



## Regular article

# Biological phosphorus removal from real wastewater in a sequencing batch reactor operated as aerobic/extended-idle regime



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## ABSTRACT

Recently, it has been reported that biological phosphorus removal (BPR) could be achieved in a sequencing batch reactor (SBR) with aerobic/extended-idle (A/EI) regime using synthetic medium. This paper first examined the feasibility and stability of the A/EI regime treating real domestic wastewater. The results showed that the A/EI-SBR removed  $1.32 \pm 0.03$ – $3.55 \pm 0.04$  mg of phosphorus per g of volatile suspended solids during the steady-state operation, suggesting that BPR from domestic wastewater could be well realised in the A/EI regime. Then, another SBR operated as the conventional anaerobic/oxic (A/O) regime was conducted to compare the soluble orthophosphate (SOP) removal with the A/EI regime. The results clearly showed that the A/EI regime achieved higher SOP removal than the A/O regime. Finally, the mechanism for the A/EI-SBR driving superior SOP removal was investigated. It was found that the sludge cultured by the A/EI regime had more polyphosphate accumulating organisms and less glycogen accumulating organisms than that by the A/O regime. Further investigations showed that the A/EI-SBR had a lower glycogen transformation and a higher PHB/PHV ratio, which correlated well with the superior phosphorus removal.

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## 1. Introduction

Enhanced biological phosphorus removal (EBPR) is currently considered to be one of the most economical and sustainable methods for removing phosphorus (P) from wastewater [1,2]. The conventional EBPR processes are based on the enrichment of activated sludge with polyphosphate accumulating organisms (PAOs) and, in the EBPR processes, biomass is subjected to alternating anaerobic and aerobic conditions so that the PAOs are favoured over other organisms. In the anaerobic phase, PAOs take up volatile fatty acids (VFAs) and store them as polyhydroxyalkanoates (PHAs). During the subsequent aerobic zone, PAOs take up excessive amounts of phosphate using the stored PHAs as both energy and carbon sources [3]. By wasting the P-rich sludge, excess P removal from wastewater can be achieved.

It is widely accepted that an anaerobic/aerobic sequence is a necessity to initiate and sustain EBPR [4]. In this regard, Comeau et al. [5], Mino et al. [6] and Arun et al. [7] developed two models

for EBPR (i.e. the “Comeau” model, and the “Mino” model). Both of the two models together with several subsequent update models all described EBPR as system with a characteristic array of an anaerobic basin followed by an aerobic phase.

The EBPR system with cyclic changes of anaerobic and aerobic conditions has an economical advantage of lower sludge production and less use of chemicals, therefore it plays an increasingly important role in controlling eutrophication of aquatic water system and is widely used all over the world [8]. Especially, it is popularly applied for treatment of domestic wastewaters, which contain typical phosphorus concentrations of 4–12 mg  $\text{PO}_4^{3-}$ -P/L [1]. But at the same time, it is a complex process when compared to biological removal of organic matter [9]. Moreover problems related to the process stability and impairment of P removal efficiencies are often reported in many full-scale applications [8,10].

In some cases, external disturbances such as nutrient limitation or excessive nitrate loading to the anaerobic reactor explained the process upsets. In other cases, microbial competition between PAOs and another group of organisms known as the glycogen (non-polyphosphate) accumulating organisms (GAOs) was considered to be the cause of the degradation in P removal [11]. Like PAOs, GAOs are able to proliferate under alternating anaerobic and aerobic conditions without performing anaerobic P release or aerobic P uptake [12]. Since GAOs consume VFAs without contributing to P removal,

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they are highly undesirable organisms in EBPR system, and minimising the growth of GAOs has the potential to be an effective strategy to promote the performance of EBPR systems [8].

Recently, it has been reported that excess P removal could be achieved without specific anaerobic period in activated sludge system if the idle period is extended properly, and this operation was defined as the aerobic/extended-idle (A/EI) regime [13,14]. Compared with conventional anaerobic/oxic (A/O) techniques, the anaerobic period was cancelled and an extended-idle phase (210–450 min) was operated in the A/EI regime. Up to now, the feasibility of using propionate as the carbon source for BPR in A/EI process has been examined [15], and the inducing metabolism of poly-P accumulation in the A/EI regime has also been addressed [16]. The previous studies suggested that the A/EI regime could achieve a good BPR performance and might be a promising alternative approach for P removal. Nevertheless, all of the experimental studies focused on the A/EI regime were conducted in simulated municipal wastewaters. In view of the complex composition and high P and nitrogen contents of real wastewater, these studies would not be able to accurately and comprehensively reflect the real situations and further examinations are needed.

This work, therefore, used domestic sewage as research object. First, a sequencing batch reactor (SBR) operated as the A/EI regime was conducted to examine the feasibility and stability for the treatment of a real domestic wastewater. Then, another SBR operated as the conventional A/O regime was conducted, and the soluble orthophosphate (SOP) removal efficiencies between the A/EI-SBR and A/O-SBR were compared. Finally, Variations of SOP and sludge glycogen as well as PHAs in a typical cycle in the two SBRs were discussed, and the mechanism for the A/EI-SBR achieving superior SOP removal performance was investigated.

