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Short Communication

Microstructural strength deterioration of aerobic granule sludge under organic loading swap

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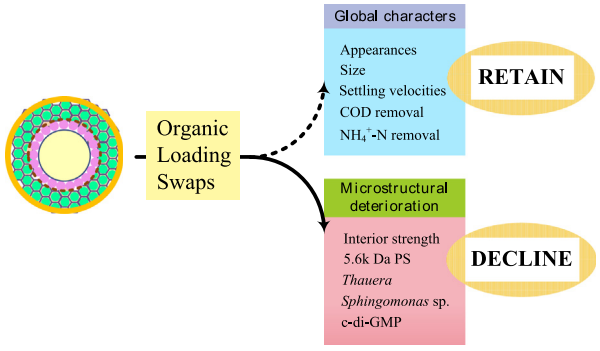
HIGHLIGHTS

- Appearances, size and bioactivities of aerobic granules were monitored.
- Organic loading swaps lead to no changes in gross indicators.
- Microstructural deterioration occurs upon loading swaps.
- Strains diminishment leads to decline in c-di-GMP and PS components.

GRAPHICAL ABSTRACT



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ABSTRACT

This study revealed that the gross indicators commonly adopted for monitoring the performance of aerobic granular sludge processes are not capable of probing the microstructural deterioration of granule interior upon organic loading swaps. These granules subjected to loading swaps retained their global characteristics: appearances, sizes and settling velocities, chemical oxygen demand (COD) and ammonia–nitrogen removal capacities. However, the granule interior strength, as determined by ultrasound method, was largely weakened upon COD switch-off and was not recovered in the subsequent COD resupply stage. In response to COD switch-off, the 5.6 kDa polysaccharides component of granule extracellular polymeric substances (EPS) was diminished. Correspondingly, two bacterial species, *Thauera* and *Sphingomonas* sp., were faded away together with the significant decline in contents of intracellular cyclic dimeric GMP (c-di-GMP). The microstructural integrity of granules was seriously deteriorated upon COD switch-off, which was not detectable by the commonly adopted gross indicators.

1. Introduction

Aerobic granular sludge refers to wastewater treatment processes with self-aggregated cells imbedded in compact three-dimensional matrix of extracellular polymeric substance (EPS) which has fast settling velocity, strong structural strength, and high tolerance to toxic and hazardous shock loads (Adav et al., 2008a; Zhang et al., 2016). The main drawback for the field applications of aerobic granular sludge is its low structural stability in long-term operation (Lee et al., 2010). The deterioration of granule structure would lead to washout of biomass from bioreactors and yield reactor failure (Wan et al., 2015b). However, there is

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commonly noting no sign in reactor operation before the occurrence of aerobic granule deterioration (as shown later).

Changes in compositions and distributions of microbial communities, EPS, surface properties or other characteristics have been used to correlate with the occurrence of aerobic granules deterioration (Wan et al., 2014b). This work demonstrated that the aerobic granules may be first weakened in structure long before the occurrence of global structural deterioration. To demonstrate this artifact, the aerobic granules were cultivated and imposed to swaps of organic loadings. The granule size, microbial community, EPS were monitored over the process. The intracellular signal molecules such as cyclic dimeric GMP (c-di-GMP) (Wan et al., 2013; Whiteley and Lee, 2016) and autoinducer-2 (AI-2) (Xiong and Liu, 2013; Sun et al., 2016) noted to correlate with the aerobic granulation process. The changes in intracellular contents of c-di-GMP and AI-2 were also reported.

2. Materials and methods

2.1. Cultivation of aerobic granules

The aerobic granules were cultivated with seed sludge at suspended solid (SS) of 6000 mg/L collected from a recycling sludge stream in a local wastewater treatment in sequencing batch reactors (SBR) of diameter 6 cm and height 180 cm (giving 2.3 L working volume) at 4 h cycles (Adav et al., 2008b). In each cycle, 1.6 L of synthetic wastewater (0.15 g/L KH_2PO_4; 0.03 g/L $CaCl_2$; 0.025 g/L $MgSO_4{\cdot}7H_2O$; 0.02 g/L $FeSO_4{\cdot}5H_2O$; 4 g/L $NaHCO_3$; 1500 mg/L chemical oxygen demand (COD) at acetate: propionate = 2:1 mol/mol; pH 7.4 ± 0.1) was fed in 3 min. Air was aerated at 5 L/min for 227 min. The suspension was settled, withdrawn at volume exchange ratio of 50%, and idle in 10 min. The NH_4^+-N concentration was 100 mg/L during the first 5 days, and then in the subsequent stage follows NH_4^+-N concentration being increased stepwise at 3-d interval from 100 mg/L to 300 mg/L, to 600 mg/L, and then back to 100 mg/L. This feeding strategy was repeated till mature granules were formed on 42 d.

