



## Regular Article

# Effect of dissolved oxygen on biological phosphorus removal induced by aerobic/extended-idle regime



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## ABSTRACT

Previous researches have suggested that biological phosphorus removal (BPR) from wastewater could be achieved by the aerobic/extended-idle (A/EI) regime. This study further investigated the effect of dissolved oxygen (DO) concentration on BPR induced by the A/EI regime. The experimental results show that 1 mg/L of DO in mixed liquor benefited the BPR performance while a higher DO level of 5 mg/L deteriorated BPR. Fluorescent in situ hybridization analysis demonstrated that the improvement at 1 mg/L of DO was due to the shift in bacterial population from glycogen accumulating organisms (GAOs) to polyphosphate accumulating organisms (PAOs). The mechanism studies revealed that DO level affected the transformations of polyhydroxyalkanoates and glycogen and the activities of exopolyphosphatase and polyphosphate kinase. In addition, the BPR performances between the A/EI regime and conventional anaerobic/oxic (A/O) process were compared. The results showed that the A/EI regime drove better BPR performance than the A/O process at both low and high DO levels. More PAO and less GAO abundances in the biomass might be the principal reason for the higher BPR efficiency in the A/EI regime. Furthermore, controlling DO at a low level of 0.5 mg/L to promote BPR was demonstrated in a real municipal wastewater. The A/EI regime showed an excellent BPR performance at the low DO levels and had a better tolerance to oxygen-limited condition as compared to the A/O regime.

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## 1. Introduction

Enhanced biological phosphorus removal (EBPR) is an effective and environmental technology to remove phosphorus (P) from wastewaters [1]. In EBPR systems, polyphosphate accumulating organisms (PAOs) are able to store P through sequential anaerobic–aerobic conditions, while another group of microorganisms known as glycogen accumulating organisms (GAOs) compete with PAOs for the available organic substrate without contributing to P removal [2]. Successful operation of the EBPR systems depends on numerous process operational factors. In some cases, process upsets and the deterioration of P removal in EBPR plants can be explained by disturbances such as the presence of nitrate in the anaerobic pools [3] whereas in still other cases, the microbial competition of GAOs with PAOs is verified to be the major reason [4].

In recent decades, factors affecting the PAO–GAO competition have been the focus of many studies, and dissolved oxygen (DO) concentration has been reported to impact the PAO–GAO competition [5,6]. On one hand, PAOs need DO or nitrate to uptake P from wastewaters. On the other hand, the inflow of DO or nitrate into anaerobic pools will inhibit the anaerobic P release. Poor P removal performances and high quantities of tetrad-forming organisms (TFOs) were often observed at very high DO concentrations of 4.5–5.0 mg/L, while DO concentrations of approximately 2.5–3.0 mg/L seemed to correlate with the dominance of PAOs [5]. Additionally, an increase in the abundance of *Accumulibacter* and a decrease in *Competibacter* were observed at a low DO level of 0.5 mg/L [7].

Recently, achieving higher P removal efficiency with less energy consumption has become a very urgent task for wastewater treatment plants (WWTPs). As aeration is a costly process and the DO level will affect the efficiency of the removal of contaminants (e.g., BPR), an important strategy to minimize energy consumption and enhance BPR in activated sludge processes is to control the DO level in the aerobic zones.

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It has been reported that BPR can be achieved in activated sludge systems without specific anaerobic pools if the idle period is extended to 210–450 min [8]. This operation was defined as the aerobic/extended-idle (A/EI) regime and the inducing mechanisms of P removal in this process were verified to be different from previous processes [9,10]. Compared to the conventional anaerobic/oxic (A/O) process, the A/EI regime has several advantages such as the higher tolerance of nitrate and greater BPR efficiency [11,12]. However, those previous studies described the general performance in terms of P removal without specifying the importance of DO effect on the competition of PAOs and GAOs and performance of BPR in the A/EI systems. Moreover, BPR induced by the A/EI regime with low DO levels, such as 1 mg/L, has never been reported. In view of the fact that the A/EI regime has no specific anaerobic period, the microbial metabolic pathway would be different from the A/O processes [9]. Thus, the effect of DO concentration on BPR performance in the A/EI regime might also be distinct from that in the A/O process.

The purposes of this work were: (1) to investigate the effect of DO concentration on BPR induced by the A/EI regime; (2) to compare the BPR performances between the A/EI and A/O regimes operated at different DO levels; (3) to evaluate the feasibility of controlling DO at low levels to improve BPR in the A/EI regime when receiving a real municipal wastewater.

## 2. Materials and methods

### 2.1. Sequencing batch reactor (SBR) operation at different DO concentrations

Experiments were carried out in three lab-scale SBRs each with a working volume of 12 L. Seed sludge was inoculated into the three SBRs concurrently. Aeration and mixing were supplied through an air diffuser placed in the bottom of the SBRs, and DO concentrations in the three SBRs were monitored by WTW Multi 340i DO meters and were kept constant at 1, 3, and 5 mg/L, respectively. All SBRs were operated as the A/EI regime with 8 h per cycle. The cyclic profile is comprised of 210 min aeration, 55 min settling, 5 min decanting, and 210 min idle periods. After the settling phase 8 L of supernatant was discharged from all the reactors and replaced with 8 L of the synthetic medium at the beginning of the aerobic phase. 1.5 L of sludge mixtures from the SBRs were discharged daily at the end of aerobic zone but before settling, resulting in a sludge retention time (SRT) of 8 d.