## 2. Materials and methods

### 2.1. Wastewater

The wastewater used in this study was collected from a residential district of Changsha, PR China. It characterized by 100–400 mg/L chemical oxygen demand (COD), 20–40 mg/L ammonia-nitrogen ( $\text{PO}_4^{3-}\text{-N}$ ), 2–8 mg/L  $\text{PO}_4^{3-}\text{-P}$ . In addition, the wastewater had typical VFAs contents of 68–186 mg/L acetic acid, 21–90 mg/L propionic acid and a few other acids. Its pH level was about 7.0.

### 2.2. Reactor setup and operation

Two identical SBRs, each with a 2.0L working volume, were seeded with activated sludge from the second wastewater treatment plant of Changsha, PR China, which routinely achieves EBPR. The initial mixed liquor suspended solids (MLSS) of activated sludge was 4000 mg/L. The two SBRs were operated with three cycles per day. The A/EI-SBR cycle consisted of a 240 min aerobic period, followed by 30 min settling, 1 min decanting and 209 min idle periods. As a control, the A/O-SBR cycle consisted of an anaerobic period (2 h), and an aerobic period (4 h), with the remainder of the cycle time for settling (30 min), decanting (1 min), and idle (89 min). After settling period 1 L supernatant was discharged from both reactors and was replaced with 1 L of domestic wastewater during the first 1 min of the aerobic period (A/EI-SBR) and the anaerobic period (A/O-SBR), respectively. The A/O-SBR was mixed using a magnetic stirrer in the anaerobic stage. During the aerobic time, air was supplied into both SBRs at a flow rate of 2 L/min. Mean cell residence time (MCRT) was controlled at approximately 14 days in both reactors by withdrawing the sludge from the reactors at the end of the aerobic period, but before settling.

### 2.3. Cycle studies

The performance of the two SBRs was monitored through cycle studies in which both liquid and solid phase samples were collected at an interval of 5–10 min during the first 30 min of the anaerobic and/or aerobic period and each 30 min afterwards for the analysis of SOP, glycogen, PHAs, as well as dissolved organic carbon (DOC). Total suspended solids (TSS) and volatile suspended solids (VSS) were determined at the end of the aerobic periods.

### 2.4. Analytical methods

Sludge samples from the two reactors were immediately filtered through a Whatmann GF/C glass microfiber filter (1.2  $\mu\text{m}$ ). The filtrate was analyzed for SOP, DOC, and the filter was assayed for TSS, VSS, sludge total phosphate (TP), PHAs and glycogen.

SOP,  $\text{NH}_4^+\text{-N}$ , nitrite, nitrate, VSS and TSS were measured according to Standard Methods [17], and DOC was determined after membrane filtration (0.45 mm cellulose nitrate filter) using a total organic carbon (TOC) analyzer (Shimadzu TOC-500, Japan) according to the literature [18]. Element analysis of sludge sample was conducted by an analyzer of scanning electron microscope with X-ray energy dispersive microanalysis (JSM-5910, Japan) after lyophilization pretreatment. The measurements of sludge glycogen, poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), and poly-3-hydroxy-2-methylvalerate (PH2MV) were the same as described in the literatures [14,19].

DAPI (4',6'-diamidino-2-phenyl indol dihydrochloride) staining was carried out to analyze the presence of intracellular poly-P granules as described previously [20]. Sludge samples taken at the end of the aerobic period were used for staining.

The fluorescence in situ hybridization (FISH) technique with 16S rRNA-targeted oligonucleotide probes was employed to quantify PAOs and GAOs in both systems. FISH analyses were the same as described by Carvalho et al. [21] and Wong et al. [22]. Sludge samples were taken from the reactors at the end of aerobic zone during steady operation period. The oligonucleotide probes used for FISH are listed in Supporting Information (SI) Table S1.

## 3. Results

### 3.1. SOP removal performance in the A/EI-SBR during long-term operation

Experiments for SOP removal were conducted and lasted for about 180 days. The efficiencies of SOP removal during this period were shown in Fig. 1.

As shown in Fig. 1, it can be clearly observed that although influent SOP concentration ranged from 2 to 8 mg/L, SOP concentration in effluent were kept among 0–0.5 mg/L mostly and the removal efficiencies were about 94%, with a highest value of 98.6% during the stable operation. This proved that SOP removal from this type of domestic wastewater could be achieved without anaerobic phase, which was conventionally considered as an absolutely necessary phase for EBPR.

Fig. 2 shows that the variations of the SOP uptake per g of volatile suspended solids (SOP/VSS) in the A/EI-SBR were consistent with the variations of influent SOP concentration during long-term operation. As influent SOP concentration changed from 2 mg/L at day 14 to 6 mg/L at day 56, the SOP/VSS progressively increased from  $0.98 \pm 0.02$  to  $2.62 \pm 0.03$  mg of P per g of VSS. The SOP/VSS ratios were maintained stably at approximately  $1.02 \pm 0.03$ – $3.69 \pm 0.05$  mg of P per g of VSS when influent SOP concentrations ranged from 2 to 8 mg/L. With the increase of SOP/VSS, TP content of the activated sludge in A/EI-SBR increased from

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