



Effect of particulate organic substrate on aerobic granulation and operating conditions of sequencing batch reactors



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ARTICLE INFO

Article history:

Received 1 April 2015

Received in revised form

17 August 2015

Accepted 18 August 2015

Available online 20 August 2015

Keywords:

Aerobic granules

Particulate organic substrate

Selection pressure

Simultaneous fill-and-draw operation

ABSTRACT

The formation and application of aerobic granules for the treatment of real wastewaters still remains challenging. The high fraction of particulate organic matter (X_S) present in real wastewaters can affect the granulation process. The present study aims at understanding to what extent the presence of X_S affects the granule formation and the quality of the treated effluent. A second objective was to evaluate how the operating conditions of an aerobic granular sludge (AGS) reactor must be adapted to overcome the effects of the presence of X_S . Two reactors fed with synthetic wastewaters were operated in absence (R1) or presence (R2) of starch as proxy for X_S . Different operating conditions were evaluated. Our results indicated that the presence of X_S in the wastewater reduces the kinetic of granule formation. After 52 d of operation, the fraction of granules reached only 21% in R2, while in R1 this fraction was of 54%. The granules grown in presence of X_S had irregular and filamentous outgrowths in the surface, which affected the settleability of the biomass and therefore the quality of the effluent. An extension of the anaerobic phase in R2 led to the formation of more compact granules with a better settling ability. A high fraction of granules was obtained in both reactors after an increase of the selection pressure for fast-settling biomass, but the quality of the effluent remained low. Operating the reactors in a simultaneous fill-and-draw mode at a low selection pressure for fast-settling biomass showed to be beneficial for substrate removal efficiency and for suppressing filamentous overgrowth. Average removal efficiencies for total COD, soluble COD, ammonium, and phosphate were $87 \pm 4\%$, $95 \pm 1\%$, $92 \pm 10\%$, and $87 \pm 12\%$ for R1, and $72 \pm 12\%$, $86 \pm 5\%$, $71 \pm 12\%$, and $77 \pm 11\%$ for R2, respectively. Overall our study demonstrates that the operating conditions of AGS reactors must be adapted according to the wastewater composition. When treating effluents that contain X_S , the selection pressure should be significantly reduced.

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1. Introduction

Aerobic granular sludge (AGS) has been extensively investigated during the last decades due to its great potential for wastewater treatment. However, most studies on aerobic granulation have

been performed in laboratory-scale sequencing batch reactors (SBR) using readily biodegradable (S_S) synthetic substrates containing mainly acetate and glucose as carbon sources. Only few studies reported successful granule cultivation with real wastewater, such as malting (Schwarzenbeck et al., 2004), brewery (Wang et al., 2007), dairy (Schwarzenbeck et al., 2005), swine slurry (Figuerola et al., 2011), soybean-processing (Su and Yu, 2005), and domestic wastewater (De Kreuk and Van Loosdrecht, 2006; Ni et al., 2009; Liu et al., 2010; Coma et al., 2012; Su et al., 2012; Wagner and Costa, 2013; Rocktäschel et al., 2015; Wagner et al., 2015). These studies have indicated that the kinetics of aerobic granulation when using real wastewaters are different from the ones obtained with synthetic wastewaters. This may be due to the fact that real

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wastewater, either from domestic or industrial origin, often contains diverse carbon sources along with a multitude of organic and inorganic compounds and also particulate matter (Lemaire et al., 2008). For example, the fraction of the particulate organic matter (X_S) in pre-settled domestic wastewaters usually accounts for around 40–60% of the total organic matter (Kappeler and Gujer, 1992; Orhon and Çokgör, 1997; Koch et al., 2000). Therefore, synthetic wastewaters composed only by S_5 cannot truly represent real and complex wastewaters. As a consequence, the formation and application of aerobic granules for the treatment of real wastewaters still remains challenging.

Previous studies suggested that the presence of X_S in the wastewater results in aerobic granules with filamentous outgrowths on their surfaces (Schwarzenbeck et al., 2004, 2005; De Kreuk et al., 2010; Peyong et al., 2012; Figueroa et al., 2015). The development of filamentous and finger-type structures can be explained by the existence of substrate gradients inside the sludge aggregates due to diffusion limitation (Martins et al., 2004; De Kreuk et al., 2010; Weissbrodt et al., 2012). Under these conditions, filamentous bacteria have a higher outgrowth kinetic because they grow preferentially in one direction and not in three directions as the floc-forming bacteria inside the aggregates (Martins et al., 2004, 2011). Particulate matter is mainly hydrolyzed at the surface of the granules. The readily biodegradable substrates produced by hydrolysis are then utilized locally, enhancing substrate gradients inside the granules and thus stimulating the outgrowth of filamentous structures (De Kreuk et al., 2010). The higher accumulation of X_S on the finger like filamentous granule structures will further enhance this phenomenon of irregular substrate uptake over the granule (De Kreuk et al., 2010). Nevertheless, to what extent the presence of X_S affects the overall granulation process (e.g. kinetics of granules formation) and substrate degradation rate still remains unclear.

