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Formation and characterisation of fungal and bacterial granules under different feeding alkalinity and pH conditions

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Abstract

Laboratory experiments were carried out using two identical bioreactors to investigate the effect of feeding alkalinity and pH on the formation of aerobic sludge granules in wastewater treatment. Both bioreactors, of 2.4 L each in working volume and seeded with activated sludge, were operated as sequencing batch reactors (SBR) and fed at the same rate with a glucose-based synthetic wastewater. The first SBR, with a low alkalinity of 28.7 mg $CaCO_3$/L in the influent, had a pH of about 3.0 in the reactor and achieved rapid formation of fungi-dominating granules in 1 week. The second SBR, with a high alkalinity of 301 mg $CaCO_3$/L from the addition of 440 mg $NaHCO_3$/L to the influent, maintained a reactor pH of around 8.1 and had a slower formation of bacteria-dominating granules, taking about 4 weeks. After granulation, both reactors performed well in organic degradation and sludge–liquid separation. However, according to examinations carried out using scanning electronic microscopy (SEM) and confocal laser scanning microscopy (CLSM), the mature fungal granules with a mean size of 7.0 mm had a loosely packed fluffy structure. Both fungi and extracellular polymeric substances (EPS) were distributed uniformly throughout the granules. The bacterial granules were smaller, with a mean size of 4.8 mm and a compact structure. EPS were distributed throughout and bacteria were mainly situated in the outer layer of the mature granules. Granulation of fungal sludge could be completed more rapidly than bacterial granulation. However, fungal granules were apparently weaker in structure and subject to more breakage and erosion than bacterial granules in aeration turbulence. The results suggest that by controlling the feeding alkalinity and reactor pH, a strategy of species selection can be developed for aerobic sludge granulation at different rates with different microbial communities and structural features.

Keywords: Aerobic granules; Biological wastewater treatment; Extracellular polymeric substances (EPS); Fungi; pH adjustment; Sequencing batch reactor (SBR)

1. Introduction

Aerobic sludge granulation is a new technology in biological wastewater treatment [1–5]. Compared to the biomass flocs used in the conventional activated sludge process, aerobic granular sludge features a number of advantages, such as a denser and stronger microbial structure, a better settling ability, more effective sludge–effluent separation, greater biomass retention and enrichment, and a much improved capability to withstand shock loadings [1,2,6,7]. An aerobic granular sludge reactor is also much easier and faster to start-up than an anaerobic granular sludge system [8,9]. From an engineering and economic point of view, aerobic sludge granulation is a promising process that has the potential to lead the next generation of biological wastewater treatment technologies.

Despite the advantages and potential of the aerobic sludge granulation process, the mechanisms of aerobic granulation are not well understood. There are a number of factors, such as the types of organic substrates, the loading rate, aeration-derived fluid shear intensity and hydraulic washing rate, that have been shown to have important influences on sludge granulation [8,10,11]. Operational adjustment is expected to manipulate a more favourable environment for the selection and growth of granule-forming species in a bioreactor. For example, a high organic loading, together with sufficient level of sludge overflow, is commonly adopted for aerobic granulation [11,12], resulting in a low-pH value in sludge suspension. pH is an important environmental factor in microbial growth. However, the effect of pH on species selection and aerobic

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granulation has not been specifically addressed. It is suggested that fungi, which are pH sensitive, have been implicated in the initial stage of granule formation [1,12,13]. Compared with bacteria, fungi can alter the pH around cells and hence create their own low-pH acidic environments [14]. Under certain conditions, fungi may become dominant in aerobic granular sludge reactors [12,13]. Nonetheless, the feeding and environmental conditions for the growth or control of fungi in sludge granulation remain to be determined. The differences between fungal granules and bacterial granules in terms of formation mechanisms, structural features and treatment performance also need to be investigated.

Most fungi prefer a low-pH medium, which is nevertheless unfavourable to the growth of most bacteria. On the other hand, fungi are particularly sensitive to HCO_3^- in the aquatic environment in which they grow [14]. A high alkalinity in HCO_3^- can prevent a drop in pH in a solution and inhibit fungal growth. These factors apparently imply that, by controlling the alkalinity in the influent, a strategy of species selection can be developed for the granulation of aerobic sludge with different microbial communities, morphological evolutions and structural properties. The main objectives of this study are therefore to determine the effect of alkalinity in the feeding substrates on the pH levels in different bioreactors, to investigate pH-based species selection in relation to the respective granulations of fungi and bacteria, as well as to characterise the different structural features and treatment performance between fungi-dominating and bacteria-dominating granules.

2. Materials and methods

2.1. Experimental set-up and the SBR operation

Two identical columns of 80 cm in height and 6 cm in diameter with working volumes of 2.4 L each were used as activated sludge bioreactors for the aerobic granulation study. Both reactors were operated as sequencing batch reactors (SBR) and were supplied during the aeration phase with an airflow rate of 4.0 L/min, equivalent to a superficial upflow air velocity of 2.4 cm/s. The two reactors were operated in a sequential mode for a 4-h cycle: 4 min of feeding, 230 min of aeration, 2 min of settling and 4 min of effluent withdrawal from the middle ports of the columns. Activated sludge from a full-scale sewage treatment plant (Stanley Sewage Treatment Works, Hong Kong) was used as the seed sludge for inoculation of the reactors.