As comparison, three reproductive SBRs were operated as the A/O process. Each cycle of the A/O SBRs consisted of 120 min anaerobic mix and 180 min aeration, followed by 55 min settling, 5 min decanting, and 120 min idle periods according to the literature [13]. 8 L supernatant was discharged from the A/O SBRs after settling period and was replaced with 8 L of the synthetic medium at the end of the idle period. The A/O SBRs were mixed with magnetic stirrers in the anaerobic period. During the aerobic stage, air was supplied into the A/O SBRs to control DO levels at 1, 3, and 5 mg/L, respectively. The SRT in the SBRs was maintained at 8 d.

The synthetic medium used as influent contained 15 mg/L  $\text{PO}_4^{3-}\text{-P}$ , 40 mg/L  $\text{NH}_4^+\text{-N}$ , and 300 mg/L chemical oxygen demand (COD). Acetate was used as the sole carbon source because it was the most common volatile fatty acid (VFA) in domestic wastewaters [14]. The concentrations of the other nutrients in the synthetic medium were as follows: 0.005 g/L  $\text{CaCl}_2$ , 0.01 g/L  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ , and 0.5 mL/L trace element solution [2].

### 2.2. Low DO concentration to improve BPR from municipal wastewater

In order to reduce the air supply during the aerobic period, it is necessary to investigate the behavior of P transformation in the

A/EI regime at low DO levels. The investigation was performed in four identical SBRs each with a working volume of 12 L. Seed sludge was inoculated into the four SBRs, two of which were operated as the A/EI regime and the other two were operated as the A/O process. The two A/EI SBRs were operated at 1 and 0.5 mg/L of DO, and the average DO concentrations in the two A/O SBRs were 3 and 0.5 mg/L, respectively. The other operational conditions were the same as those described in Section 2.1 except that the SBRs in this study received real municipal wastewaters which were collected from the inlet well of a WWTP in Changsha, China. The main characteristics of the municipal wastewater are as follows: total phosphate (TP) 6.5–9.7 mg/L, soluble orthophosphate (SOP) 4.6–7.8 mg/L, total nitrogen (TN) 32.4–45.8 mg/L, ammonia nitrogen ( $\text{NH}_4^+\text{-N}$ ) 23.9–36.2 mg/L, soluble COD 120–216 mg/L, pH 7.0–7.2.

### 2.3. Analytical methods

$\text{NH}_4^+\text{-N}$ ,  $\text{NO}_2^-\text{-N}$ ,  $\text{NO}_3^-\text{-N}$ , SOP, TP, COD, volatile suspended solids (VSS), and total suspended solids (TSS) were measured using the standard methods for the examination of wastewaters [15]. Sludge glycogen, poly-3-hydroxybutyrate (PHB), poly-3-hydroxyvalerate (PHV), and poly-3-hydroxy-2-methylvalerate (PH2MV) were measured according to the literature [8]. The total polyhydroxyalkanoates (PHAs) were calculated as the sum of measured PHB, PHV, and PH2MV.

4',6'-diamidino-2-phenylindole dihydrochloride (DAPI) staining was carried out to analyze intracellular poly-P granules [16]. Sludge samples used for staining were taken at the end of the aerobic period. Analysis of exopolyphosphatase (PPX) and polyphosphate kinase (PPK) activities was performed according to the literature [2].

Fluorescence in situ hybridization (FISH) technique was the same as described in the literature [9]. The following oligonucleotide probes used for hybridization are listed in Supporting Information (SI) Table S1. 30 microscopic fields were analyzed for the hybridization of individual probes using a confocal scanning laser microscope (FV 500). FISH quantification was performed with image database software (VideoTest Album3.0).

## 3. Results and discussion

### 3.1. Variations of DO and pH during one cycle in A/EI SBRs

To verify the effect of DO concentration on pH variations, pH was not held constant in this study so that pH varied as they would in a full-scale wastewater treatment plant. DO and pH were monitored continuously and the cyclic profiles of pH and DO variations in A/EI SBRs are shown in Fig. 1. At low DO level, the pH increased sharply from 7.1 to 7.5 in the initial 45 min of the aerobic period and decreased to 7.2 at the end of aeration, then further decreased to the initial level of 7.1 during the subsequent idle period. Similar variations were observed at moderate and high aeration, indicating little difference in pH variations.

### 3.2. BPR performances in the A/EI SBRs at different DO concentrations

It took about 21 d before effluent SOP concentration became stable in all SBRs, then the data were reported. Table 1 summarizes the reactor performances of the three SBRs during the steady-state operation. From Table 1, it can be concluded that COD and total nitrogen (TN) removal efficiencies were insensitive to DO concentration. However, the data of effluent SOP concentrations and SOP removal efficiencies showed that BPR performance depended

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