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The ability of PAOs to conserve their storage-driven phosphorus uptake activities during prolonged aerobic starvation conditions

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ABSTRACT

A post-denitrification process, known as enhanced biological phosphorus removal and recovery (EBPR-r), was recently developed to facilitate phosphorus (P) recovery from municipal wastewater. This process utilises a biofilm containing phosphate-accumulating organisms (PAOs) to capture P from wastewater and then release the captured P into a separate smaller stream for recovery. The addition of external carbon in the EBPR-r process is expected to be a main operating cost. Hence, it is important to ensure that the added carbon, which is stored internally as poly-β-hydroxy-alkanoates (PHA) within PAOs, is predominately used for P uptake. This study explored the ability of PAOs to conserve their storage-driven P uptake activities following exposure of the biofilm to oxidising and P-deficient conditions for extended periods (up to 7 days). Even after 2 days of exposure the biofilm retained a similar ability to up take P (1.20 ± 0.09 mg-P/g total solids). Beyond 2 days of exposure, a decline in P uptake activity was noted, with only 15% activity remaining by day 7. This study provides the first evidence of the ability of PAOs to conserve their storage-driven P uptake activities. This unique behaviour of PAOs may enable flexible operational strategies, such as infrequent carbon replenishment, to be implemented (i.e. facilitate multiple P uptake phases before an anaerobic carbon replenishment). Such flexibility may reduce the capital and operational costs of the EBPR-r process, increasing the economic incentive for P recovery from wastewater.

1. Introduction

The recycling of phosphorus (P) from municipal wastewater is an environmentally sustainable initiative because P is a scarce resource [1]. Sewage treatment plants are potential sites for P recovery, but municipal wastewater typically has a low P concentration (< 10 mg-P/L), making P recovery from this source technically and economically challenging [2]. Generally, a concentration > 50 mg-P/L is recommended for P recovery [3,4].

A post-denitrification process, termed as enhanced biological phosphorus removal and recovery (EBPR-r), was recently developed to facilitate P recovery from municipal wastewater [5,6]. Similar to conventional enhanced biological phosphorus removal (EBPR), the EBPR-r process uses phosphorus accumulating organisms (PAOs) to uptake and release P from wastewater. However, unlike conventional EBPR, which utilises suspended cells and a single wastewater stream, EBPR-r makes use of biofilms to facilitate P recovery in a two-step process, and is operated using two streams that are hydraulically separated. In the first step the PAOs uptake phosphate (PO_4^{3-}) from low P-containing wastewater using nitrate (NO_3^-) and/or oxygen (O_2) as final electron acceptors, and P is stored intracellularly as polyphosphate (Poly-P). The internal carbon storage polymers (poly-β-hydroxy-alkanoates; PHAs) of PAOs are used as the energy source for this process. In the second step the P-enriched PAO biofilm is exposed to a recovery stream of smaller volume under anaerobic conditions. An external carbon source (acetate) is introduced to the recovery stream and the carbon reserves (e.g. PHA) of PAOs are replenished via acetate uptake. The energy requirements for this process are fulfilled by the hydrolysis of Poly-P, which in turn releases PO_4^{3-} into the recovery stream. The capture of P from wastewater in a concentrated recovery stream provides an opportunity to recover P as a fertiliser, which could generate a revenue stream for the wastewater industry.

In the EBPR-r process the replenishment of carbon is performed in a separate recovery stream by dosing an external carbon source. As the requirement for carbon incurs a substantial operational cost, it is important to ensure that the added carbon, which is stored intracellularly by PAOs, is predominately used for P uptake. If carbon storage polymers are not conserved (e.g. are depleted during the decanting and

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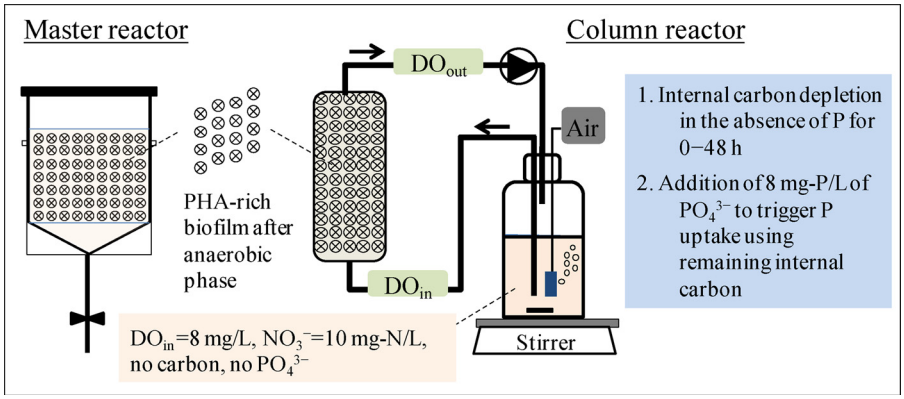

Fig. 1. A schematic diagram of the short-term (0–48 h) P- and C-starvation test. The EBPR-r biofilm fully loaded with internal carbon reserves was removed from the master reactor, immediately transferred to the column reactor, and exposed for various times (0, 3, 12, 24 and 48 h) to oxidising conditions in the absence of soluble PO_4^{3-} and carbon. Thereafter, PO_4^{3-} was added to trigger storage-driven P uptake.

filling of the two streams) and are rapidly oxidised without facilitating the uptake of P, the wastage of carbon can lead to higher operational costs.

