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Physical and hydrodynamic properties of aerobic granules produced in sequencing batch reactors

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ABSTRACT

The structural and hydrodynamic properties of aerobic granules, such as their morphology, fractal dimension, porosity, size distribution, settling velocity, permeability, and shear strength, were characterized. Microbial granules were formed in two sequencing batch reactors (SBRs) that are used to treat glucose-based synthetic wastewater. The first SBR (R1) had a low pH of about 3.0, due to low influent alkalinity (28.7 mg $CaCO_3$/L), and produced fungi-dominated granules. The second SBR (R2) had a pH of around 8.1, due to high influent alkalinity (301 mg $CaCO_3$/L), and produced bacteria-dominated granules. The fungal granules were larger and weaker, with a loosely packed fluffy structure, whereas the bacterial granules were smaller and stronger and had a compact structure. The granules from both R1 and R2 were fractal aggregates, and they had fractal dimensions of 2.23 and 2.42, respectively. The settling velocities in water for the granules from R1 ranged from 0.38 to 2.67 cm/s. Those from R2 ranged from 0.42 to 3.21 cm/s. This is in good agreement with the settling velocities predicted by Stokes' law for porous but impermeable spheres. The fungal granules were almost completely impermeable, with an average fluid collection efficiency of 0.006, whereas the bacterial granules were slightly permeable, with an average fluid collection efficiency of 0.052. The results demonstrate that biomass enrichment in bioreactors can be achieved by the generation of dense and fast-settling sludge granules. By controlling the feeding condition, different types of aerobic granules can be produced with different structural features and hydrodynamic properties.

1. Introduction

Aerobic sludge granulation is a novel biotechnology that has been developed in recent years for biological wastewater treatment [1–4]. Compared to the conventional activated sludge process, granular bioreactors offer several advantages, such as a denser and stronger microbial aggregate structure, excellent sludge settleability and ensured solid–effluent separation, a higher biomass concentration, and the ability to withstand shock loadings. It has been reported that the concentration of granular sludge can reach 12 g/L in aerobic sequencing batch reactors (SBRs) [5,6]. With a concentrated biomass, granular SBRs can have a treatment loading as high as 8 kg/m^3 d for the removal of chemical oxygen demand (COD) [5]. Aerobic sludge granulation is a new microbial immobilization technology that has the potential to advance fundamentally the practice of biological wastewater treatment [1,3,4,7]. Biomass granules have unique structural features that differ greatly from those of conventional sludge flocs. Therefore, a detailed characterization of the physical and hydrodynamic properties of aerobic granules is needed.

The aggregates formed by microorganisms have a porous and fractal structure [8–12]. The fractal dimension provides a valuable indication of the formation mechanisms and the structural features of bio-aggregates. A lower fractal dimension value usually suggests a looser and more porous aggregate structure with better mass transfer characteristics, and a higher fractal dimension suggests a denser and stronger structure formed in a highly sheared environment or by microbial growth. Diatom aggregates in marine waters have been found to be highly porous, with a fractal dimension of around 1.5 [13], whereas microbial flocs in activated sludge collected from wastewater treatment plants have a fractal dimension of about 2.25 [10,14]. A somewhat lower fractal dimension of 2.09 has been reported for bacterial flocs produced in a laboratory SBR [10]. Anaerobic granules formed in biohydrogen production are reported to have a fractal dimension that increased from 2.11 to 2.48 with an increasing substrate load [11], and the aggregates of yeast produced in rotating test tubes were found to have a higher fractal dimension of 2.66 [13]. Biofilms formed by attached bacterial growth may have an even higher fractal dimension of up to 2.8 [15]. However, the fractal features of aerobic sludge granules have yet to be characterized.

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The porous structure of fractal aggregates allows the development of an interior flow [10,14,16,17]. The highly permeable inorganic aggregates of latex microspheres settle more than four times faster than predicted by Stokes' law [17]. In contrast, biological aggregates, such as activated sludge flocs and biohydrogen-producing granules, have been found to be less permeable, and the advective flow through these aggregates is not sufficiently large to affect their settling behavior [11,18]. However, even this limited internal permeation can still enhance the mass transport rate through microbial flocs and granules [11,18,19]. The hydrodynamic properties of aerobic granules, including their permeability and settling velocity–size relationship, remain to be determined.

The laboratory study reported herein was carried out to investigate the physical and hydrodynamic characteristics of aerobic sludge granules formed in biological wastewater treatment systems. Two bioreactors were operated with glucose-based wastewater influent. Due to a difference in feeding alkalinity between the two reactors, bacteria-dominated granules were produced in one reactor, and fungi-dominated granules were produced in the other. Individual granules from the two types of granular sludge were measured for size, mass, porosity, and settling velocity, based on which their fractal dimension, permeability, and interior flow were determined. In addition, the shear strength of the two types of granules was also measured.

