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# Stability and performance of two GSBR operated in alternating anoxic/aerobic or anaerobic/aerobic conditions for nutrient removal

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#### ABSTRACT

Two granular sludge sequencing batch reactors (GSBR) with alternating anoxic/aerobic (R1) and anaerobic/aerobic (R2) conditions were operated with a 4-carbon-source synthetic influent. The physical properties of the granular sludge were very good ( $SVI \approx 20 \text{ mL g}^{-1}$ ) and high solid concentrations (up to  $35 \text{ gL}^{-1}$ ) were obtained in the bioreactor operated with a pre-anoxic phase with additional nitrate (R1). In contrast, performance and granule settleability were lower in R2 due to the development of filamentous heterotrophic bacteria on the surface of granules. These disturbances were linked to the fact that a fraction of COD remained during the aerobic phase, which was not stored during the anaerobic period. To stabilize a GSBR with a mixture of organic carbon sources, it is thus necessary to maximize the amount of substrate used during the non-aerated, anaerobic or anoxic, phase. Comparable phosphate removal efficiency was observed in both systems; enhanced biological P removal being greater in anaerobic conditions, while the contribution of precipitation (Ca–P) was more significant in anoxic/aerobic conditions.

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#### 1. Introduction

The aerobic granular sludge process has been proposed as a promising approach to biological wastewater treatment [1]. Thanks to their dense structure, aerobic granules have very good settling ability that allows high biomass retention in the bioreactor. This enables the process to withstand high-strength wastewater and results in the biological reactor having a smaller volume than conventional activated sludge systems [2]. The size and density of the granules allow simultaneous nitrification, denitrification and phosphorus removal, SNDPR [3,4] to be maintained. However, the operating conditions that improve the stability of the reactor's performance and the physical properties of aerobic granular sludge still need consideration. Instability and poorer properties of granular sludge have been reported for real sewage, for example, compared to the ideal results reported with purely acetate fed granules [5–7].

Various operating parameters have been identified that influence granule formation in aerobic systems. They include the

aeration rate, substrate feeding mode, organic loading rate, and settling time [8–12]. In granular sludge sequencing batch reactors (GSBR), the aeration rate plays two major roles: firstly, it imposes the hydrodynamic conditions in the reactor and, secondly, it controls the oxygen mass transfer in the aggregates. High aeration rate has been shown to provide high shear force, which erodes the surface of granules; to stimulate bacterial strains to secrete more extracellular polymeric substances (EPS), thus enhancing structural integrity; to reduce substrate transfer resistance in the liquid boundary layer at the granule surface; and to provide sufficient oxygen for organic substrate degradation [8,13]. Various studies have demonstrated that a high aeration rate (expressed by the superficial air velocity SAV) accelerates the formation of stable aerobic granules. Beun et al. [14] showed that smooth, stable granules could be obtained only with an SAV above 2.0 cm s<sup>-1</sup>. Tay et al. [13] found that regular, rounder, compact aerobic granules could be formed only above a minimum aeration rate (SAV =  $1.2 \text{ cm s}^{-1}$ ). Hence, the development of stable aerobic granules in pure aerobic systems is limited because of the high energy demand involved in aeration [15] and because efficient nitrogen and phosphorus removal requires the presence of anaerobic or anoxic and aerobic conditions [16].

Alternating anoxic/aerobic and anaerobic/aerobic conditions have both been reported to be helpful for granulation. Possible



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Nomenclature				
ACP	amorphous calcium phosphate (Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> ·XH <sub>2</sub> O)			
AOB	ammonium oxidizing bacteria			
COD	chemical oxygen demand (mg L <sup>-1</sup> )			
DO	dissolved oxygen concentration (mg $L^{-1}$ )			
EBPR	enhanced biological phosphorus removal			
EPS	extracellular polymeric substance			
GSBR	granular sequencing batch reactor			
HAP	hydroxy-apatite Ca <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> (OH)			
MLSS	mixed liquor suspended solid (gL <sup>-1</sup> )			
MLVSS	mixed liquor volatile suspended solid (gL <sup>-1</sup> )			
NOB	nitrite oxidizing bacteria			
OLR	organic loading rate (kg COD m $^{-3}$ d $^{-1}$ )			
PAO	polyphosphate accumulating organism			
SAV	superficial air velocity (cm s <sup>-1</sup> )			
SND	simultaneous nitrification denitrification			
SVI	sludge volume index (mLg <sup>-1</sup> )			
VFA	volatile fatty acid			

explanations for this are that conversion of readily biodegradable COD to internal stored biopolymers limits the substrate utilization rate during the aerobic phase [17] or that anoxic growth inside the granule improves aggregate density [18,19]. Another reason is that alternating non-aerated feast periods and aerated famine periods encourages the selection of slow-growing bacteria, which are supposed to be positive for the densification of bio-aggregates [9]. The work of Wan et al. [18] showed that the alternating anoxic feast and aerobic famine regimes allowed the formation of stable aerobic granules and simultaneous nitrification denitrification (SND) at a reduced air flow rate (SAV =  $0.6 \text{ cm s}^{-1}$ ). On the other hand, the alternation of anaerobic and aerobic conditions has been widely reported to promote internal biopolymer storage and enhance biological phosphorus removal by polyphosphate-accumulating organisms [20,21].

Therefore, the aim of this study was to compare the effect of alternating anoxic/aerobic and anaerobic/aerobic conditions on the performance and stability of an aerobic granular sludge process for simultaneous carbon, nitrogen and phosphorus removal. For this purpose, two reactors were run in parallel. They were fed with a mixture of organic substrates and operated with a similar aeration rate. The first reactor (R1) was operated with alternating anoxic/aerobic conditions, the second one (R2) was operated with alternating anaerobic/aerobic conditions. Process performance and the microscale structure of granules were investigated for both reactors.

