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Performance and microbial population dynamics during stable operation and reactivation after extended idle conditions in an aerobic granular sequencing batch reactor

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HIGHLIGHTS

- Extended idle conditions decayed the aerobic granular system.
- Granlues structure and physical properties could be fully restored.
- EPS, especially PN contributed to storage and reconstruction of aerobic granules.
- Prolonged idle conditions irreversibly shifted microbial populations.

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ABSTRACT

The evolution of removal performance and bacterial population dynamics of an aerobic granular sequencing batch reactor were investigated during stable operation and reactivation after prolonged storage. The system was run for a period of 130 days including the stable condition phase, storage period and the subsequent reactivation process. Excellent removal performance was obtained during the stable operation period, which was decayed by the extended idle conditions. The removal efficiencies for both carbon and nitrogen decayed while phosphorus removal remained unaffected. Both granules structure and physical properties could be fully restored. Microbial populations shifted sharply and the storage perturbations irreversibly altered the microbial communities at different levels. Extracellular polymeric substances (especially protein) and key groups were identified as contributors for storage and re-startup of the aerobic granular system.

1. Introduction

Aerobic granular sludge has been recognized as an efficient and innovative approach for wastewater treatment due to its advantages in compact and strong microbial structure, excellent settling velocity, high biomass retention and the capacity to withstand high loading rates and resistance to toxicities (Adav et al., 2008). The aerobic granules are facilitated by bacterial consortia composed of both heterotrophs and autotrophs capable of oxic, anoxic and anaerobic metabolisms, which often reside within different layers of aerobic granules (He et al., 2016a). Therefore, the loss of microbial activity and removal capacity due to long-term extended idle conditions or storage is of concerns hindering the application of this technology, during which aerobic granules are under anaerobic and starvation conditions (Adav et al., 2007; Gao et al., 2012). However, large variations of flow and concentration of wastewater may occur in industrial and touring plants, when the biological reactors have to operate in an idle mode (Morgenroth et al., 2000; Zhu and Wilderer, 2003). The long-term storage with capacity for maintaining appropriate physicochemical characteristics and reactivation efficiency are significant for the practical application of aerobic granules from the perspective of engineering viewpoint (Wang et al., 2008).

The successful application by seeding anaerobic granules into a USAB reactor for startup shades light on the reactivation of stored aerobic granules (Yuan et al., 2012). Previous conducted on biological reactor have confirmed that both activated sludge and aerobic granules hold the capacity for storage and reactivation (Morgenroth et al., 2000; Wang et al., 2008). Numerous studies have focused on the impact of long-term extended idle conditions on aerobic granules in terms of morphological structure, physical characteristics and microbial activity (Adav et al., 2007; Wang et al., 2008; Zhu and Wilderer, 2003). Massive studies have focused

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on the changes of granular structure, microbial activity and biomass evolution. But few have correlated these attributes with the biological reactor performing simultaneous carbon, nitrogen and phosphorus removal. A fewer researchers have also examined the microbial community dynamics caused by storage, and identified the main contributors for storage and subsequent reactivation processes (Wan et al., 2014a,b), however, they just emphasized the groups for constructing stability of granules and those involved in carbon and nitrogen removal. Up to date, none has explored the microbial communities involved in phosphorus removal suffering long-term starvation.

Although sufficient knowledge about the microbial structure, activity and removal performance in storage and recovery of aerobic granules is available, the reactor performance and microbial population dynamics in response to the extended idle perturbations from the stable operation period, storage period to the recovery period have not been reported. Therefore, the main goal of the present study was to investigate the long-term operation of an aerobic granular system throughout the stable operation, extended idle conditions and the subsequent reaction process in terms of granules structure, physical properties, and microbial communities. The evolution of attributes of granules including settleability, microbial activity, cellular relative hydrophobicity (RH) and extracellular polymeric substances (EPS) secretion was careful investigated and their functions for storage and reactivation processes were identified. Focuses were on the dynamics of bacterial populations and phylogenetic classification of key groups related to simultaneous carbon, nitrogen and phosphorus removal. It is expected that this research could provide some useful information about the practical application of aerobic granular sludge technology.

