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Research article

Development of aerobic granular sludge under tropical climate conditions: The key role of inoculum adaptation under reduced sludge washout for stable granulation

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ABSTRACT

Aerobic granular sludge (AGS) is a promising technology for wastewater treatment. However, the success of the process depends on the formation of stable granular biomass, which is associated with the microbiological aspects of the sludge and reactor operating conditions. In this study, the development of AGS from a poor nitrifying flocculent sludge obtained in a sewage treatment plant designed only for organic matter removal was assessed in a sequencing batch reactor (SBR) under tropical climate conditions (temperatures of 28 ± 4 °C). The results showed that, despite the alternating anaerobic-aerobic conditions during the granules selection phase under high sludge washout rates (low settling time), readily biodegradable organic matter was mainly removed aerobically. The formed granules were unstable, exhibiting a substantial amount of filaments and pasty consistency. The biomass characteristics (e.g., sludge volume index, density, diameter and settling velocity) were negatively impacted as complete granulation was reached, while biomass loss and degranulation became inevitable. Poor nitrification and no enhanced biological phosphate removal (EBPR) were observed. Implementation of a new operational strategy incorporating an adaptation of the seed sludge under reduced washout conditions (high settling time) prior to the granules selection stage enabled most of the influent organics to be removed anaerobically. Besides allowing a feast-famine regime to be established in the reactor, the sludge acclimation phase favoured the development of slow-growing organisms and suppressed the appearance of filamentous-like structures. Fast-settling granules with regular shape remained stable in the long-term, while high ammonium (> 95%) and total nitrogen removal (> 90%) was obtained. However, EBPR activity was very unstable, most likely due to the high temperatures. The findings of this study are important for the spreading of the AGS technology worldwide, especially in developing countries where the conditions are different in all aspects.

1. Introduction

Biological wastewater treatment processes have been substantially improved over the years thanks to the dedication of environmental researchers and engineers. Since 1914, when the suspended growth-based activated sludge (AS) process has been developed, many other treatment technologies have been proposed (Angelakis and Snyder, 2015).

Recently, a new process called aerobic granular sludge (AGS) has been developed in the Netherlands (Heijnen and van Loosdrecht, 1998). It relies on the formation of granular shaped particles from flocculent sludge in a reactor environment subjected to controlled selection pressures (De Kreuk and De Bruin, 2004). The granulation process is most often achieved in sequentially operated batch reactors where poor settling flocs are forced to grow in the form of spherical shaped granular sludge by gradual reduction of the settling time (Beun et al., 1999). This operational strategy selects for fast setting biomass while promoting washout of light flocs. Consisting of compact and dense microbial structures, aerobic granules exhibit high settling velocity and are often characterized by low sludge volume index (SVI) as compared with flocculent sludge. These characteristics facilitate solid-liquid separation and allow high concentrations of biomass to be obtained in the reactor (Bassin, 2018). The volumetric treatment capacity of the plant can then be increased and the construction of compact and decentralized

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wastewater treatment systems becomes feasible (Liu and Liu, 2006). The physical strength of granular microbial aggregates also makes them less vulnerable to adverse environmental conditions and refractory compounds (Zhang et al., 2017). Moreover, the coexistence of different redox zones within the granular structure enables several processes (e.g., chemical oxygen demand (COD), nitrogen and phosphorus removal) to be accomplished in a single reactor tank (De Kreuk and De Bruin, 2004). Given these attractive features shown by AGS, the formation of granules has been subject of many previous investigations (Beun et al., 1999; De Kreuk et al., 2005a; Pronk et al., 2015a).

Aerobic granulation is affected by several factors, among which are the substrates, organic load, shear stress and microbial community of the sludge (Adav et al., 2008). The latter influence the duration of the granulation process and affects several important properties of the granular biomass, such as density and bioactivity (Ivanov et al., 2006; De Kreuk and van Loosdrecht, 2004). Moreover, the stability of the granular biomass agglomerates is strongly dependent on the bacterial consortia and its potential to produce extracellular polymeric substances (EPS) (Zhang et al., 2015). In this context, the sludge used to inoculate the bioreactor seems to have a significant role on the biogranulation process.

In most studies on the formation of aerobic granular biomass, the sludge used as inoculum to promote granulation was obtained from wastewater treatment plants designed for biological nutrient removal (BNR) (Beun et al., 1999; De Kreuk and van Loosdrecht, 2004; Barr et al., 2010). This seems reasonable since nutrient removal is mandatory in many countries, especially in North America and Europe (Oleszkiewicz and Barnard, 2006). It is then expected that many different microbial functional groups such as ordinary heterotrophs, nitrifiers, denitrifiers, and polyphosphate-accumulating organisms (PAOs) are present in the seed sludge. However, many wastewater treatment facilities, especially in developing countries, are only designed for COD abatement (Mara, 2004), given that nitrogen and phosphorus discharge limits are not regulated by local environmental legislations. This is the case of Brazil, where the treatment requirements are much less stringent than in developed nations (Von Sperling, 2008). In this sense, many key players in the nitrogen and phosphorus cycle may be lacking in the microbial community of non-BNR systems.

