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Aerobic granulation of protein-rich granules from nitrogen-lean wastewaters

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HIGHLIGHTS

- The protein-rich granules were cultivated from nitrogen-lean wastewaters.
- The yielded granules can survive in sequential batch reactor mode.
- The yielded granules can survive in continuous-flow operation for 216 d.
- The nitrate-only feed would not produce stable PN-rich granules.

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ABSTRACT

Proteins (PN)-rich granules are stable in structure in long-term reactor operations. This study proposed to cultivate PN-rich granules with PN/polysaccharides (PS) >20 from nitrogen lean wastewater, with ammonia-nitrogen as sole nitrogen source at chemical oxygen demand (COD)/N of 153.8. The yielded granules can sustain their structural stability in sequencing batch reactor mode for sufficient treatment of wastewaters up to 7000 mg/L COD and with COD/N < 500 and in continuous-flow reactor for successful 216-d treatment of wastewaters up to organic loading rate (OLR) of 39 kg/m^3-d. The produced granules were enriched with *Firmicutes* and β-proteobacteria as dominating strains. More than 58% of the nitrogen fed in the nitrogen-lean wastewater is converted to the PN in the granules. The replacement of ammonia by nitrate as sole nitrogen source led to granules enriched with γ-proteobacteria which are easily deteriorated at low OLR.

1. Introduction

Aerobic granular sludge processes have the advantages over conventional activated sludge processes by their higher sludge settleability, higher biomass retention rates, and greater tolerances to shock loadings or feed toxicity (Adav et al., 2008a; Khan et al., 2013; Zhang et al., 2016). Aerobic granules are a matrix of extracellular polymeric substances (EPS), inorganic salts and other residues with living cells embedded in it (Chen and Lee, 2015; Lee and Chen, 2015). The EPS can have high ion exchange capability and high buffer to environmental threat for the intra-granular cells (Annadurai et al., 2002; Ramesh et al., 2005; Liu and Lee, 2014). The quantity and compositions of EPS (including proteins (PN) and polysaccharides (PS)) of granules correlate with the structural stability of the granules under storage or reactor operation (Lee et al., 2010; Ding et al., 2015).

The PN/PS ratio for activated sludge is generally not far exceeding unity (Tsai et al., 2008; Sheng et al., 2008, 2013); while aerobic granulation commonly increases the PN/PS ratio with enriched extracellular proteins (McSwain et al., 2005; Adav and Lee, 2008). Certain studies noted not-too-high PN/PS ratio (0.8–2.1) for their mature aerobic granules (Li et al., 2011; Kong et al., 2015; Adav et al., 2008b; Long et al., 2016; Liu et al., 2016a). Most recent studies demonstrated high PN/PS ratios for the cultivated aerobic granules. Deng et al. (2016) cultivated aerobic granules from 641 to 1281.9 mg/L acetate wastewaters with 95.6–191.1 mg/L NH_4Cl in sequential air-lift reactors. The PN/PS ratio of the cultivated granules was around 3.2. Wei et al. (2014, 2016) cultivated granules using feed of 600–2000 mg/L chemical oxygen demand (COD) as glucose and 200 mg/L NH_4^+-N. These authors measured the PN/PS ratio of cultivated granules at 5.0–6.4. Sun et al. (2016) cultivated granules using alternating organic loadings shifting between 4 and

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17 kg COD/m^3-d with excess nitrogen supply (mainly from peptone) and noted that the PN/PS of the mature granules ranged 4.0–5.5. Liu et al. (2016b) found that starvation could accelerate aerobic granulation and enhance granule stability for reaching PN/PS > 5.0 from 585 mg/L COD and 55 mg/L NH_4^+-N wastewaters. Aqeel et al. (2016) cultivated granules at PN/PS of 2.8–5.7 using 300 mg/L glucose wastewater with 57.3 mg/L NH_4^+. When filamentous bulking occurred, the ratio was reduced to 1.5. Li et al. (2014) cultivated 0.5 mm aerobic granules from full-scale SBR treating 50,000 m^3/d municipal wastewater. The PN/PS ratio of the granules was about 12.5, a very high ratio noted in literature studies. These authors claimed that the high PN contents of granules are a result of biological response to toxic substances in feed wastewaters.

As mentioned above, the nitrogen-rich (COD/N < 25) wastewaters were adopted for cultivation of protein-enriched granules. This study demonstrated that, conversely, aerobic granulation for high PN/PS granules can occur with nitrogen-lean (COD/N = 153.8) wastewaters. Without sufficient nitrogen supply, the yield coefficient of cells would be significantly reduced, producing low biomass during operation. Additionally, the high PN/PS granules yielded with NH_4^+-N rather than with NO_3^--N were noted be stably existing in 216-d treatment for high-strength wastewaters in a continuous-flow reactor. The results reported herein provide a possible scenario for practice: cultivating aerobic granules off-line using nitrogen-lean medium and then feeding the cultivated granules to reactors for treating nitrogen-rich or nitrogen-lean wastewaters.

