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Drying and recovery of aerobic granules

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HIGHLIGHTS

- Aerobic granules were dried via seven protocols to bone-dry form.
- Dried granules are recovered in size and microbial activity in reactor operation.
- All dried granules remains structure intact after volume shrinkage of >80%.
- Strains that can survive over the drying and storage periods were identified.

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ABSTRACT

To dehydrate aerobic granules to bone-dry form was proposed as a promising option for long-term storage of aerobic granules. This study cultivated aerobic granules with high proteins/polysaccharide ratio and then dried these granules using seven protocols: drying at 37 °C, 60 °C, 4 °C, under sunlight, in dark, in a flowing air stream or in concentrated acetone solutions. All dried granules experienced volume shrinkage of over 80% without major structural breakdown. After three recovery batches, although with loss of part of the volatile suspended solids, all dried granules were restored most of their original size and organic matter degradation capabilities. The strains that can survive over the drying and storage periods were also identified. Once the granules were dried, they can be stored over long period of time, with minimal impact yielded by the applied drying protocols.

1. Introduction

Aerobic granules are a relatively novel wastewater treatment technology proposed in 1997 (Morgenroth et al., 1997). The aerobic granules are aggregates of living cells embedded in matrix of extracellular polymeric substances (EPS), inorganic salts and other impurities for providing them capability of handling high-strength or toxic wastewaters (Adav and Lee, 2008; Zhang et al., 2016). The structural stability of aerobic granules is easily lost in long-term operations or storage (Lee et al., 2010), which limits the applicability of aerobic granular process in full-scale applications (Ni et al., 2009; Liu et al., 2010; Lin et al., 2010).

Successful granule storage can temporarily cease intra-granular biological activities without major structural deterioration or cell damage in the stored environment (Adav et al., 2009; Yuan et al., 2012). Also, the biological activities of the stored granules should be recoverable in the subsequent recovery stage (Tay et al., 2002; Zhu and Wilderer, 2003; Wang et al., 2008; Gao et al., 2012). Since microbial activities will cease under bone-dry condition, Lee et al. (2010) proposed an innovative idea to store granules in dry form. Lv et al. (2013) latter demonstrated that their granules dehydrated by acetone solutions could be stored and then fully recovered with minimal loss of structural integrity and microbial activities in reactor operation. These findings are welcome since the dry granules are much lighter in weight and easier to handle than those in conventional storage solutions. However, acetone adopted by Lv et al. (2013) is not an environmentally friendly solvent that needs recovery after its use. The use of more easily implemented protocols, such as simple air drying or sun drying, for granule drying has not been justified in its performance for granule storage.

This study is a continuation work for Lee et al. (2010) and Lv et al. (2013) compared the granule characteristics after being dried by seven protocols and then tested their appearances and biological activity for wastewater treatment after recovery.

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2. Materials and methods

2.1. Cultivation of aerobic granules

Aerobic granules were cultivated in a column-type sequencing batch reactor (SBR) of 5 cm diameter and 100 cm height, yielding 1.55 L working volume. The seed was from the reflux stream of waste sludge in a wastewater treatment plant at Zhongli Industrial Park, Taiwan. The cultivation medium was composed of 3.7:1 mol/mol propionate:ethanol mix for 2850 mg/L chemical oxygen demand (COD), 40 g/L KH_2PO_4, 20 g/L K_2HPO_4, 1.2 g/L $CaCl_2$, 0.75 g/L $MgSO_4{\cdot}7H_2O$, 0.6 g/L $FeSO_4{\cdot}5H_2O$, 0.4 g/L $NaHCO_3$, and 200 mg/LNH_4Cl. The reactor was operated at 6 cycles per day while each cycle was composed of 5 min feeding, 227 min aeration, 1 min settling, and 7 min drainage. The reactor was operated at exchange rate of 0.75 and hydraulic retention time of 6.6 h. The pH and temperature of reactor suspension were maintained at 7.1 and 25 °C, respectively. Air was pumped into the reactor bottom at 5 L/min. Mature granules could be harvested after 40-d cultivation.

2.2. Drying and recovery experiments

The mature aerobic granules were washed with distilled water and were separated equally into seven parts with each part of granules being dehydrated by one of the following protocol and was then stored at room temperature for 22 d before recovery tests. The seven drying protocols are as follows:

(1) The mature granules after briefly removing surface moisture by tissue paper were placed at 60 °C oven for one hr.
(2) The mature granules after briefly removing surface moisture by tissue paper were placed at 37 °C thermostat for six hr.
(3) The mature granules after briefly removing surface moisture by tissue paper were placed in 5 °C freezer for 25 h.
(4) The mature granules after briefly removing surface moisture by tissue paper were placed under direct sunlight for 6 h (6th, Nov., 2009, 13:30–19:30 at 25°01′05.1″N 121°32′19.0″E).
(5) The mature granules after briefly removing surface moisture by tissue paper were placed in a dark room at 25 °C for 25 h.
(6) The mature granules after briefly removing surface moisture by tissue paper were placed in an air stream of velocity of about 10 m/s at 25 °C for 10 h.
(7) The mature granules after briefly removing surface moisture by tissue paper were placed sequentially in 50% w/w, 80% w/w and 100% w/w acetone, each for 2 h.