2. Materials and methods

2.1. Experimental setup

A SBR with a diameter of 100 mm and an effective volume of 3.6 L was operated in the present study as described in He et al. (2016b) (Fig. S1). The effluent withdrawal point was set at the height of 250 mm, giving an exchange ratio of 50% per cycle. Each cycle consisted of 6-h cycle including 2 min of feeding, 120 min of anaerobic phase, 90 min of oxic phase, 144 min of anoxic phase, 2 min of settling time and 2 min of effluent discharge periods. The composition of the synthetic wastewater used was according to the previous research (He et al., 2016a). The reactor was aerated with a fine bubble aerator with a flow rate of 2.5 L/min from the bottom of the reactor. A mechanical stirrer was positioned on the top of the reactor with a speed of 250 rpm.

The aerobic granular SBR was run for a period of 130 days in order to simulate three periods over time defining a stable operation (A, 50 days), an extended idle conditions (B, 58 days), and a reactivation period (C, 22 days). The SBR was operated on an anaerobic/oxic/anoxic (A/O/A) mode as the previous research (He et al., 2016a) during phase A and C, while feeding, aeration, stirring and effluent were stopped during the phase B. The extended idle condition lasted for over 8 weeks, when the water temperature was not controlled (15 ± 2 °C).

2.2. MiSeq pyrosequencing

Three aerobic granular sludge samples were collected at the end of each phase (day 50, day 108 and day 130, referred to as E1, E2 and E3, respectively) from the SBR. After appropriate treatment, the aerobic granules were used for DNA extraction, PCR amplification, and pyrosequencing using the primer sets 338F (5′-ACTCC TACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTC TAAT-3′) (Liu et al., 2015) as the procedures by our previous research (Wang et al., 2015). The Illumina MiSeq platform (PE300, CA, USA) was applied for sequencing of the complete genome of collected samples following the manufacturer's instructions. The statistical and bioinformatics analysis was conducted according to our previous procedures (Wang et al., 2015).

2.3. Analytical methods

The COD, nitrogen (including NH_4^+-N, nitrate (NO_3^--N), nitrite (NO_2^--N)), TP, MLSS, sludge volume index at 5 min (SVI_5) were measured according to the standard methods (APHA, 2005). Total inorganic nitrogen (TIN) was regarded as the sum of NH_4^+-N, NO_3^--N, NO_2^--N (He et al., 2017). The pH and DO were measured using a pHS-25 meter and YSI5000 meter. Aerobic granules after appropriate pretreatment were used for EPS extraction with a modified heat extraction method, protein (PN) content was determined by a modified Lowry method and polysaccharides (PS) content was analyzed using a phenol-sulfuric acid method (Long et al., 2014). EPS was regarded as the sum of PN and PS. The specific oxygen uptake rate (SOUR) were determined using a respiration method (He et al., 2015) at 20 °C. RH was determined by the weight method by Rosenberg (1984).

3. Results and discussion

3.1. Granules structure and characteristics

The mature aerobic granules during phase A (Fig. S2A) remained spherical, compact and dense in structure and brown yellow in color, which was in accordance with the typical characteristics of aerobic granular sludge cultivated with synthetic or real domestic wastewater from our previous researches (He et al., 2015; He et al., 2016a,b). Subject to an extended idle conditions during phase B, the granules (Fig. S2B) turned black though no obvious disintegration in the structure was observed. Zhu and Wilderer (2003) reported that it was caused by the sulfide generated by sulfate-reducing bacteria under long-term anaerobic conditions. This was in accordance with the offensive rotten egg odor during storage and first several days of reactivation. During phase C, the granules recovered gradually to a brown-yellowish color (Fig. S2C and D). The aerobic granules settled as individual particles during the settling period and did not disintegrate over operation. A thin layer of flocs was observed on the top of the settled granules during the first several days during phase C. As a result of the extremely short settling time of 2 min, the flocculent sludge that could not settle instantaneously was washed out from the reactor, thus selecting the remaining granules with fast settling velocity (Long et al., 2014; Wan et al., 2014b) (Table 1). Therefore, the settling velocity and SVI_5 got fully recovered over operation during phase C, along with the concentrations of sludge. The biomass decrease in the reactor was probably resulted by three aspects: washing out of flocs with worse settleability, degradation of intracellular components by endogenous metabolism and solid depletion of cells (Oviedo et al., 2003). It could be seen from Table 1 that sharp decline in MLVSS/MLSS occurred after storage, which recovered rapidly after reactivation. Yuan et al. (2012) revealed that the decrease in MLVSS/MLSS ratio was due to the cell hydrolysis and anaerobic fermentation within aerobic granules, which resulted in the evolution of granules color.

Though no obvious integration and discharge of aerobic granules were observed, it should be noted that the settleability of sludge increased considering the recovery of settling velocity and SVI_2 (Table 1) (He et al., 2016b). The prolonged storage significantly