2. Materials and methods

2.1. Granule cultivation

Three sequential batch reactors (SBR, R1–R3) of size 180 cm × 5 cm and a working volume of 2.0 L were used to investigate the effects of organic loading rate and nitrogen species to aerobic granulation (Chen, 2010). Seed sludge was the waste activated sludge collected from an industrial wastewater treatment handling 500 m^3/d bakery wastewater of average COD of around 500 mg/L. The SBR were operated at 4-h cycles. At the beginning of each cycle, 1.2 L synthetic wastewater was pumped into the reactors, and 1.2 L effluent was discharged at the end of each cycle. The synthetic influent contained propionate:ethanol at 3.7:1 mol/mol to make COD up to 2850 mg/L. The organic loading rates of the SBR's were adjusted by changing the feed concentrations of propionate and ethanol. The NH_4^+-N/NO_3^--N for R1–R3 were 100/0, 30/70 and 0/100, respectively, at COD/N = 153.8. Discussions in latter sections will reveal that this nitrogen dosage is insufficient to maintain high cell yield in organic degradation.

In parallel tests, the cultivated R1 granules were tested in SBR for treating wastewater with 2300–7000 mg/L COD with NH_4^+-N at COD/N = 2500, 500, 100, or 50 (n1–n4) to reveal the capabilities of the R1 granules for handling these high strength but with very high COD/N wastewaters.

The feed compositions are listed in Supplementary materials.

2.2. Continuous-flow reactor

A continuous-flow reactor of size 0.8 L identical to the one adopted in Chen and Lee (2015) was applied herein. The stable granules cultivated in Section 2.1 were operated in this reactor at 2.2–19 h hydraulic retention time (HRT) and 25 °C. The feed concentrations were the same as those used in cultivation stage, with organic loading rate (OLR) of 6–39 COD kg/m^3-d. The air was pumped into the reactor bottom at 5 L/min. The total testing time was 216 d.

2.3. Microbial community analysis and strain isolation

The bacterial community of aerobic granules was analyzed by PCR-DGGE technology according to Wan et al. (2011). Briefly, the genomic DNA for samples was extracted by protocol of PowerSoil DNA isolation kit (Mobio Inc., USA). The PCR primers 8F and 518R (containing GC clamp) were used to amplify the variable V1-V3 region of bacterial 16S rRNA gene using initial denaturing step at 94 °C for 10 min; then 30 cycles of denaturing at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min 30 s; final extension at 72 °C for 10 min. The PCR products were run on 8% polyacrylamide gels with a linear gradient of 30–60% denaturant at 140 V for 10 h at 60 °C. DNA collected from the target bands was sequenced via BLAST searches of the National Center for Biotechnology Information (NCBI) database.

Some cultivated granules were ground and was diluted with medium up to 10^9 folds. The dilutions were spread onto agar plates containing the cultivation medium for 7-d incubation at 30 °C. The visible colonies appeared were selected and replating to the agar medium for a few times. The isolates from the granules were checked with scanning electron microscopy (SEM) observation and were identified by DNA sequence.

2.4. Other analyses

The collected granules were fixed with 2.5% glutaraldehyde, and then dehydrated by successive passages of 50, 70, 80, 90, 100% ethanol solution followed by critical point drying for SEM observation. The extracellular polymeric substances (EPS) of granules, including quantities of polysaccharides (PS) and proteins (PN) were extracted based on ultrasonic treatment + formamide + NaOH protocol by Adav and Lee (2008). The concentrations of COD, NH_4^+, NO_3^-, NO_2^- in collected samples were tested based on Taiwan EPA Standard Methods, W518.50C, W457.5B, W419.51A, and W418.53C, respectively. The suspended solids (SS) and volatile suspended solids (VSS) concentrations of samples were measured based on NIEA W211.58A and R212.02C, respectively. The aggregation index is defined as (OD(0)-OD(30))/OD(0), where OD(0) is the initial optical density of suspension (600 nm) and that of suspension after 30-min settling. High aggregation index denotes a strong tendency for cell agglomeration for fast settling.

3. Results and discussion

3.1. Cultivated granules

The SS concentrations of R1–R3 were increased with OLR with most VSS/SS ratios exceeding 85% (Fig. 1). The R1 and R2 suspen-

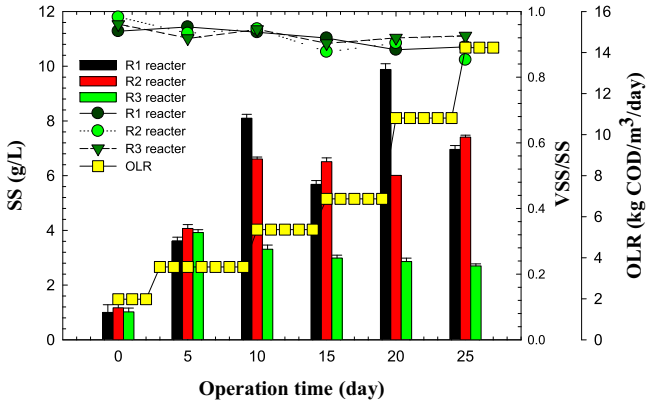

Fig. 1. The SS and VSS/SS ratios of R1–R3 granules during cultivation.