Six original and 22-d stored granules dried under protocol #1–#7 were individually placed into 125-mL vials containing 50-mL cultivation medium (as in Section 2.1) for recovery tests under 25 °C and 250 rpm shaking up to 48 h. This recovery tests were repeated twice. The tests with original granules and those with only medium (with no granules) were used as controls.

2.3. Microbial community analysis and strain isolation

The bacterial community of aerobic granules was analyzed by polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) technology according to Chen (2010). The genomic DNA for samples was extracted by protocol of UltraClean Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The PCR primers 341f-GC and 534r were used to amplify the variable V6–V8 regions of bacterial 16S rRNA gene using initial denaturing step at 95 °C for 6 min; then 25 cycles of denaturing at 95 °C for 1 min, 52 °C for 1 min, 72 °C for 1 min 30 s; final extension at 72 °C for 10 min. The PCR products were purified using Gene-Spin-V3 1-4-3 DNA Extraction Kit (Protech Technology, Taipei, Taiwan) and were run on 8% polyacrylamide gels with DCode Universal Mutation Detection System (BIO-RAD) at linear gradient of 40–65% denaturant at 70 V for 16.5 h at 60 °C. DNA collected was sequenced via BLAST searches of the National Center for Biotechnology Information (NCBI) database.

2.4. Other analysis

The extracellular polymeric substances (EPS) of granules, including quantities of polysaccharides (PS) and proteins (PN), were measured based on methods by Adav and Lee (2008). The concentrations of COD, in collected samples were tested based on Taiwan EPA Standard Methods, W518.50C. The suspended solids (SS) and volatile suspended solids (VSS) concentrations of samples were measured based on NIEA W211.58A and R212.02C, respectively.

3. Results and discussion

3.1. Appearances of tested granules

The appearances of original, acetone-dried and recovered granules were shown in Fig. S1. The appearances of granules dried under the rest six protocols were shown in Fig. S2. The original granules were yellow-brown in color and 5.1 ± 0.6 mm in diameter; after drying, the granules became dark brown in color and 2.5 ± 0.3 mm in diameter (Fig. 1). Hence, the volume shrinkage was exceeding 80%.

The aerobic granules can be recovered to almost their original size (4.8 ± 0.5 mm) and to dark yellow in color. The free settling velocity of the original aerobic granules was 159 ± 16 m/h. In recovery stage, only 10-min hydration (“10 min” in Fig. 1) could increase the granule size from about 2.5 mm to more than 3 mm. After 24-h recovery the granule size (“first” in Fig. 1) were back to about 5 mm of all granules after three repeated drying-recovery cycles, with settling velocities being 107–118 m/h, about 29% lower than the original granules. Repeated recoveries led to similar granule sizes (“second” and “third”) and similar free settling velocities. These observations revealed that the tested gran-

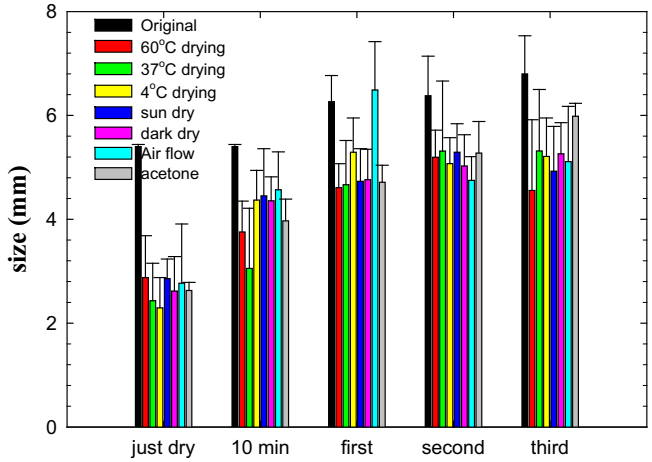

Fig. 1. Sizes of tested aerobic granules. Just dry: the granules just after drying; 10 min: the granules just after 10 min recovery for the 1st drying-recovery cycle; first: the granules just completed the 1st drying-storage-24-h recovery stage; second: the granules just completed the 2nd drying-storage-24 h recovery stage; third: the granules just completed the 3rd drying-storage-24 h recovery stage.