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Accelerated aerobic granulation using alternating feed loadings: Alginate-like exopolysaccharides

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HIGHLIGHTS

- Alternating OLR feeding is applied to accelerate aerobic granulation.
- Increase in OLR stimulates the cells to secret c-di-GMP to produce ALE.
- Excess ALE with subsequent shearing forms aerobic granules.
- Fraction of building blocks in ALE does not affect granulation.

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ABSTRACT

Alginate-like exopolysaccharides (ALE) likely contribute markedly to strength of aerobic granules. This study cultivated aerobic granules from propionate wastewaters using strategies with different organic loading rates (OLRs) (4.4–17.4 kg/m^3-d). When the OLR increased suddenly, the constituent cells (*Pseudomonas*, *Clostridium*, *Thauera* and *Arthrobacter*) were stimulated to secret extracellular cyclic diguanylate (c-di-GMP) and produced excess ALE, which formed a large quantity of sticky materials that served as the precursor of aerobic granules. Formation of excess ALE was the prerequisite for accelerated granulation. Conversely, this study observed no enrichment of poly guluronic acid blocks in ALE during granulation.

1. Introduction

Aerobic granulation, a biological wastewater treatment technology, is typically suitable for handling toxic high-strength industrial effluent (Adav et al., 2008). Aerobic granules were typically cultivated from wastewater at high organic loading rates (OLRs) (Lopez et al., 2009). Factors affecting aerobic granulation are seed sludge, substrate composition, OLR, feeding strategy, reactor design and its hydrodynamics, settling time, exchange ratio, and aeration intensity (Show et al., 2012). Wang et al. (2012) compared the granulation process of two sequential batch reactors (SBRs): for one reactor, wastewater was fed in one batch at the start of each cycle; in the other reactor, half of the wastewater was fed at the beginning of each cycle and the other half was fed sometime within the same cycle. They determined that two-step feeding had adverse effects of granulation. Gao et al. (2011) showed that high OLRs enhance granulation rates. The OLR affects the granulation rate and the microbial communities in formed granules (Li et al., 2008). Zhang et al. (2013) cultivated granules in less than 24 h with an OLR of 24 kg/m^3-d and settling time of 1 min. However, the cultivated granules had poor stability. Khan et al. (2013) determined that aerobic granulation is insensitive to OLR based on their literature review.

Liu and Tay (2004) showed that granule formation was a four-step sequential process: (1) cell-to-cell contact; (2) initial attachment to aggregates; (3) enhancement in aggregate strength by accumulating extracellular polymeric substances (EPS); and (4) hydrodynamic shear force to compact the aggregate structure. Conversely, by observing the exchange of constituent fine aggregates between the granule surfaces and surrounding suspensions, Zhao et al. (2014) determined that granulation is a random coagulation process. Aerobic granules have a layered structure with spatial distributions of polysaccharides, proteins, lipids and different functional strains (Adav et al., 2010). Gonzalez-Gil and Holliger (2014) applied imaging approaches to investigate the detailed

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structure of aerobic granules, indicating that granules mainly grow via biomass outgrowth and not by small particle aggregation. Lee et al. (2010), who applied a multiple color staining technique to explore the layered structure of aerobic granules, proposed that granulation is biological processes that comprises formation of initial aggregates with live cells, followed by EPS secretion, limiting oxygen transfer (Chiu et al.,, 2007a,b), such that aerobic strains were lysed to form anaerobic strains with anaerobic protein-rich cores.

Cell–cell interaction is the essential link in the intra-granular structure (Lee et al., 2010). Bacteria can use cyclic diguanylate (c-di-GMP) molecules as second messenger to regulate cellulose synthesis (Ross et al., 1987) and to drive the transition between motile status and the sessile state of cells (Tamayo et al., 2006). Wan et al. (2013) was the first to confirm that synthesized intracellular c-di-GMP enhances the long-term stability of aerobic granules. Wan et al. (2014a) isolated X9, a flocculating consortium, from aerobic granules, noting that X9 had high levels of c-di-GMP at a late stage of granulation to promote the formation of large aerobic granules. Wan et al. (2014b) determined that when starving, cells in aerobic granules secreted excess quantities of c-di-GMP and pentaphosphate (ppGpp), forcing the cells into viable but non-culturable (VBNC) status, such that granules can survive and be stored for long periods.

Excess EPS form the interiors of aerobic granules that are secreted by bacteria under environmental stress (McSwain et al., 2005). Chen et al. (2007a) elucidated the distribution of EPS (proteins, α- and β-polysaccharides and lipids) and cells (total and dead) in aerobic granules using a novel sixfold staining scheme and the confocal laser scanning microscopy (CLSM) technique. The 3D structures of EPS in aerobic granules were visualized (Chen et al., 2007b). Alginate is composed of poly mannuronic acid blocks (MM blocks), poly guluronic acid blocks (GG blocks) and their mix (MG blocks) (Seviour et al., 2012). Lin et al. (2010) extracted the alginate-like exopolysaccharides (ALE) from aerobic granules and identified a high percentage of the GG blocks (approx. 69%). These authors suggested that ALE plays a significant role in granule strength. Lin et al. (2013) also noted that their granules had ALE with enriched GG blocks, resulting in stronger blocks than those from aerobic flocculent sludge with fewer GG blocks.