Although the ability of PAOs to use their internal carbon storage polymers to facilitate P uptake has been widely demonstrated, no studies have investigated whether storage-driven P uptake activity can be sustained in the event of a prolonged absence of P in the bulk water. Several studies have investigated the effect of electron donor (carbon) starvation on the activities and endogenous processes of PAOs [7–11]. These findings indicate that PAOs exhibit a higher decay rate when exposed to aerobic conditions compared to anaerobic conditions. However, the PAO biomass used in these studies was obtained at the end of a P uptake phase (i.e. at the end of an aerobic or anoxic period), when the internal carbon storage reserves of PAOs are known to be minimum [12]. Hence, whether PAOs can conserve their storage-driven P uptake activity under aerobic and P limiting conditions has remained unknown until the present study.

The aim of this study was to undertake the first assessment of the ability of PAOs to conserve their storage-driven P uptake activity under aerobic and P limiting conditions. This was achieved by first exposing the EBPR-r biofilm to the recovery stream to enable them to replenish their internal carbon reserves (i.e. PHAs) via acetate uptake. Subsequently, the biofilm was exposed to a highly oxidising conditions (a wastewater stream containing > 7 mg/L O_2 and 10 mg-N/L NO_3^-) in the absence of soluble P and C. Subsequently, P was added to the bulk water at various time intervals to investigate the ability of EBPR-r biofilms to carry out storage-driven P uptake. Another aim of the study was to investigate how long PAOs can tolerate such a highly oxidising environment without losing their storage-driven P uptake ability. The findings of this study will be useful for evaluating the economic feasibility of the EBPR-r process.

2. Materials and methods

2.1. Reactor configuration and synthetic wastewater

A laboratory-scale sequencing batch biofilm reactor (master reactor) was operated continuously in EBPR-r configuration for more than 2 years, as described previously [5]. In brief, 1000 biofilm carriers (Kaldnes® K1 polyethene carrier) 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) facilitating P uptake and denitrification, and for 2 h to a separate recovery stream (1.8 L; 25% of the volume of the wastewater stream) to enable anaerobic P release and replenishment of carbon reserves in PAOs.

Both the wastewater and recovery streams contained a mineral salts growth medium consisting (per L) of: 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 trace element solution. The trace element 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 ethylenediaminetetraacetic acid (EDTA). The trace element solution pH was adjusted to 7.0. The wastewater stream also contained 8 mg-P/L of PO_4^{3-} (as 1 M phosphate buffer: 46 g KH_2PO_4 and 115 g K_2HPO_4 per L) and 10 mg-N/L of NO_3^- (as $NaNO_3$). To restore intracellular carbon reserves during anaerobic release of P, 375 mg/L acetate (as $C_2H_3NaO_2$) was added to the recovery stream; this corresponded to a 400 mg chemical oxygen demand (COD). 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.

The experiments described below were carried out during steady state operation of the master reactor, which showed stable total solid (TS) and PO_4^{3-} effluent concentrations.

2.2. Short-term (0–48 h) exposure to P- and C-deficient, and highly oxidising conditions

A series of batch experiments was performed, during which the EBPR-r biofilm was exposed to P- and C-deficient conditions and an oxidising environment (presence of O_2 and NO_3^-) for various periods (0–48 h). At the end of each starvation period PO_4^{3-} was introduced to assess the impact of P- and C-starvation on storage-driven P uptake.

The procedure described below was followed to ensure all batch experiments were initiated using biofilms that contained a high level of internal carbon storage polymers. To achieve this the biofilm carriers (approximately 330; taken from two compartments) were removed from the master reactor at the end of an anaerobic P release phase and gently washed with deionised water to remove any residual acetate and P. These carriers were immediately transferred into a separate column reactor (440 mL working volume; Fig. 1), through which a wastewater stream (containing neither soluble C nor P) was recirculated (1.2 L; 130 mL/min). Ion chromatography analysis showed the absence of acetate and P in the wastewater, confirming that there was no carryover of residual C and P from the biofilm. To create a highly oxidising environment NO_3^- was added to the wastewater to give an initial concentration of 10 mg-N/L, and dissolved oxygen (DO_{in}) was maintained throughout the experiment at a concentration of 7.8 ± 0.2 mg/L by purging air into the recirculation line of the column reactor. During each batch experiment the inflow (DO_{in}) and outflow (DO_{out}) oxygen concentrations in the column reactor were monitored online respectively using two luminescent DO probes (PDO_2; Barben Analyser Technology, USA). Monitoring and control of the DO levels were achieved using a programmable logical controller (PLC; National Instruments, USA) and LabVIEW software (National Instruments, USA).

Following exposure of the biofilm to P- and C-deficient wastewater in an oxidising environment for various lengths of time (0, 3, 12, 24, 48 h), PO_4^{3-} (8 mg-P/L) was added to the wastewater to assess the