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Short Communication

Saline storage of aerobic granules and subsequent reactivation

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HIGHLIGHTS

- Saline storage was for the first time revealed its promise for granule storage.
- Saline-stored granules were successfully reactivated and operated for 85 d.
- Reactivated granules were operated in SBR and CFR at varying COD.

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ABSTRACT

Loss of structural stability and bioactivity during long-term storage and operation is primary challenge to field applications of aerobic granular processes. This study for the first time stored aerobic granules in 5% w/w NaCl solution at 4 °C for 187 d. The stored granules were then successfully reactivated and used for 85 d in sequencing batch reactors (SBR) and continuous-flow reactors (CFR) at varying levels of chemical oxygen demand (COD). High-throughput sequencing results reveal that *Thauera* sp., *Paracoccus* sp., and *Nitrosomonas* sp. were the predominant in the stored aerobic granules, and *Pseudoxanthomonas* sp. accumulated during the reactivation process. Saline storage, in which cells are in an unculturable state by saline stress, is a promising storage process for aerobic granules.

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1. Introduction

The use of aerobic granules for industrial wastewater treatment is studied (Adav et al., 2008). Cells in aerobic granules are embedded in a compact matrix of extracellular polymeric substances (EPS), and thereby have excellent settling ability and high tolerance to toxic pollutants in wastewaters (Zhu et al., 2013). Loss of the structural stability of aerobic granules during use is a major operational challenge (Lee et al., 2010). One strategy to overcome instability is to replenish deteriorated granules with fresh granules that can be cultivated elsewhere and then used when needed. Extended storage of cultivated granules for subsequent reactivation can take a central role in supply chain of aerobic granules for wastewater treatment (Liu et al., 2005).

Aerobic granules typically lose their bioactivity during storage (Tay et al., 2002). While one study successfully reactivated its granules after long-term storage in idle water (Zhu and Wilderer, 2003), another reported poor structural stability for aerobic granules stored in tap water (Zeng et al., 2007). Adav et al. (2007) and Lv et al. (2013) froze aerobic granules at −20 °C and then successfully reactivated the granules in 1 d. Conversely, Gao et al. (2012) noted that 4 °C was superior to subfreezing temperatures for retaining the bioactivity of stored granules. To suppress anaerobes in the granules' core and thereby protect the EPS matrix from consumption, a phenol solution was used by Adav et al. (2007) for long-term storage of aerobic granules. Wan et al. (2014) noted that partially nitrifying aerobic granules had excellent tolerance to wastewater salinity. The use of saline waters, such as naturally occurring saltwater is both cost effective and of environmentally benign. To the best of our knowledge, using a high concentration of salt in a solution to preserve aerobic granules has not been investigated.

This study stored aerobic granules in 5% w/w NaCl solution at 4 °C for 6 months and then reactivated the stored granules in a sequencing batch reactor (SBR) and continuous-flow reactor (CFR). Granules commonly lose their structural stability mush faster in a CFR than in an SBR (Lee et al., 2010). This study also shows that the stored granules that are then reactivated can operate stably in a CFR for 85 d.

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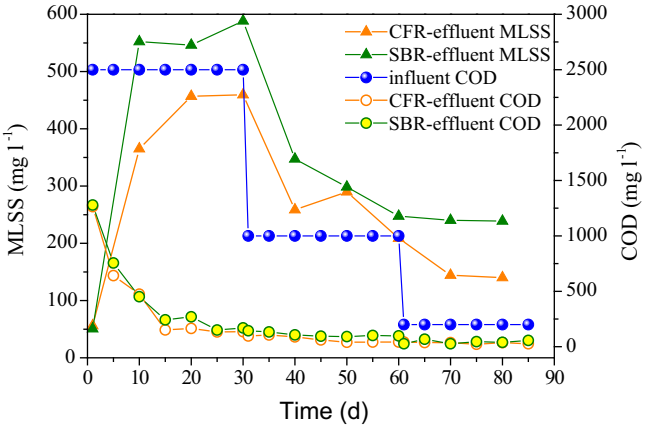

Fig. 1. Time courses of influent and effluent COD and MLSS of reactivation SBR and CFR.

2. Methods

2.1. Cultivation and storage strategies of aerobic granules

The aerobic granules were cultivated in a column SBR (6 cm × 180 cm, the cultivation SBR) with a working volume of 2.3 l. The feed was synthetic wastewater of compositions as follows (per liter): NH_4Cl 0.2 g, KH_2PO_4 0.66 g, K_2HPO_4 1.22 g, $CaCl_2$ 0.03 g, $MgSO_4{\cdot}7H_2O$ 0.025 g, $FeSO_4{\cdot}5H_2O$ 0.02 g, $NaHCO_3$ 0.013 g, peptone 0.4 g, yeast extract 0.25 g, pH 7.2 ± 0.1, chemical oxygen demand (COD) at sodium acetate: sodium propionate = 3:1. The total COD was 1000 mg l^{-1}, 2500 mg l^{-1}, and 5000 mg l^{-1}, corresponding to the loading rates of 6 kg m^{-3} d^{-1}, 15 kg m^{-3} d^{-1}, and 30 kg m^{-3} d^{-1}. The seed was activated sludge of suspended solids (SS) of 6000 mg l^{-1} from recirculating sludge stream of a wastewater treatment plant in Shanghai, China. The aeration rate to the SBR was fixed at 5 l min^{-1}. The SBR were operated at 4 h cycles with 3 min of filling, 227 min of aeration-settling, 5 min of decanting, and 5 min of idling. The settling time was decreased from 30 min to 2 min, judged based on reactor performances, with the aeration time being correspondingly increased. The temperature was 28 ± 1 °C.

