of these electron acceptors. Hence, in the present study batch experiments were conducted to assess the influence of each of O_2 and NO_3^- as sole electron acceptors on P removal. The EBPR-r biofilm could readily use either O_2 or NO_3^- for P uptake, or both (Figure S2 of supporting information). The highest PUR was observed when O_2 was provided, either alone (0.038 mmol-P/gTS*h) or in combination with NO_3^- (0.043 mmol-P/gTS*h). When NO_3^- was used as the sole electron acceptor, the PUR was markedly reduced by 30%, from 0.043 to 0.030 mmol-P/gTS*h (Table 1). According to Kuba *et al.* (1996), the energy (adenosine triphosphate) production during oxidative phosphorylation with NO_3^- as the electron acceptor is approximately 40% less than occurs with O_2 as the acceptor. While the reduced energy from oxidative phosphorylation based on NO_3^- could have contributed to the 30% reduction in PUR, the possible role of a low-abundance denitrifying PAO population in the biofilm should not be overlooked.

As expected, the highest NO_x removal rate (0.076 mmol-N/gTS*h) was observed when NO_3^- was supplied as sole electron acceptor. When supplemented with DO (8 mg/L), 70% of the denitrification efficiency of the biofilm was retained (Table 1), indicating that the enriched EBPR-r biofilm could denitrify under a very high DO concentrations.

The oxygen gradient across the biofilm enabled denitrification in the presence of DO

To elucidate the effect of DO and NO_3^- loading on the P uptake and denitrification behaviour of the EBPR-r biofilm, two sets of experiments were conducted in which the concentration of one electron acceptor was maintained constant while the concentration of the other was varied. The concentration profiles for each experiment set are illustrated in the supplementary data Figure S3.

At a NO_3^- concentration of 10 mg-N/L, increasing the bulk DO concentration from 0 to 8 mg/L increased the PUR by 43% (from 0.030 to 0.043 mmol/gTS*h) (Figure 2(a)). A linear relationship ($R^2 = 0.999$) was obtained between the OUR and the applied bulk DO concentration (Figure 2(b)).
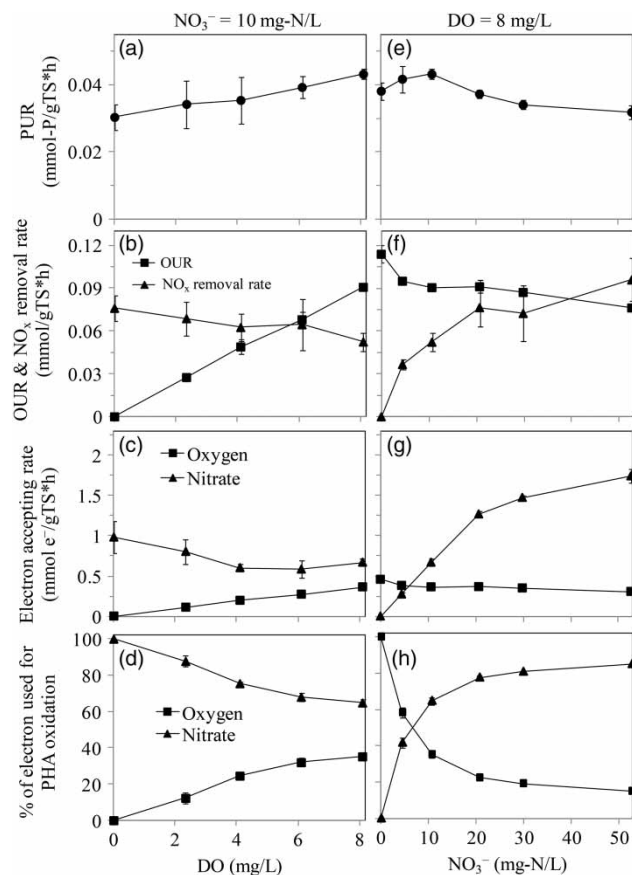
Table 1 | The result for the intact EBPR-r biofilm under three electron acceptor scenarios

	Electron acceptors		
Rates	O_2	$O_2 + NO_3^-$	NO_3^-
Phosphate uptake rate (mmol-P/gTS*h)	0.038 ± 0.002	0.043 ± 0.002	0.030 ± 0.004
NO_x removal rate (mmol-N/gTS*h)	n.a.	0.052 ± 0.007	0.076 ± 0.009
P removal efficiency (%)	72 ± 6	77 ± 1	53 ± 1
N removal efficiency (%)	n.a.	38 ± 5	54 ± 1
P/N ratio (g-P/g-N as NO_3^-)	n.a.	1.36 ± 0.05	0.54 ± 0.03

Figure 2 | Effect of bulk DO (0–8 mg/L) and initial NO_3^- (0–50 mg-N/L) concentration on: (a) and (e) phosphate uptake rate; (b) and (f) OUR and NO_x-N removal rate; (c) and (g) electron accepting rate for O_2 and NO_3^-; and (d) and (h) the percentage of electrons used for PHA oxidation using electrons generated by O_2 and NO_3^- reduction.