No study has assessed whether a correlation exists between extracellular c-di-GMP, ALE contents (and blocks), and the aerobic granulation process. This study cultivated aerobic granules from propionate wastewater at alternating OLRs. The ALE contents and constituent blocks and those of extracellular c-di-GMP were quantified. The microbial communities of cultivated granules were also identified.

2. Methods

2.1. Reactor setup and tests

Two identical SBRs (RS and RC) of height 150 cm and 8 cm in diameter with working volume of 4.78 l were adopted. Effluent was drained off by a valve 40 cm above reactor bottom to lead to a volume exchange rate of 57.9%. Seed sludge was obtained from a wastewater treatment plant of Uni-President Ind. Co., Jhongli, Taiwan, and settled to total suspended solids (TSS) of 6% w/w. All reactors were operated at 4-h cycles, consisting of filling (6 min), aeration, settling and effluent (6 min). The settling time was decreased from 20 min to 5 min in cultivation stage with the remaining being aeration stage. Air was supplied from the bottom of the reactors via diffusers located at the bottom of the reactor. Reactors were supplied with air at a constant flowrate of 8 l/min which is equal to a superficial velocity of 2.65 cm/s. The experiment was performed at 28–30 °C.

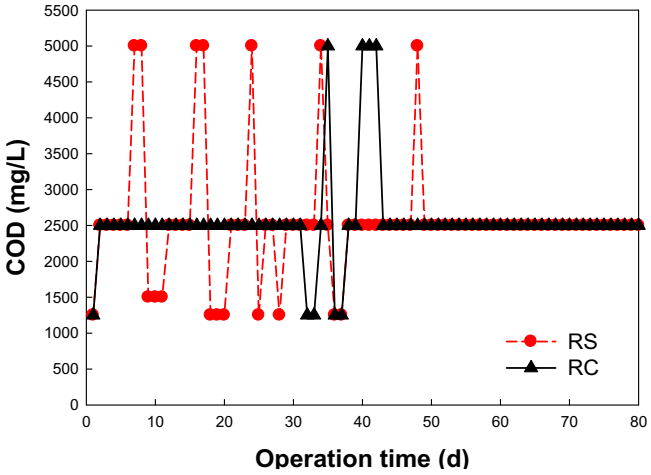

Fig. 1. Alternating feeding loadings in RS and RC during 80 d operation. COD of 1250, 1500, 2500 and 5000 mg/l correspond to OLR of 7.5, 9, 12 and 30 kg/m^3-d, respectively.

All reactors were fed with the same synthetic wastewater consisting of propionate as the sole carbon source and the other main nutrients were as follows (in mg/l): peptone, 400; meat extract, 250; NH_4Cl, 200; KH_2PO_4, 660; $CaCl_2$, 40, $MgSO_4{\cdot}7H_2O$, 25; $FeSO_4{\cdot}5H_2O$, 20; $(NH_4)_2SO_4$, 1330; $NaHCO_3$, 13. RS was operated at alternating COD feed at OLR between 4.4 and 17.4 kg/m^3-h while RC was at constant COD at OLR of 15 kg/m^3-h. The test scenario was shown in Fig. 1.

2.2. Analytical methods

Total suspended solid and volatile suspended solid of aerobic granules were measured using Standard Methods (APHA, 1998). The EPS of granule samples was extracted using ultrasound–formamide–sodium hydroxide method (Adav and Lee, 2008). The protein contents in the EPS were determined using the modified-Lowry method with bovine serum albumin as the standard. The polysaccharides content in the EPS was measured using the Anthrone method with glucose as the standard. The COD of collected samples was measured by COD reagent vials produced by CHEMetrics company, including reacting with an acidic solution of potassium dichromate in the presence of a catalyst (silver) and digested for 2 h at 150 °C, then the oxidizable organic compounds was oxidized by dichromate ion. The pH of samples was measured by WTW pH meter pH-315.

The ALE of granule samples were extracted using method modified from Lin et al. (2013). In brief, 2.0 g of dried biomass was extracted by 100 ml 0.2 M Na_2CO_3 at 80 °C for 210 min. After centrifugation at 15,000 rpm for 30 min, the pellet was discarded. pH of the supernatant was then adjusted to 2 by adding 1.0 M HCl. The precipitate was collected by centrifugation (15,000 rpm, 30 min) and was then dissolved in 1.0 M NaOH. The ALE in the supernatant was precipitated by addition of 99.5% ethanol to a final concentration of 80% (v/v). The precipitate was collected by centrifugation (15,000 rpm, 20 min) and dehydrated via successive passages through 85% and 95% ethanol (2 h), and then lyophilized.

The collected ALE was further fractionated according to Lin et al. (2010). 0.25 g of ALE was dissolved in 9 ml of deionized water and heated at 100 °C for 0.5 h after addition of 1 ml 3.0 M HCl. After cooling, the mixture was centrifuged at 15,000 rpm for 30 min with the supernatant being neutralized with 1.0 M NaOH and then was added with 80 ml of ethanol to form precipitate (MG blocks);