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# The effect of oxygen supply on nitrogen removal via nitrite using stored substrate (PHB) as the electron donor in SBRs



Lei Miao<sup>a</sup>, Shuying Wang<sup>a,\*</sup>, Rulong Zhu<sup>a,b</sup>, Tianhao Cao<sup>a</sup>, Yongzhen Peng<sup>a</sup>

<sup>a</sup> Engineering Research Center of Beijing, Beijing University of Technology, Beijing, China

<sup>b</sup> Power China Huadong Engineering Corporation Limited, Hangzhou, China

#### A R T I C L E I N F O

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#### ABSTRACT

This study investigated the effect of oxygen supply on nitrogen removal performance, by applying different oxygen supplies to four SBRs during aeration. That resulted in four different volumetric oxygen transfer coefficients ( $K_L a$ ), which were  $3.64 h^{-1}$ ,  $10.56 h^{-1}$ ,  $16.82 h^{-1}$ ,  $28.17 h^{-1}$ . The research showed that the overall specific nitrogen removal load increased from 0.64 to  $3.03 \text{ mmolN Cmol}X^{-1} h^{-1}$ , with the increased oxygen supply. Total nitrogen removal reaching above 97%, even with  $K_L a$  of  $10.56 h^{-1}$ . When oxygen supply increased ( $K_L a$  increased from 3.64 to  $16.82 h^{-1}$ ), the percent of SND decreased, while the rate of SND increased. However, they both decreased when oxygen supply continued to increase ( $K_L a$  increased to  $28.17 h^{-1}$ ). Besides, stronger oxygen supply in aeration preserved more PHB at the start of anoxic stage, which facilitated endogenous denitritation rate from 0.58 to  $3.68 \text{ mmolN Cmol}X^{-1} h^{-1}$ . In summary, stronger oxygen supply benefits nitrogen removal performance.

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#### 1. Introduction

Sequencing batch reactor (SBR) technology is considered as one of the most effective systems for improving nutrient removal because of its minimal space requirements, simple management, and flexible modifications through on-line control [1–3]. Additionally, SBR technology can adopt emerging and novel biological nitrogen removal processes, such as anaerobic ammonium oxidation (Anammox) [4,5], denitrifying phosphorus removal [6,7], nitritation/denitrifaction process [8,9], and simultaneous nitrification and denitrification (SND) processes [10,11]. In comparing a nitritation/denitritation process and a nitrification/denitrification process, the former reduces the aeration consumption in aerobic stage by 25%, and also saves the carbon source requirement in the anoxic stage by 40% [12].

The most apparent difference between SBR and a conventional continuous system is the dynamic periodicity feature, which exposes biomass to feast and famine conditions (driven by whether an external carbon source is present or not). Microorganisms store considerable fractions of soluble substrate as storage polymers (e.g., poly- $\beta$ -hydroxybutryic acid (PHB)) in dynamic conditions [13]. In SBR technology, microorganisms capable of storing exogenous organics have a competitive advantage because of their ability to accommodate changing conditions. Meanwhile, in the absence of oxygen, PHB serves as electron donor for denitritation after the external substrate is depleted [13]. Moreover, the PHB respiration rate is much slower than that of the soluble substrate [9]. This means that the rapid oxidation of external organics into CO<sub>2</sub> can be slowed by PHB storage; PHB then remains available when the external substrate is depleted in the aerobic and anoxic stages. Consequently, PHB can be used as the internal carbon source for SND during the aerobic stage, and for endogenous denitritation during the anoxic stage. With SBR technology, up to almost 100% nitrogen can be removed without carbon supplementation through SND and endogenous denitritation [9].

Reducing power can be preserved through PHB storage. Third et al. [14] found that when DO was lower (0.5 mg/L), the percentage of nitrogen removal via SND increased with slower rate of nitrogen removal. A moderate DO concentration (1 mg/L) was optimal for both SND percent and rate. However, very little literature exists about the effect of oxygen supply on nitrogen removal performance in SBRs using stored substrate (PHB) as the electron donor. This is particularly the case for nitrogen removal via nitrite. To address this issue, this study used four similar SBRs with different oxygen supplies to explore two problems. First, we studied how different oxygen supply (different transfer coefficients ( $K_La$ )) affect PHB synthesis and degradation. Second, we studied the relationship

<sup>\*</sup> Corresponding author. Fax: +86 10 67392627.