Uncorrected Proof

5   P. Y. Wong *et al.* | Phosphorus uptake and denitrification under aerobic condition   **Water Science & Technology** | in press | 2015

Such a first order kinetic behavior suggested that the biofilm was limited by O_2 (Meyer *et al.* 2005). Increasing the bulk water DO concentration from 0 to 8 mg/L could result in deeper penetration of O_2 into the biofilm, triggering the higher OURs (from 0 to 0.091 mmol/gTS*h) and the lower denitrification rates (1.5-fold reduction from 0.076 to 0.052 mmol-N/gTS*h) (Figure 2(b)). This result confirms the presence of an oxygen gradient in the biofilm, with the inner anoxic environment facilitating the observed denitrification despite the bulk water being saturated with oxygen.

In contrast, when the bulk DO concentration was fixed at 8 mg/L, increasing the NO_3^- concentration from 0 to 50 mg-N/L resulted in an increase in the denitrification rate (from 0 to 0.096 mmol-N/gTS*h) (Figure 2(f)). This result was expected because increased NO_3^- availability in the bulk water could facilitate the penetration of NO_3^- into the deeper anoxic layers of the biofilm, as observed in conventional EBPR process under anoxic conditions (Ahn *et al.* 2001; Yuan & Oleszkiewicz 2010; Zhou *et al.* 2010). In terms of P uptake, only a slight decrease in the PUR was observed with increasing NO_3^- concentration (16% of overall inhibition, from 0.038 to 0.032 mmol/gTS*h) (Figure 2(e)). No increase in PUR and a continuous increase in denitrification (evident in Figure 2(f)) could be a result of some NO_3^- being utilised by denitrifying glycogen accumulating organisms (denitrifying GAOs) in the biofilm. The slight decrease in PUR is consistent with the findings of Yuan & Oleszkiewicz (2010), who observed an increased anoxic PUR and a decreased aerobic PUR with increasing NO_3^- concentrations in the bulk water. It is also possible that when the NO_3^- concentration increased in the bulk water, some PAOs were able to switch from using O_2 as electron acceptor to use of NO_3^-. With more PAOs using NO_3^- as electron acceptor, the OUR and PUR may have decreased. Whether the elevated level of O_2 inhibited the activity of denitrifying PAOs remains unclear, and should be the subject of further research.

More than half of the stored reducing power was used for denitrification at 8 mg/L of DO

In the absence of a soluble carbon, the observed P uptake and denitrification activities were driven by internal carbon storage (e.g. PHAs) in the EBPR-r biofilm. To compare the reduction kinetics of NO_3^- and O_2 for the biofilm, the OUR and NO_x removal rates (Figure 2(b) and 2(f)) were transformed into a common unit (electron accepting rate) (Figure 2(c) and 2(g)), and were also expressed as a percentage of the electrons used for internal carbon oxidation (Figure 2(d) and 2(h)).

At a bulk DO concentration of 8 mg/L and a NO_3^- concentration of 0 mg-N/L, the electrons stored in the biofilm were predominantly used to reduce O_2 at a maximum electron accepting rate of 0.46 mmol e$^-$/gTS*h (Figure 2(g)). With increasing bulk water NO_3^- concentration the electron reduction rate for O_2 decreased only slightly, whereas the electron reduction rate for NO_3^- increased dramatically. At NO_3^- concentration of approximately 8 mg-N/L, the biofilm appeared to be transferring electrons at a similar rate to both O_2 and NO_3^-. At a bulk water NO_3^- concentration exceeding 8 mg-N/L, NO_3^- became the dominant electron acceptor (>50%) (Figure 2(h)). Thus, a NO_3^- concentration of >8 mg-N/L in the wastewater is favourable for efficient NO_3^- reduction.