2.2. Organic loading swap test

The mature aerobic granules were placed in the identical reactor in Section 2.1 and were fed with cultivating medium with 300 mg/L NH_4^+-N. The reactor was operated in 4 h cycles at 50% volume exchange ratio. The COD was suddenly dropped from 1500 mg/L to zero on day 1 and maintained as such for a total of 10 days. On day 11, the COD was reverted back to 1500 mg/L.

2.3. Analytical methods

2.3.1. Microbial community

The similarity of bacteria communities were analyzed using the PCR-DGGE technology (Wan et al., 2015a), and the microbial communities were further analyzed by high-throughput sequencing technology (Wan et al., 2014a). The POCHE Emulsion-PCR involved in high-throughput sequencing was taken single genetic molecule as template. The high-throughput sequencing was accomplished using Iontorrent PGM (Thermo Fisher Scientific. Waltham, MA, USA).

2.3.2. Signal molecules

The aerobic granular samples (initial, 10 d, and 20 d) were firstly lyophilized, and then 0.05 g of lyophilized sample was

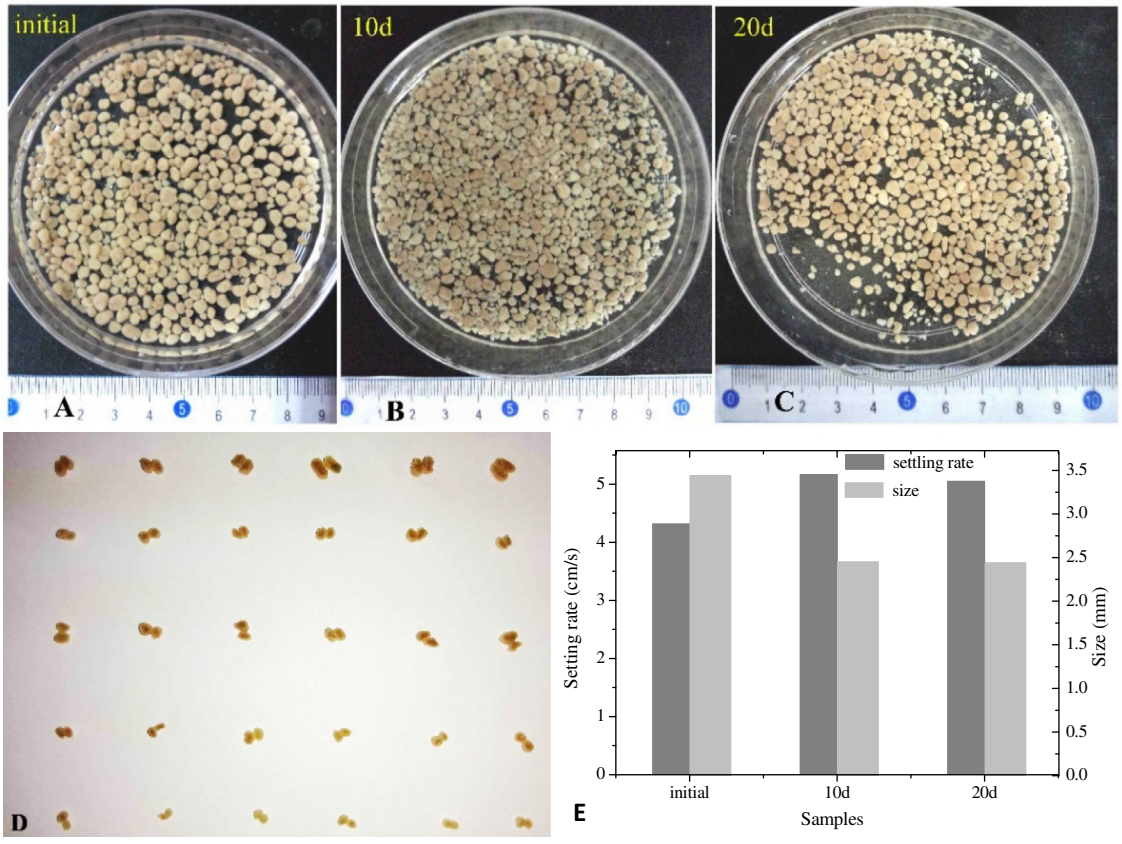

Fig. 1. Morphology and bacteria communities of aerobic granules. (A–C) are appearances of initial, 10-d, and 20-d aerobic granules; (D) is sectional picture of 30 randomly picked initial aerobic granules; (E) is DGGE profile for three randomly picked aerobic granules from initial, 10-d and 20-d granules.