*E-mail addresses*: miaolei2819@emails.bjut.edu.cn (L. Miao), wsy@bjut.edu.cn (S. Wang).

Notation							
K <sub>L</sub> a	Volumetric oxygen transfer coefficient (h-1)						
OUR	Oxygen uptake rate (mmol $O_2 L^{-1} h^{-1}$ )						
NO <sup>-</sup> <sub>2</sub> -N <sub>accum</sub> Nitrite accumulation ratio (%)							
SAOR	Specific	ammonium	oxidation	rate			
	(mmolN Cmo	$p (X^{-1} h^{-1})$					
TDNR	Theoretical	specific	denitritation	rate			
	(mmolN Cmo	$p (X^{-1} h^{-1})$					
SNRL	Specific	nitrogen	removal	load			
	(mmolN Cmo	$p (X^{-1} h^{-1})$					
R <sub>PHB-syn</sub>	PHB synthesis rate (Cmmol Cmol $X^{-1}$ h <sup>-1</sup> )						
$R_{\rm PHB-ox}$	PHB oxidation rate (Cmmol Cmol $X^{-1}$ h <sup>-1</sup> )						
R <sub>Ac</sub>	Acetate uptake rate (Cmmol Cmol $X^{-1}$ h <sup>-1</sup> )						
f <sub>PHB</sub>	PHB fraction of activated biomass (Cmol Cmol $X^{-1}$ )						
Y <sub>PHB/Ac</sub>	Yield of PHB from acetate (Cmol Cmol <sup>-1</sup> )						
OTR	Oxygen transfer rate (mmol $O_2 L^{-1} h^{-1}$ )						
SOUR	Specific	oxygen	uptake	rate			
	$((mmol O_2 C))$	$mol X^{-1} h^{-1}$ )					
%SND	Nitrogen removal ratio from SND (%)						
R <sub>SND</sub>	Rate of SND (mmolN Cmol $X^{-1}$ h <sup>-1</sup> )						
%TN	Total nitrogen removal ratio (%)						
K <sub>PHB</sub>	The rate constants in the PHB degradation kinetics						

between PHB metabolism and nitrogen removal performance (i.e., SND and endogenous denitritation performance). The goal of both of these study elements was to help improve oxygen management at SBR wastewater treatment plants.

#### 2. Materials and methods

#### 2.1. Synthetic influent composition and sludge

The standard composition of the nutrient medium was: CH<sub>3</sub>COONa: 3000 mg/L, NH<sub>4</sub>Cl: 765 mg/L, KH<sub>2</sub>PO<sub>4</sub>: 44 mg/L, NaHCO<sub>3</sub>: 125 mg/L, MgSO<sub>4</sub>·7H<sub>2</sub>O: 51 mg/L, CaCl<sub>2</sub>·2H<sub>2</sub>O: 300 mg/L, FeSO<sub>4</sub>·7H<sub>2</sub>O: 6.25 mg/L, and 1.25 mLL<sup>-1</sup> of trace element solution. The medium was autoclaved before each experiment, preventing bacterial activity in the feed tank. After autoclaving, the trace element solution was added to the influent; the solution contained: ethylene-diamine tetraacetic acid (EDTA): 15 g/L, ZnSO<sub>4</sub>·7H<sub>2</sub>O: 0.43 g/L, CoCl<sub>2</sub>·6H<sub>2</sub>O: 0.24 g/L, MnCl<sub>2</sub>·4H<sub>2</sub>O: 0.99 g/L, CuSO<sub>4</sub>·5H<sub>2</sub>O: 0.25 g/L, NaMoO<sub>4</sub>·2H<sub>2</sub>O: 0.22 g/L, NiCl<sub>2</sub>·6H<sub>2</sub>O: 0.19 g/L, NaSeO<sub>4</sub>·10H<sub>2</sub>O: 0.21 g/L, H<sub>3</sub>BO<sub>4</sub>: 0.014 g/L, and NaWO<sub>4</sub>·2H<sub>2</sub>O: 0.050 g/L.

Sludge from a conventional pilot-scale SBR treating municipal wastewater was used as inoculums. The pilot-scale SBR had previously realized partial nitrification (nitritation) with a nitrite accumulation ratio of more than 90%.