At a NO_3^- concentration of 10 mg-N/L and a DO concentration of 0 mg/L, the electrons stored in the biofilm were solely used to reduce NO_3^- at a maximum electron accepting rate of 0.98 mmol e$^-$/gTS*h (Figure 2(c)). When the DO concentration increased from 0 to 8 mg/L, the proportion of electrons accepted by O_2 gradually increased from 0 to 35% (Figure 2(d)). It is noteworthy that even at such a high bulk water DO concentration the biofilm was able to channel approximately 65% of the electrons to NO_3^- reduction. This unique ability of the biofilm to reduce NO_3^- in the presence of DO is critical for the EBPR-r process, and is probably a consequence of the presence of an oxygen gradient across the biofilm, as discussed above.

The biofilm structure is essential for denitrification in the presence of O_2

To determine whether the cells in the biofilm could continue to denitrify when the DO gradient was disrupted, the biofilm was removed from the carriers and physically disturbed to form suspended aggregates (mean size 185 ± 11 μm). The aerobic and anoxic ratio difference of P uptake (PUR_{aer}/ PUR_{anx}) and denitrification activities of the suspended biomass (MLSS of 1.03 ± 0.05 g/L) were compared with that of the intact biofilm.

Similar to the intact biofilm, the suspended biomass showed the highest PUR (0.53 mmol-P/gMLSS*h) when both O_2 and NO_3^- were provided as electron acceptors (Table 2). When NO_3^- was the sole electron acceptor the PUR decreased by 38%, which is consistent with the observed decline in the previous experiments with the intact biofilm (~30% in Table 1). Thus, the decreased PUR observed with NO_3^- was not caused by the biofilm structure, but rather appeared to be influenced by the type of electron acceptor.

Uncorrected Proof

6   P. Y. Wong et al. | Phosphorus uptake and denitrification under aerobic condition   Water Science & Technology | in press | 2015

Table 2 | The result for the dispersed biofilm under four conditions tested

	Rates	Aerobic	Anoxic
With P	PUR (mmol-P/gMLSS*h):	0.53	0.33
	NO_x removal rate (mmol-N/gMLSS*h):	0.16	1.00
	NO_3^- removal rate (mmol-N/gMLSS*h)	n.a.	2.23
	NO_2^- formation rate (mmol-N/ gMLSS*h)	n.a.	2.39
No P	PUR (mmol-P/gMLSS*h):	n.a.	n.a.
	NO_x removal rate (mmol-N/gMLSS*h):	0.00	0.78
	NO_3^- removal rate (mmol-N/gMLSS*h)	n.a.	1.58
	NO_2^- formation rate (mmol-N/ gMLSS*h)	n.a.	1.89

As with the intact biofilm, the highest level of denitrification by the suspended culture was observed in the absence of DO (Table 2). However, when O_2 (DO > 6 mg/L) was introduced into the bulk water a 6-fold decrease in the denitrification rate (from 1.00 to 0.16 mmol-N /gMLSS*h) was observed. This was remarkably different from the intact biofilm, for which the respective decline in the denitrification rate was only 1.5-fold (Table 1). One plausible explanation for the decrease in denitrification activity after the disruption of oxygen gradient is the inhibition of nitrate reductase, which is a membrane-bound enzyme that catalyses the reduction of NO_3^- to NO_2^-, and is sensitive to O_2 (Ogunseitan 2005). Under anoxic conditions, the rate of reduction of NO_3^- (2.23 mmol-N/gMLSS*h) far exceeded that of NO_2^-, resulting in the accumulation of the NO_2^-

observed in this study (an accumulation rate of 2.39 mmol-N/gMLSS*h, Figure 3(c)). Only when NO_3^- became limiting was an overall reduction in NO_x observed. Accumulation of NO_2^- is a common observation during denitrification and has been extensively discussed in the literature (Ahn et al. 2001; Zhou et al. 2010). Under aerobic conditions the nitrate reductase enzyme was exposed to O_2, resulting in inhibition of the enzyme and a significant decrease in the NO_3^- reduction rate (Figure 3(a)). These results demonstrate that maintenance of the biofilm structure for bacterial growth is critical for the EBPR-r process to achieve satisfactory rates of denitrification, particularly when strict anoxic conditions cannot be maintained.

The dependency of denitrification on P

The observed denitrification in suspended biomass could be performed by either PAOs or other non-PAO bacteria, including GAO. By definition, GAOs do not require storage of P under either aerobic or anoxic conditions (Oehmen et al. 2006). To investigate the denitrification activities of non-PAO organisms, the suspended biomass experiment was conducted aerobically and anoxically with no P in the bulk water.