Synthetic wastewater was used as the feeding influent, which consisted of glucose as the sole carbon source, NH_4Cl and other necessary nutrients in accordance with the chemical composition given by Tay et al. [3]. The chemical oxygen demand (COD) concentration in the influent was 1000 mg/L, and the COD:N:P ratio was kept at 100:5:1. The only variation between the two reactors was the alkalinity level in the influent. For the first SBR, R1, there was no addition of $NaHCO_3$ to the feeding solution, which had a low alkalinity of 28.7 mg $CaCO_3$/L. For the second SBR, R2, 440 mg/L of $NaHCO_3$ was added to the feeding solution, increasing the influent alkalinity to 301 mg $CaCO_3$/L. The reactors were operated at room temperature, and the water temperature was 20–22 °C.

2.2. Analytical methods

The sludge concentration in terms of the mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and effluent suspended solids (ESS) was measured in accordance with the Standard Methods [15]. The organic content in the effluent was measured for COD concentration, following the Standard Methods, and for total organic carbon (TOC) using a TOC analyser (TOC-5000A, Shimadzu, Japan). The extracellular polymeric substances (EPS) of the sludge were extracted using a heating method at 60 °C for 30 min, and the extract was further analysed for its TOC and the contents of proteins, polysaccharides and humic-like substances in accordance with the methods used by Li and Yang [9]. The evolution of aerobic granules in the reactors was observed regularly under an optical microscope (BX60, Olympus) equipped with a digital camera (Infinity 3, Lumenera Scientific, Ottawa, Canada). Photographs of aerobic granules were also taken with a DSLR camera (Nikon D70s, Nikon, Japan) for size estimation. The microstructure of mature granules was observed with a scanning electron microscope (SEM) (Leica Stereoscan 360, Leica Instruments, Cambridge, UK), following the sample treatment procedure detailed previously [16,17].

2.3. Fluorescence staining and CLSM examination

Upon the completion of sludge granulation after 70 days, the 3D structure of the mature granules, particularly the distribution of microbial cells and EPS within the granules, was examined using confocal laser scanning microscopy (CLSM) (LSM 5 Pascal, Zeiss, Jena, Germany). For fluorescent staining of both cells and EPS, two probes were applied collectively: SYTO9 (25 μM, Molecular Probe, Eugene, OR) to target all microbes, and ConA-TRITC lectin (250 mg/L, Sigma) to target the polysaccharides with D-glucose or D-mannose [7,18]. When excited by a laser at proper wavelengths, the SYTO9 and ConA-TRITC probes emit green light and red light, respectively. The granules were placed and stained in slide wells and were incubated in a moisture chamber (a 50 mL conical centrifuge tube) in dark conditions at room temperature for 20 min. After incubation, the samples were carefully rinsed with filtered phosphate buffered saline (PBS) three times to remove any unbound probes. The stained samples were visualized on two channels with corresponding excitations and emissions for SYTO9 (488 nm, BP 515-530) and ConA-TRITC (543 nm, LP560). A stack scanning was performed on whole granules to render the distribution of cells and polysaccharides at each depth.

In addition to the observation of intact granules, the granules were also cryosectioned for examination of their interior details. The granules were rinsed with PBS and embedded in Tissue-Tek OCT compound (Miles, Elkhart, IN) and frozen overnight at −40 °C. The frozen granules were sectioned to a thickness of 50 μm using a rotary cryomicrotome (CM 1510-Cryostat, Leica, Germany). The section specimens were collected on the wells of glass slides coated with 0.01% poly-L-lysine solution. The slides were immersed in PBS for 10 min to remove the OCT compound and then air-dried. Following the procedures described above, the granule sections were stained and examined with the CLSM.

3. Results and discussion

3.1. Formation of aerobic granules

Granulation of the aerobic sludge was satisfactorily achieved in both reactors R1 and R2 under the same operational conditions but with different feeding alkalinities. Granules began to form in R1 after 7 days and became dominant and stable after 30 days. In R2, granules began to develop after 20 days and became dominant and stable after 45 days. The mature granules in R1 and R2 after 70 days had mean sizes of 7.0 and 4.8 mm, respectively. The granular sludge was completely different from the seed of activated sludge, which had a loose and irregular floc structure. The aerobic granules cultivated in R1 and R2 were round in shape with a clear contour boundary (Fig. 1). With the achievement of aerobic granulation, sludge settleability was greatly improved.

The respective morphologies of the aerobic granules formed in R1 and R2 were rather different (Fig. 1). The granules in R1 were fluffy and relatively loose in structure, while the microbes in R1 appeared to be fungi rather than bacteria. In contrast, the