Given that the seed biomass is often obtained from local wastewater treatment facilities (Pronk et al., 2015b), mainly due to transport constraints, it is important to understand the formation of aerobic granular sludge under different environmental and operational scenarios. Besides using seed sludge from BNR systems, most of the studies on AGS development were performed at moderate to low temperatures (De Kreuk et al., 2005a; Pronk et al., 2015b), but information on aerobic granulation under tropical climate conditions is still limited (Halim et al., 2016). To contribute for the expansion of AGS technology worldwide, further studies on the formation of granules at different inoculum and temperature conditions are necessary. In this contribution, we describe the challenges in achieving stable granulation using seed biomass from an AS-based wastewater treatment plant designed for COD removal with poor nitrification capability. Long-term experiments were carried out for more than 300 days in order to evaluate the granular biomass development and determine the best experimental approach for achieving stable granulation under tropical climate conditions (over 28 °C). Concomitantly, the performance of the system in terms of COD, nitrogen and phosphate removal was followed in the long-term and the results were associated to the implemented operational strategies.

2. Material and methods

2.1. Experimental set-up and operating conditions

In this work, the formation of aerobic granular sludge was investigated in a bubble column sequencing batch reactor (SBR). A schematic drawing of the experimental apparatus is shown in Fig. S1. The reactor was made of transparent plexiglass with internal diameter (D) of 5 cm and useful height (H) of 79 cm, giving an H/D of around 15. Large H/D ratios are commonly used in lab-scale studies aiming at aerobic granulation as they facilitate the retention of fast settling biomass and washout of light flocculent sludge at short settling times (Bassin et al., 2012a). The useful volume of the SBR was 1.5 L. In each SBR cycle, 0.95 L of influent was fed to the reactor and the same volume of treated effluent was drained from the discharge port located 30 cm from the reactor bottom. Thus, the volume exchange ratio (VER) was 63%.

Mixing and aeration were provided by a porous diffuser located at the bottom of the reactor, which was connected to an air compressor. The airflow rate was controlled at around 4 L/min with the aid of pressure regulating gauge and an airflow meter. Dissolved oxygen concentration (DO) varied from 4 to 6 mg/L during the SBR cycle. The reactor system was operated at room temperature (28 ± 4 °C). pH was maintained within 7.0–7.5 by either adding 1 M NaOH or 1 M HCl. The influent wastewater was synthetically prepared in the laboratory to ensure substrate controlled conditions. Based on previous work (Bassin et al., 2012b), two solutions were prepared: solution 1 (containing the organic carbon source) and solution 2 (containing the nitrogen and phosphorus source). Their composition is displayed in Table 1. A trace element solution (Vishniac and Santer, 1957) was also added to solution 2 in a proportion of 5 m$props_value{literPattern}/L. To obtain the desired influent COD (~400 mg/L), ammonium (~50 mgN/L) and phosphate (~15 mgP/L) concentrations, typically found in domestic sewage (Metcalf and Eddy, 1991), 150 mL of solutions 1 and 2 was mixed with 650 mL of demineralized water before being fed to the reactor. Small deviations from the theoretical concentrations of different influent components resulted from the preparation procedure of the synthetic medium. The SBR was inoculated with sludge from an AS plant treating municipal wastewater of the city of Rio de Janeiro, Brazil. The plant was only designed for COD removal, i.e., there was no separate anaerobic-anoxic zones intended for nitrogen and phosphorus removal. Biomass was collected in the sludge recycle line from the secondary clarifier.

To enable biological phosphorus removal, implementation of alternating anaerobic and aerobic conditions is essential. Therefore, the SBR cycle consisted of a non-mixed anaerobic feeding phase from the bottom of the tank through the settled sludge bed, followed by aeration, settling and effluent withdrawal. The duration of each phase and the actuation of the influent/effluent peristaltic pumps and influent/effluent/air valves were controlled by a programmable logic controller (PLC) linked to a computer software interface. The total cycle time was set at 3 h and, given the VER of 63%, the hydraulic retention time (HRT) corresponded to around 4.8 h. The feeding period was always maintained at 60 min while the effluent withdrawal phase was kept invariable at 5 min in all experiment conditions. The settling and aeration times were varied, as detailed below.

Two different operational strategies were employed, designated

Table 1
Composition of solutions 1 and 2 used to feed the SBR.

Solution 1[a]		Solution 2[a]	
Component	Concentration (mg/L)	Component	Concentration (mg/L)
NaAc $3H_2O$	4385	NH_4Cl	900.7
$MgSO_4$ $7H_2O$	445	K_2PO_4	368
KCl	175	KH_2PO_4	143.5
$CaCl_2$	364		

[a] 250 mL of each solution was mixed with 650 mL of demineralized water to obtain influent COD, ammonium and phosphate concentrations of 400 mg/L, 50 mgN/L and 15 mgP/L. Therefore, the organic, nitrogen and phosphorus load applied to the bioreactor corresponded to 2 kgCOD/(m^3.d), 0.25 kgN/(m^3.d), and 0.075 kgP/(m^3.d), respectively.