Under anoxic conditions the absence of phosphate decreased the NO_x removal rate only by 22%, from 1.00 mmol-N/gMLSS*h in the presence of P to 0.78 mmol-N/gMLSS*h in the absence of P (Table 2). This confirmed
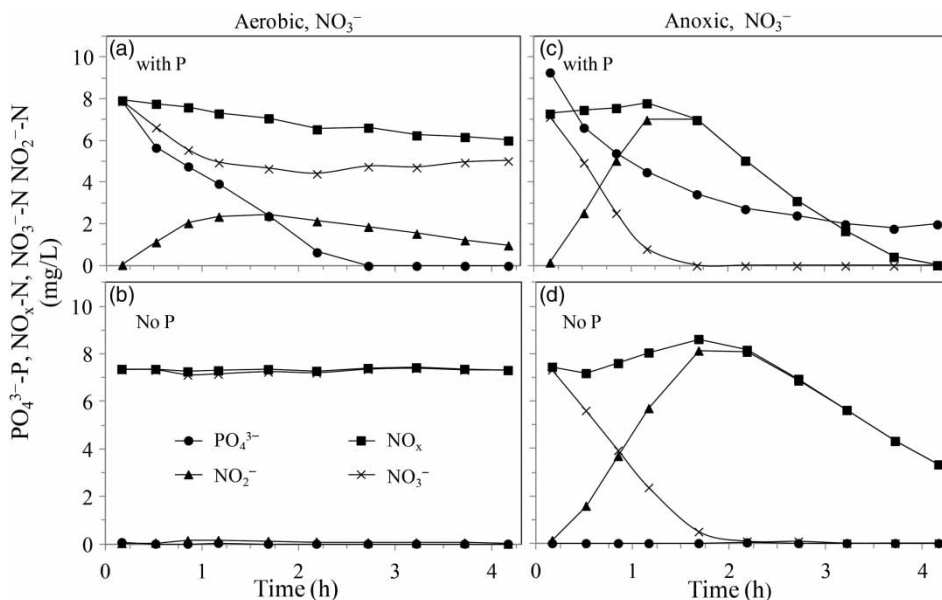

Figure 3 | Concentrations of soluble PO_4^{3-}-P, NO_x-N (NO_3^--N + NO_2^--N), NO_3^--N and NO_2^--N over time associated with suspended biomass incubated with 10 mg-N/L of NO_3^- under four conditions: (a) oxygenated, with phosphate; (b) oxygenated, without phosphate; (c) anoxic, with phosphate; and (d) anoxic, without phosphate.

Uncorrected Proof

7   P. Y. Wong *et al.* | Phosphorus uptake and denitrification under aerobic condition   **Water Science & Technology** | in press | 2015

the role of non-PAO organisms (e.g. denitrifying GAOs) in biofilms carrying out denitrification. However, exposing the suspended biomass to aerobic conditions in the absence of phosphate resulted in a complete inhibition of denitrification (Figure 3(b)), indicating that the non-PAO denitrifiers in the EBPR-r culture were unable to denitrify when exposed to O_2. It is plausible that they had reduced affinity for NO_3^- than did the PAOs, or that they were more sensitive to O_2. Alternatively, it is possible that the denitrifying GAOs predominately occupied the inner parts of the biofilm, where the penetration of O_2 was reduced (Lemaire *et al.* 2008), as has been reported for the granules enriched in the SNDRP process.

Implications of the study

EBPR-r is a novel post-denitrification process that enables P recovery. The success of this strategy depends on whether denitrification can be efficiently driven by the reducing power stored in the biofilm. The lack of soluble carbon and ammonia in the influent of this process could result in an elevated bulk water DO, which might affect the denitrification process. Our previous study suggested that the EBPR-r process can facilitate denitrification without the need to maintain a strictly anoxic environment in the bulk water (Wong *et al.* 2013). This was confirmed in the current study, where 60% of the reducing power stored in the biofilm was found to be expended on denitrification, even when the bulk water DO concentration was near saturation (8 mg/L). However, the denitrifying ability of the EBPR-r process was remarkably compromised when the biofilm structure was physically disturbed, implying that maintenance of the biofilm structure is critical for the success of EBPR-r as a post-denitrification strategy when oxygen intrusion occurs.

CONCLUSION

The results of this study suggest that:

- the EPBR-r biofilm facilitated P and N removal in a process that was not sensitive to oxygen intrusion;
- at a NO_3^- concentration of 10 mg-N/L, increasing the DO concentration (from 0 to 8 mg/L) increased the PUR by 43% and decreased the denitrification rate by 31%;
- at a DO concentration of 8 mg/L, increasing the NO_3^- concentration (from 0 to 50 mg-N/L) increased the denitrification rate (from 0 to 0.096 mmol-N/gTS*h).