Aerobic granular sludge was successfully cultivated in a lab-scale reactor treating malting wastewater with a high content of particulate organic matter (Schwarzenbeck et al., 2004). The removal X_S by aerobic granules resulted from two different mechanisms (Schwarzenbeck et al., 2004): (1) during initial granule formation and growth, particulate substrates adsorbed onto the biofilm matrix of the granules; (2) for the completely granulated sludge bed with mature granules, X_S is removed as a result of the presence and metabolic activity of a dense protozoa population covering the granules' surface. De Kreuk et al. (2010) demonstrated using starch as a model particulate substrate that X_S is mainly removed by adsorption at the granule surface, followed by hydrolysis and consumption of the hydrolyzed products. Unlike soluble substrates, particulate organic matter cannot pass through cell membranes and need to undergo extracellular hydrolysis prior to adsorption (Orhon and Çokgör, 1997). The hydrolysis processes is responsible for the conversion of particulate into readily biodegradable substrate that can serve as a necessary carbon source for denitrification or biological phosphorus removal (Morgenroth et al., 2002). Therefore, nutrient removal can be limited by the extent and kinetics of hydrolysis processes of the particulate organic matter (Morgenroth et al., 2002). If hydrolysis of the organic substrate in the particulate form is a slow process, it can then be hypothesized that the reactor operation should be adapted so that the hydrolysis rate and ultimately the granule formation are encouraged.

The present study therefore aimed at (i) better understanding to what extent the presence of X_S in the wastewater affects granule formation and the reactor performances and at (ii) evaluating how the operating conditions of an AGS reactor can be adapted to overcome the effects of the presence of X_S in the wastewater. Two SBRs fed with synthetic wastewater were operated in parallel over several weeks in absence (R1) or presence (R2) of particulate

substrate. Different operating conditions were applied in order to evaluate their effects on granulation and treatment performances. The physical properties of the sludge (size fraction of granules, sludge volume index, etc.) as well as the microbial activities (COD, nitrogen and phosphorus removal) were followed.

2. Materials and methods

2.1. Experimental set-up

Two reactors of 10.2 L (0.12 m internal diameter and 1.2 m height) were used for cultivation of aerobic granules in absence (R1) or presence (R2) of particulate substrate. Synthetic substrates were used in order to operate the reactors under controlled conditions (stable influent composition). The reactors were operated in SBR mode with cycles of 4 h and at a volume exchange ratio of 50%. The pH was monitored but not controlled. During the aeration phase, air was continuously introduced at a superficial upflow velocity of 1.2 cm s⁻¹. The solids retention time (SRT) was not controlled and was calculated taking into account the amount of biomass washed-out during the supernatant removal.

R1 was inoculated with 5 L of conventional activated sludge taken from an aeration tank of a municipal wastewater treatment plant operated for full biological nitrogen and phosphorus removal (ARA Thunersee, Switzerland). The seeding sludge had a total suspended solids concentration (TSS) of 3.4 g_{TSS} L⁻¹ and a sludge volume index (SVI₃₀) of 103 mL g_{TSS}⁻¹. The SBR cycle of R1 was divided into the following phases: 60 min of anaerobic feeding from the bottom of the reactor, 167 min of aeration, 8 min of settling, 3 min of effluent withdrawal, and 2 min of idle period. After 25 days of acclimation with a synthetic wastewater containing only soluble substrate (S_5), half of the sludge volume from R1 was added in R2 and the two systems were started in parallel with the same biomass concentration (3.5 g_{TSS} L⁻¹).

2.2. Experimental approach

The experimental approach was divided in four different phases to evaluate the effect of operating conditions on granulation and reactors performances (Table 1). In *Phase I*, the effect of the presence or absence of X_S on the aerobic granulation was evaluated. The SBR cycle consisted of 60 min of anaerobic feeding from the bottom of the reactor, 167 min of aeration, 8 min of settling, 3 min of effluent withdrawal, and 2 min of idle period. The effluents were extracted at the half of the maximum working heights of the reactors, resulting in operations with variable working volume. Biomass with settling velocity larger than 4.5 m h⁻¹ was therefore selected. This value corresponds to the height of supernatant (0.6 m) and the settling time (8 min).

Phase II aimed at evaluating to what extent an extension of the anaerobic phase favors the formation of the granules and the reactor performance. The anaerobic period of R2 was therefore extended to 90 min, with 60 min of anaerobic feeding and 30 min of anaerobic reaction without mixing. Due to some technical failures, the operation of R1 had to be discontinued during *Phase II*. R1 was re-inoculated with the same inoculum and operated under the same conditions previously applied in *Phase I*, in order to reestablish the biomass characteristics. No data are thus presented for R1 during *Phase II*.

In *Phase III*, the effect of an increased selection pressure for fast-settling biomass, i.e. granules, was investigated. The settling time in both reactors was therefore reduced from 8 min to 4 min in order to wash-out the flocs with the effluent and to retain in the reactor only aggregates with good settling ability, i.e. granules. As a result, biomass with settling velocity larger than 9.0 m h⁻¹ was selected.

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