#### 2.2. Experimental lab-scale reactor and procedure

This study was carried out in four lab-scale SBRs of identical design each with a working volume of 10 L. A pH probe, a dissolved oxygen (DO) probe, an oxidation reduction potential (ORP) probe were installed in each SBR. An air flow-meter controlled air flow rate.

The nitritation in lab-scale reactor was achieved under the inhibition of high FA (free ammonia) since the ammonia concentration in the influent was high. To facilitate sludge adaptation to the synthetic water, an operational mode of SBR<sub>2</sub> was applied to domesticate the inoculums until they reached steady state. Steady state was assumed to be achieved when biomass concentration and

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Operational mode of the SBRs over the standard cycle.

Sequence	Steps	Duration (hours or minutes)		Note
		SBR1	$SBR_2$ , $SBR_3$ , and $SBR_4$	
1	Fill	3 min	3 min	-
2	Aeration	Real-time control	Real-time control	Aerobic stage
3	Stir	12 h	Real-time control	Anoxic stage
4	Settling	30 min	30 min	-
5	Withdraw	5 min	5 min	-

nutrient removal efficiency remained constant for five repetitive cycles.

Four different oxygen supplies (SBR<sub>1</sub>:20Lh<sup>-1</sup>, SBR<sub>2</sub>:80Lh<sup>-1</sup>, SBR<sub>3</sub>:160Lh<sup>-1</sup>, SBR<sub>4</sub>:220Lh<sup>-1</sup>) were used for four SBRs, respectively. In view of DO concentration varies during the aerobic stage, the oxygen supplies were used as the control parameters. One unique oxygen supply level was used in each SBR to assess the impact of four different oxygen levels in otherwise identical conditions. This experimental design allowed for four different  $K_La$  levels (SBR<sub>1</sub>:3.64 h<sup>-1</sup>, SBR<sub>2</sub>:10.56 h<sup>-1</sup>, SBR<sub>3</sub>:16.82 h<sup>-1</sup>, SBR<sub>4</sub>:28.17 h<sup>-1</sup>). One standard experimental cycle consisted of five steps, summarized in Table 1. Real-time control referred to the application of pH and ORP variations to indicate the completion of nitritation and denitritation, respectively [9]. We fixed the duration of the SBR<sub>1</sub> anoxic stage at 12 h, because of the failure of thorough nitrite removal through endogenous denitritation.

For each SBR, volumetric exchange ratios were set as 20%. Temperatures were maintained at 25 °C. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were maintained at 4100–4300 mg L<sup>-1</sup> and 3700–3900 mg L<sup>-1</sup>, respectively, resulting in the sludge retention times of 16 days. No extra carbon sources were added at the start of the anoxic stage.

Each test was carried out in parallel three times. Test results are reported as average values with 95% confidence values. Before each test, all the sludge from across all four SBRs was mixed and aerated to eliminate the residual PHB from the previous test. Sludge was then evenly distributed to each SBR.

#### 2.3. Analytical methods

The ammonia nitrogen  $(NH^+_4-N)$ , nitrate  $(NO^-_3-N)$ , nitrite  $(NO^-_2-N)$ , total phosphorus (TP), and MLSS/MLVSS were each measured using standard methods [15]. DO, pH, and ORP were monitored using a pH/Oxi 340i analyzer (WTW Company, Germany). PHB and acetate were determined using a method described by Zeng et al. [16]. The acetate measurements in the reactor were ultimately not used, because of the very fast biomass uptake of acetate.

### 2.4. Measurement of the volumetric oxygen transfer coefficient $(K_La)$

The  $K_La$  was measured before each test to establish the specific aeration rate. For the measurement, the culture was aerated until DO concentration reached a steady state. Under that condition, the oxygen transfer rate (OTR) into solution is equal to the oxygen uptake rate (OUR) of the biomass. To determine the biomass OUR, aeration was then switched off and the DO rate was measured as it dropped. Therefore, the  $K_La$  could be calculated as [14]:

$$K_{\rm L}a({\rm h}^{-1}) = {\rm OUR}/(C_{\rm S} - C_{\rm L}) \tag{1}$$

where the OUR (mmol  $O_2 L^{-1} h^{-1}$ ) was the oxygen uptake rate;  $C_L$  (mmol  $O_2 L^{-1}$ ) was the oxygen steady state concentration before switching off the aeration; and  $C_s$  (mmol  $O_2 L^{-1}$ ) was the saturating concentration of oxygen in the gas phase.

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