In summary, this study highlights the importance of the EBPR-r biofilm structure in enabling denitrification to take place at the same time as P removal for recovery. The data also suggest some operational boundaries (e.g. specific DO and NO_3^- concentrations in the influent) necessary for the EBPR-r biofilm to reduce P and N to acceptable levels in the effluent.

REFERENCES

Ahn, J., Daidou, T., Tsuneda, S. & Hirata, A. 2001 Metabolic behavior of denitrifying phosphate-accumulating organisms under nitrate and nitrite electron acceptor conditions. *Journal of Bioscience and Bioengineering* **92** (5), 442–446.

American Public Health Association, American Water Works Association, Water Environment Federation (U.S.) 1995 *Standard methods for the examination of water and wastewater*. APHA-AWWA-WEF, Washington, DC.

Boltz, J. P., Morgenroth, E., Daigger, G. T., DeBarbadillo, C., Murthy, S., Sorensen, K. H. & Stinson, B. 2012 Method to identify potential phosphorus rate-limiting conditions in post-denitrification biofilm reactors within systems designed for simultaneous low-level effluent nitrogen and phosphorus concentrations. *Water Research* **46** (19), 6228–6238.

Bond, P. L., Erhart, R., Wagner, M., Keller, J. & Blackall, L. L. 1999 Identification of some of the major groups of bacteria in efficient and noneficient biological phosphorus removal activated sludge systems. *Applied and Environmental Microbiology* **65** (9), 4077–4084.

Cordell, D., Rosemarin, A., Schröder, J. J. & Smit, A. L. 2011 Towards global phosphorus security: a systems framework for phosphorus recovery and reuse options. *Chemosphere* **84** (6), 747–758.

Gerardi, M. H. 2003 *Nitrification and Denitrification in the Activated Sludge Process*. John Wiley & Sons, Inc., Hoboken, p. 1 online resource (207 p.).

Ginestet, P., Audic, J. M., Urbain, V. & Block, J. C. 1998 Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. *Applied and Environmental Microbiology* **64** (6), 2266–2268.

Uncorrected Proof

8   P. Y. Wong *et al.* | Phosphorus uptake and denitrification under aerobic condition   **Water Science & Technology** | in press | 2015

Kuba, T., Murnleitner, E., van Loosdrecht, M. C. M. & Heijnen, J. J. 1996 A metabolic model for biological phosphorus removal by denitrifying organisms. *Biotechnology and Bioengineering* **52** (6), 685–695.

Lemaire, R., Yuan, Z., Blackall, L. L. & Crocetti, G. R. 2008 Microbial distribution of Accumulibacter spp. and Competibacter spp. in aerobic granules from a lab-scale biological nutrient removal system. *Environmental Microbiology* **10** (2), 354–363.

Meyer, R. L., Zeng, R. J., Giugliano, V. & Blackall, L. L. 2005 Challenges for simultaneous nitrification, denitrification, and phosphorus removal in microbial aggregates: mass transfer limitation and nitrous oxide production. *FEMS Microbiology Ecology* **52** (3), 329–338.

Oehmen, A., Saunders, A. M., Vives, M. T., Yuan, Z. & Keller, J. 2006 Competition between polyphosphate and glycogen accumulating organisms in enhanced biological phosphorus removal systems with acetate and propionate as carbon sources. *Journal of Biotechnology* **123** (1), 22–32.

Ogunseitan, O. 2005 *Microbial Diversity: Form and Function in Prokaryotes*. Blackwell Pub., Malden, MA.

Wei, N., Shi, Y. H., Wu, G. X., Hu, H. Y., Wu, Y. H. & Wen, H. 2014 Tertiary denitrification of the secondary effluent by denitrifying biofilters packed with different sizes of quartz sand. *Water* **6** (5), 1300–1311.

Wong, P. Y., Cheng, K. Y., Kaksonen, A. H., Sutton, D. C. & Ginige, M. P. 2013 A novel post denitrification configuration for phosphorus recovery using polyphosphate accumulating organisms. *Water Research* **47** (17), 6488–6495.

Yuan, Q. Y. & Oleszkiewicz, J. 2010 Interaction between denitrification and phosphorus removal in a sequencing batch reactor phosphorus removal system. *Water Environment Research* **82** (6), 536–540.

Zeng, R. J., Lemaire, R., Yuan, Z. & Keller, J. 2003 Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. *Biotechnology and Bioengineering* **84** (2), 170–178.

Zhou, S., Zhang, X. & Feng, L. 2010 Effect of different types of electron acceptors on the anoxic phosphorus uptake activity of denitrifying phosphorus removing bacteria. *Bioresource Technology* **101** (6), 1603–1610.