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Research article

# Improvement of carbon usage for phosphorus recovery in EBPR-r and the shift in microbial community

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ABSTRACT

Enhanced biological phosphorus removal and recovery (EBPR-r) is a biofilm process that makes use of polyphosphate accumulating organisms (PAOs) to remove and recover phosphorus (P) from wastewater. The original process was inefficient, as indicated by the low P-release to carbon (C)-uptake ($P_{rel}/C_{upt}$) molar ratio of the biofilm. This study successfully validated a strategy to improve the $P_{rel}/C_{upt}$ ratio by at least 3-fold. With an unchanged supply of carbon in the recovery stream, an increase in the hydraulic loading in stages I, II and III (7.2, 14.4 and 21.6 L, respectively) resulted in a 43% increase in the $P_{rel}/C_{upt}$ ratio (0.069, 0.076 and 0.103, respectively). The ratio further increased by 150% (from 0.103 to 0.255) when the duration of the P uptake period was increased from 4 h (stage III) to 10 h (stage IV). Canonical correspondence analysis showed that, correlated to the 3-fold increase in the $P_{rel}/C_{upt}$ ratio, there was an increase in the abundance of PAOs ("*Candidatus Accumulibacter*" Clade IIA) and a decrease in the occurrence of glycogen accumulating organisms (GAOs) (family *Sinobacteraceae*). However, the four stage operation impaired denitrification, resulting in a 5-fold reduction in the $N_{den}/P_{upt}$ ratio. The decline in denitrification was consistent with a decrease in the abundance of denitrifiers including denitrifying PAOs (family *Comamonadaceae* and "*Candidatus Accumulibacter*" Clade IA). Overall, a strategy to facilitate more efficient use of carbon was validated, enabling a 3-fold carbon saving for P recovery. The new process enabled up to 80% of the wastewater P to be captured in a P-enriched stream (>90 mg/L) with a single uptake/release cycle of recovery.



## 1. Introduction

Recycling of phosphorus (P) is essential because P is a non-renewable resource (Rittmann et al., 2011). One potential source of P for recovery is municipal wastewater. However, municipal wastewater typically contains only 7–10 mg-P/L, making P recovery from this source challenging (Parsons and Smith, 2008). For P recovery to be chemically and economically viable, a wastewater stream having a P concentration of >50 mg-P/L is generally required (Cornel and Schaum, 2009).

To address this challenge, several approaches based on enhanced biological phosphorus removal (EBPR) have recently been developed (Acevedo et al., 2015; Kodera et al., 2013; Valverde-Pérez et al., 2015; Wong et al., 2013; Xia et al., 2014). Among these, a post-denitrification process termed enhanced biological phosphorus removal and recovery (EBPR-r) was proposed by Wong et al. (2013). The EBPR-r process is designed to remove nitrogen (N) and recover P from secondary effluent that has low COD/N and COD/P ratios. The EBPR-r is a two-step process involving the use of a biofilm containing polyphosphate accumulating organisms (PAOs) to facilitate N removal and P recovery from wastewater. The first step facilitates storage-driven denitrification and P uptake by PAOs from wastewater. The second step involves exposure of the P-enriched PAOs biofilm to an anaerobic environment to facilitate replenishment of carbon reserves (via acetate uptake) and a release of the stored P into a separate recovery stream. As the volume of the recovery stream is only a small fraction of the volume of the wastewater stream, P is both

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recovered and concentrated in this separate stream.

Wong et al. (2013) reported that an EBPR-r reactor having a wastewater: recovery stream volumetric ratio of 4:1 was able to achieve a 4-fold concentration of P, from 8 mg-P/L in the wastewater stream (7.2 L) to 28 mg-P/L in the recovery stream (1.8 L). Moreover, by repeated release of P into the same recovery stream, a final P concentration of 100 mg-P/L was achieved in the recovery stream (Wong et al., 2013). However, this mode of operation resulted in a $P_{rel}/C_{upt}$ ratio (the amount of P released per carbon substrate taken up by PAOs under anaerobic conditions) of only 0.08 mol-P/mol-C, which was substantially lower than the 0.50–0.75 mol-P/mol-C typically reported for PAO biomass in conventional EBPR reactors (Filipe et al., 2001; Lopez-Vazquez et al., 2007). This low ratio implies that a large portion of the consumed carbon is used for processes not necessarily involving P recovery; for example, uptake by glycogen accumulating organisms (GAOs) (Bond et al., 1995). As carbon addition represents a substantial operational cost, carbon use in processes not involving P recovery should be minimised. Thus, development of a strategy to improve the $P_{rel}/C_{upt}$ ratio of EBPR-r biofilm was warranted.

Optimisation of the $P_{rel}/C_{upt}$ ratio has been extensively studied in conventional EBPR processes. Several factors are known to favour the growth of PAOs over GAOs, contributing to an increase in the $P_{rel}/C_{upt}$ ratio. These include pH (>7.25), temperature (<25 °C), the organic carbon: P ratio in the influent (10–25 mg-COD/mg-P), the type of carbon source (propionate), and the mode of carbon feeding (slow feeding rate) (Oehmen et al., 2007; Tu and Schuler, 2013). For instance, Oehmen et al. (2006) reported a higher $P_{rel}/C_{upt}$ ratio (0.30–0.45) in a propionate-fed EBPR reactor, while in an acetate-fed reactor the $P_{rel}/C_{upt}$ ratio decreased from 0.40 to 0.05 after 120 days of operation. In addition, Tu and Schuler (2013) reported an 11-fold increase in the $P_{rel}/C_{upt}$ ratio (from 0.05 to 0.55) when the carbon feeding rate was decreased from 1200 mg/L.h (over 10 min) to 100 mg/L.h (over 120 min).