2. Materials and methods

2.1. Bioreactors and sludge granulation

Microbial granules were cultivated in two identical sequencing batch reactors. Each SBR had a working volume of 2.4 L and dimensions of 80 cm in height and 6 cm in diameter. The two reactors were operated in a sequential mode for a 4 h cycle that consisted of 4 min of feeding, 230 min of aeration at an airflow rate of 4.0 L/min, 2 min of settling, and 4 min of effluent withdrawal from the middle ports of the columns. Activated sludge from a local sewage treatment plant – the Stanley Sewage Treatment Works in Hong Kong – was used as the seed sludge for the inoculation of the reactors.

A synthetic substrate that consisted of glucose, NH_4Cl, and other nutrients, in accordance with the chemical composition given by Tay et al. [20], was used as the feeding influent. The influent had a COD concentration of 500 mg/L and a COD:N:P ratio of 100:5:1. The feeding influents for the two SBRs were the same except for their alkalinity. The influent in the first SBR (R1) contained no $NaHCO_3$ and had a low alkalinity of 28.7 mg $CaCO_3$/L. In the second SBR (R2), 440 mg/L of $NaHCO_3$ was added to the influent to increase the alkalinity to 301 mg $CaCO_3$/L. Both reactors were operated at room temperature, and the water temperature was 20–22 °C.

2.2. Settling experiments and characterization of the granules

2.2.1. Settling experiments

Biomass granulation was well achieved in both bioreactors. Two months after complete granulation, suspensions of mature granules were sampled from the reactors for granule characterization. Following procedures detailed previously [10,11,17,21], settling experiments were conducted on individual granules in a settling column, using an acrylic settling column of 90 cm in height and 8.1 cm in diameter with a corn bottom and a valve. This column was filled with tap water that had a density of 0.997 g/cm^3 at 22 °C. During the test, a granule was placed gently into the column from the top, and the time that it took for the granule to settle through the middle 70 cm distance in the column was recorded to calculate its terminal settling velocity in water. The granule was then released and retrieved from the bottom for subsequent analysis.

Before the settling test, each granule was placed in a Petri dish (9.5 cm) to measure its size. Each granule was photographed using a Digital Single Lens Reflex (DSLR) camera (D70s, Nikon, Japan), and the photos were then processed with a computer-based image analysis system (Scion Image, Frederick, MD). Granule size d was calculated in terms of equivalent diameter by $d = (4A/\pi)^{1/2}$, where A is the projected area of the granule [10,11]. After the settling test, the dry mass of the recovered granule was measured. The granule was dried at 105 °C for 1.5 h on a pre-weighted polycarbonate membrane filter (0.4 μm), and its dry mass, W_d, was measured using an electronic microbalance (AEM-5200, Shimadzu, Japan). More than 70 individual granules from each SBR were tested and analyzed.

2.2.2. Shear strength of the granules

It is difficult to measure directly the strength of an individual granule. The integrity coefficient suggested by Ghangrekar et al. [22] has been used as an index for the strength of microbial aggregates. The strength of granular sludge, therefore, can be defined as its capability to resist the disintegration that is caused by elevated shear stress [6,22,23]. To determine the shear strength, a 5 ml suspension of sludge granules was withdrawn from the bioreactor and diluted with tap water to 30 ml in a 50 ml polypropylene conical tube (BD, Faclon). The sludge mixture was sheared by a vortex mixer (Maxi Mix II, Thermolyne) for 2 min, and after settlement for 1 min, the supernatant was decanted and its suspended solids (SS) were measured. The remaining granular sludge of around 5 ml was again diluted to 30 ml, and the suspension was sheared by the vortex mixer for another 3 min. After 1 min of settlement, the SS in the supernatant was determined, and the amount of settled sludge was measured. The strength of the granular sludge can be quantified by the integrity coefficient (IC), that is,

$$\mathrm{IC}_t = \left(1 - \frac{\mathrm{SS}_s}{\mathrm{SS}_0}\right) \times 100\% \;, \tag{1}$$

where SS_0 is the total amount of granular sludge and SS_t is the amount of the sludge solids in the supernatant after t minutes of the vortex shear test. Although the integrity coefficient does not give the exact strength of a granule, it provides an overall and practical indication of the shear strength of the sludge particles in a dynamic fluid condition.

2.2.3. Size distribution and extracellular polymeric substances (EPS) of the granules

The particle size distribution of the granules in each reactor was determined using the DSLR camera and the image analysis system. The granule samples were collected from the sludge suspension and placed in a glass Petri dish (Fig. 1). The projected photo images of the granules were then analyzed using image software for particle sizing and counting, as in procedures detailed elsewhere [12,24]. Ten samples for each reactor, and at least 50 views of each sample in the dish, were analyzed to obtain the size distribution and the corresponding mean size of the granules in each reactor. In addition, the granule microstructure was observed with a scanning electron microscope (SEM) (Leica Stereoscan 360, Leica Instruments, Cambridge, UK).

EPS were extracted from the sludge by heat extraction at 60 °C for 30 min [25]. The total organic carbon (TOC) of the EPS extraction was then measured using a TOC analyzer (TOC-5000A, Shimadzu, Japan).