#### 2. Materials and methods

#### 2.1. Reactor operating conditions

The experimental set-up included two geometrically identical granular sequencing batch reactors (GSBR), each with a working volume of 17 L (internal diameter = 15 cm, total height = 105 cm, H/D ratio = 7). Both reactors were inoculated with the same concentration of a stabilized hybrid sludge (containing both flocs and granules) cultivated in alternating anoxic/aerobic conditions. The initial MLSS and MLVSS concentrations were 19.5 g L<sup>-1</sup> and 13.1 g L<sup>-1</sup> respectively. The initial SVI was 22 mL g<sup>-1</sup><sub>MLSS</sub>. Reactor R1 was operated with alternating anoxic/aerobic conditions, whereas R2 was operated with alternating anaerobic/aerobic conditions. Each reactor was operated sequentially with a cycle time of 4 h including 15 min of feeding, 20 min of anoxic or anaerobic reaction (nitrogen gas injection); 145 min of aerobic reaction; 30 min of

#### Table 1

Operating condition	s of both reactors.
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Parameter	R1		R2
Volumetric exchange ratio (%)		47	
Hydraulic retention time (h)		8.5	
Organic loading rate (kg COD m <sup>-3</sup> d <sup>-1</sup> )		2.8	
Ammonia loading rate (kg N–NH4 m <sup>-3</sup> d <sup>-1</sup> )		0.14	
Nitrate loading rate (kg N-NO <sub>3</sub> m <sup>-3</sup> d <sup>-1</sup> )	0.28		0
Phosphorus loading rate (kg P-PO <sub>4</sub> m <sup>-3</sup> d <sup>-1</sup> )		0.08	
Superficial upflow velocity of N <sub>2</sub> (cm s <sup>-1</sup> )	$1.1\pm0.1$		$0.6\pm0.1$
Superficial upflow velocity of air (cm s <sup>-1</sup> )		$1.0\pm0.1$	
Temperature (°C)		$20\pm 2$	
pH (not regulated)	7.5-9.2		7.2-8.5

settling and 30 min of discharge (with a volumetric exchange ratio of 47%). The aeration rate was similar in both reactors, with a superficial air upflow velocity (SAV) of  $1.0 \pm 0.1$  cm s<sup>-1</sup>. Both reactors were fed at the bottom of the column when aeration was stopped (static fill). The feed consisted of a synthetic substrate [18] having the following composition: COD of  $1000 \text{ mg L}^{-1}$  (25% contribution each of glucose, acetate, propionic acid and ethanol),  $[PO_4^{3-}] = 30 \text{ mg P L}^{-1}, \quad [Ca^{2+}] = 46 \text{ mg L}^{-1}, \quad [HCO_3^{--}] = 100 \text{ mg L}^{-1},$  $[MgSO_4 \cdot 7H_2O] = 12 mg L^{-1}, [NH_4^+] = 50 mgN L^{-1}. A COD/N-NH_4^+$ ratio of 20 was maintained. Nitrate was dosed in R1 in order to maintain anoxic conditions after feeding ( $[NO_3^-] = 100 \text{ mgN L}^{-1}$ ). The pH probe and DO probe were installed online and the data were acquired by the computer every 30 s. pH fluctuated naturally during a reactor cycle, from 7.5 to 9.2 in R1 and from 7.2 to 8.5 in R2. The temperature was maintained at  $20 \pm 2$  °C with a water jacket. The reactor performance was monitored through weekly cycle studies, in which samples were analysed at regular intervals during an SBAR cycle. Table 1 summarizes the main operating conditions of the two reactors.

Due to annual closure, the supply of influent to the reactors was interrupted for two consecutive weeks and the cycle of operation was modified: the new 2-h cycle consisted of 15 min aeration and 105 min settling. This period (from day 105 to day 120) is referred to as the "starvation period".

#### 2.2. Analytical characterization of the liquid and solid phases

Analyses were conducted according to standard methods (AFNOR) [22]: COD (NFT 90-101), MLSS (NFT 90-105) and MLVSS (NFT 90-106). NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> concentrations were analysed by ion chromatography (IC25, 2003, DIONEX, USA) with prior filtering of the samples through a 0.2  $\mu$ m poresize acetate filter. The sludge volume index (SVI) was measured in the reactor after 30 min of settling and showed less than ±10% of difference relative to the standard procedure in a graduated test tube. Microscopic observations of sludge samples were made with a Biomed-Leitz<sup>®</sup> binocular photonic microscope. Particle size distribution was measured with a Malvern 2000 Mastersizer<sup>®</sup> analyzer and with statistical image processing.

The proportion of granules by mass and by volume was estimated using Eqs. (1) and (2) respectively:

Percentage of granules by mass = 
$$\frac{\text{MLSS}_{\text{granule}} \times 100}{\text{MLSS}_{\text{hybrid sludge}}}$$
 (1)

Percentage of granules by volume = 
$$\frac{V_{\text{granule}} \times 100}{V_{\text{hybrid sludge}}}$$
 (2)

where MLSS<sub>granule</sub> and MLSS<sub>hybrid sludge</sub> are the mixed liquor suspend solids in granules and hybrid sludge respectively. In order to assess the MLSS of granules, sieving at 315  $\mu$ m was performed as described in Filali et al. [23]. V<sub>granule</sub> and V<sub>hybrid sludge</sub> represent the apparent volume of granules and hybrid sludge, respectively, in the reactor after 30 min of settling.

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