Another approach to improve the $P_{rel}/C_{upt}$ ratio is by increasing the P-loading (by increasing the P concentration) (Choi et al., 2011; Converti et al., 1993; Liu et al., 1997; Panswad et al., 2007). Choi et al. (2011) reported an increase in the $P_{rel}/C_{upt}$ ratio from 0.01 to 0.02 with an increase in the P concentration in wastewater from 20 to 80 mg-P/L. Panswad et al. (2007) showed a similar trend of increase in the $P_{rel}/C_{upt}$ ratio (from 0.07 to 0.13) with an increase in the P concentration from 6 to 14.4 mg-P/L. However, increasing the P concentration is impractical in the EBPR-r process because municipal wastewater is used as the process influent. An alternative approach to achieving a higher P-loading is to increase the hydraulic loading. For example, P-loading to the EBPR-r process could be doubled if the volumetric ratio (4:1) of the wastewater : recovery stream could be increased to 8:1. As an increase in the P-loading does not change the P concentration in the wastewater, it is unclear whether increasing the hydraulic loading would enhance the $P_{rel}/C_{upt}$ ratio of the EBPR-r biofilm. To achieve a higher $P_{rel}/C_{upt}$ ratio, an increase in P uptake by the biofilm is required at higher hydraulic loadings. In the event that an increase in P uptake rate is not achievable, the duration of P uptake could be extended to facilitate additional uptake of P (hence achieving a higher $P_{rel}/C_{upt}$ ratio). The impact of an increase of P-loading and the duration of P uptake on the $P_{rel}/C_{upt}$ ratio is yet to be investigated, but is of particular relevance to optimisation of the EBPR-r process. Hence, to optimise the EBPR-r process the aim of this study was to assess the effect of increasing the hydraulic loading and the period of P uptake on the $P_{rel}/C_{upt}$ ratio. We used 454 pyrosequencing of 16S rRNA genes to monitor bacterial community changes caused by changes to the hydraulic loading and uptake period.

## 2. Materials and methods

### 2.1. Wastewater stream and P recovery stream

Both the wastewater and recovery streams contained a standard growth medium consisting of (per L): 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 0.3 mL of a nutrient solution. The nutrient solution contained (per L) 1.5 g $FeCl_3 \cdot 6H_2O$, 0.15 g $H_3BO_3$, 0.03 g $CuSO_4 \cdot 5H_2O$, 0.18 g KI, 0.12 g $MnCl_2 \cdot 4H_2O$, 0.06 g $Na_2MoO_4 \cdot 2H_2O$, 0.12 g $ZnSO_4 \cdot 7H_2O$, 0.15 g $CoCl_2 \cdot 6H_2O$ and 10 g EDTA. The wastewater stream contained 8 mg-P/L of phosphate (supplemented as 1 M phosphate buffer: 46 g $KH_2PO_4$ and 115 g $K_2HPO_4$ per L) and 10 mg-N/L of nitrate (as sodium nitrate). Because EBPR-r is a post-denitrification process, the wastewater stream was formulated to represent secondary effluent, which is limiting in both soluble carbon and ammonium. In contrast, the recovery stream contained 350 mg/L of acetate (as sodium acetate). This carbon supply corresponded to 370 mg/L of chemical oxygen demand (COD). Concentrated stock solutions (15 ×) of these two streams were prepared and the pH was adjusted to 7.0 ± 0.2 using 2 M HCl. Defined volumes of the stock solutions and deionised (DI) water were simultaneously pumped into the reactor at the beginning of each phase to achieve the desired concentrations.

### 2.2. Reactor configuration, automated operation and online monitoring

A laboratory-scale reactor (the master reactor) was operated at 22 ± 2 °C to enrich an EBPR-r biofilm, as described previously (Wong et al., 2013). In brief, 1000 biofilm carriers (Kaldnes® K1 polyethene) were equally distributed in the master reactor (internal diameter of 130 mm) among eight adjoining stainless steel mesh compartments. Over a 6-h cycle the biofilm carriers were alternately exposed to a wastewater stream (7.2 L, containing 8 mg-P/L $PO_4^{3-}$ and 10 mg-N/L $NO_3^-$) for 4 h to enable P uptake, and to a separate recovery stream (1.8 L, containing 350 mg/L acetate) for 2 h to facilitate anaerobic P release. The wastewater stream (which was stored in a separate tank because of its relatively large volume) and the recovery stream were continuously recirculated (337 mL/min) through the master reactor to facilitate P uptake and P release, respectively. Peristaltic pumps (Masterflex®, USA) were used for recirculation and exchange of liquid. The operation of the reactor was automated using control devices and software (LabVIEW), and dissolved oxygen (DO), pH and redox potential (ORP) of the bulk water were recorded online. A schematic diagram of the master reactor configuration is shown in Wong et al. (2013).

The master reactor was seeded using biomass from another laboratory-scale EBPR-r reactor in which stable P recovery had been achieved (Wong et al., 2013). The master reactor was operated in four stages over 450 days (Table 1). The effect of increasing hydraulic loading was examined during the first three stages and the effect of increasing P uptake duration was investigated during the final stage. Different hydraulic loadings were achieved by increasing the volume of the wastewater stream from 7.2 L (stage I) to 14.4 L (stage II) and to 21.6 L (stage III). A 4-h P uptake phase was maintained during the initial three stages. During stage IV the P uptake phase was increased from 4 h to 10 h, while maintaining the same volumetric loading used in stage III. In all stages the amount of carbon supplied to the recovery stream (1.8 L with 350 mg/L acetate) and the duration of P release (2 h) were maintained constant. On achieving a stable operation at each stage, the $P_{rel}/C_{upt}$ ratio and the P and N removal efficiencies of the reactor were determined.