The granules were started to form since 20 d, and mature granules were observed on 30 d. The mature granules were then transferred to another column SBR (16 cm × 70 cm) operated in 6 h cycles with 10 min of filling, 290 min of aeration, 45 min of anoxic mixing with internal reflux, 5 min of settling, 8 min of decanting, and 2 min of idling. The compositions of synthetic feed were as follows (per liter): NH_4Cl 0.2 g, KH_2PO_4 0.026 g, $CaCl_2$ 0.01 g, $MgSO_4{\cdot}7H_2O$ 0.05 g, $NaHCO_3$ 0.5 g, peptone 0.02 g, pH 7.2 ± 0.1. The organics were also consisted of acetate and propionate (3:1). The aeration rate to the SBR was fixed at 5 l min^{-1}. The 2SBR was performed for 50 d. Then the aerobic granules stayed in the SBR at 30 s settling were selected. This settling process was repeated for 5 times to screen off flocculated sludge and tiny granules. Finally, the collected granules were stored in 5% NaCl solution at 4 °C for 187 d.

2.2. Reactivation experiments

The stored granules were reactivated and then operated in an SBR (the cultivation SBR) and a continuous-flow reactor (CFR) tested in Wan et al. (2014). In brief, the CFR is a column (6 cm × 105 cm) reactor with a three-phase separated at the top to recover overflow granules. Stored granules were fed to each reactor at dry weight of 1.0 ± 0.1 g. The flow rate of CFR was 9.6 l d^{-1}. The HRT of SBR for each cycle was 4 h. The feed was the same as the cultivation feed of 1 SBR in Section 2.1. The reactors were operation at identical conditions: Phase I (1–30 d) at COD = 2500 mg l^{-1}, Phase II (31–60 d) at COD = 1000 mg l^{-1}, and Phase III (61–85 d) at COD = 200 mg l^{-1}. The step-decline in influent COD is trying to allow the granules to adopt to low strength environment and to be feasible for applications to low-strength wastewaters. The aeration rates for both reactors were kept at 5 l min^{-1}. The temperatures were maintained at 28 ± 1 °C.

2.3. High throughput sequencing

The total genomic DNA (100 μl) was extracted using Mo-Bio kit (MoBio Laboratories, Inc., USA) according to the manufacturer's manual. The 1% agarose gels were used to evaluate DNA validity, and then the Qubit 2.0 kit was adopted for DNA quantification. The extracted DNA was magnified by PCR and was sequenced by the ROCHE Emulsion-PCR technology and by ion torrent PGM (life Technology, American) (Wan et al., 2014).

2.4. Other analysis

The specific oxygen utilization rate (SOUR) was determined using (Tas, 2010) at 20 ± 1 °C. The granular samples were placed on a filter and naturally dried for 5 min. The aerobic granules of 4 ± 0.1 g were taken into a 1-l column reactor. The nutrient solution was firstly aerated. The column was sealed and the oxygen probe was taken to observe the dissolved oxygen (DO) variation. The mixture was sampled at intervals, and centrifuged at 10,000 g for 10 min. The pellets were collected. The DO concentration was measured by a WTW 340i Portable Meters (WTW, Germany). The tests were taken for three times, and the average

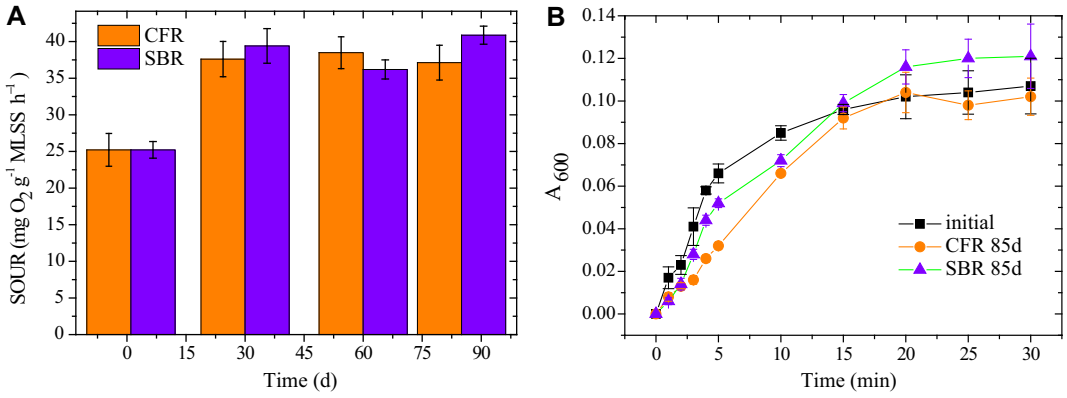

Fig. 2. Tests of bioactivities (left) and strengths (right) of aerobic granules in two reactors.

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