Size-dependent calcium carbonate precipitation induced microbiologically in aerobic granules

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HIGHLIGHTS

- Calcium carbonate precipitated in aerobic granules.
- Calcium carbonate precipitation was significantly dependent on granule size.
- Aragonite is the only polymorph of carbonate in granules.
- Needle shaped aragonite was embedded in EPS matrix of granule.
- No any specific type of bacteria was responsible for aragonite precipitation.

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ABSTRACT

Calcium and ash accumulation in aerobic granules treating organic wastewater was investigated systematically in this study. It was found that ash content of granular sludge reached 43% at stable state of reactor operation with calcium carbonate precipitation as the main cause. Since granular sludge in the reactor at the steady state was the mixture of granules with different sizes, granules in the reactor on operation days 90, 120 and 150, respectively, were sorted into 10 categories by size to study the inorganic substances. It was found that calcium was selectively enriched in granules and the precipitation of calcium carbonate was just dependent on granule size instead of operation duration at the steady state. More calcium carbonate was precipitated in bigger aerobic granules, but both calcium and ash contents reached stable values when granule size was bigger than 700 μm. Analysis by X-ray diffractometer disclosed that aragonite was the only polymorph of calcium carbonate detected in aerobic granules with size bigger than 300 μm. Scanning electron microscope revealed clearly for the first time that bundles of needle shaped aragonite was embedded in extracellular polymeric substances of aerobic granules. A mechanism on calcium carbonate precipitation was proposed to deepen the understanding of biomineralization.

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1. Introduction

Aerobic granule is a kind of unique self-immobilized microbial aggregates without carrier material, which is distinctly different from activated sludge and conventional biofilm. Because of good biomass settleability, high biomass retention in bioreactor, and ability to sustain high organic loading rate, aerobic granulation as a novel technology for biological wastewater treatment has attracted considerable attention recently as a hot research topic.

Metal ions such as Ca^{2+}, Mg^{2+}, and Al^{3+} were believed to play an important role for granulation and they have been purposely dosed to stimulate and enhance the formation of aerobic granules,

anaerobic granules, and biofilms [1,3]. Basically there is no doubt that Ca^{2+}, Mg^{2+}, or Al^{3+} augmentation is beneficial to microbial aggregation. The mechanism of this metal ion augmentation is not fully understood, but it has been accepted that nucleus formation of inorganic precipitates could be the main reason as metal precipitates were observed in the enhanced granular sludge. In addition, it has been evidenced that inorganic precipitates in granular sludge could improve the structure stability and strength of aerobic granules [4–6]. Meanwhile, however, a very high ash content in aerobic granules up to 50–84% [7,12] was reported at some circumstances, in which calcium, magnesium, or iron precipitation was observed [14–16]. Although these metal precipitates in granules are favorable in terms of granule structure and stability, the negative impact was also reported. For example, bioactivity of granule sludge was reduced by Ca accumulation in granules [7], which could result

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in the incompetence with suspended sludge for grabbing substrate and the instability of granular sludge reactor operation [8]. In addition, the accumulation of inorganic precipitates and excessive high ash content in sludge mean that effective biomass concentration is low. This might pose challenge to meet the reactor design criteria from the aspect of effective biomass concentration. At the same time, it also might require a higher aeration rate for fluidization of granules or a complete mixing of granular sludge. Given both positive and negative effects that inorganic precipitates on aerobic granular sludge, it is very necessary to investigate the mechanism of inorganic precipitation and develop strategies to employ the advantages but prevent the disadvantages of precipitates in granules.

So far, there are some studies on inorganic precipitates in aerobic granules, but they mainly focused on the augmentation of aerobic granulation [1,3], identification of main inorganic precipitates in granules [16] and the relationship between inorganic precipitates with granule structure [4–6]. The mechanism of inorganic precipitation in granules and how granule size affects inorganic precipitation especially at steady state are still not clear. Therefore, this paper aims to identify metal precipitation in aerobic granules in a sequencing batch reactor without an excessive Ca^{2+} and Mg^{2+} addition in the wastewater and investigate the main factors leading to metal precipitation in aerobic granules at steady state. A relationship between granule size and calcium precipitation was established to explore the mechanism of carbonate precipitation.

2. Materials and methods

2.1. Experimental setup

An internal loop airlift reactor with a working volume of 4.75 L was used to cultivated aerobic granules in this study. The internal diameter of the reactor column was 8 cm with a ratio of working height to the internal diameter of column of 12. The draft tubes had an internal diameter of 5 cm. The reactor was operated sequentially with a cycle time of 4 h, which included 5 min of influent filling, 215–227 min of aeration, 3–15 min of settling and 5 min of effluent discharging from the middle port of the reactor with a volumetric exchange ratio of 50%. This operation resulted in a 8 h hydraulic retention time (HRT). Fine air bubbles for aeration were supplied through a ceramic dispenser at the reactor bottom with an airflow rate of 300 L/h. A time controller was installed to achieve the reactor automatic operation. The reactor was placed into a temperature controlled room at 25 °C. Influent wastewater was stored in a refrigerator at 4 °C to avoid the deterioration of water quality. Thus, the water temperature in the cycle ranged from around 15 to 25 °C. Meanwhile, influent wastewater was prepared every two days to avoid degradation.

2.2. Medium and inoculum

The synthetic wastewater was prepared to simulate the real wastewater in Jurong Water Reclamation Plant [40] without considering excessive ammonium for nitrification and denitrification. It consisted of: NaAc (C source) 1665 mg/L, NH_4Cl (N source) 150 mg/L, K_2HPO_4 (P source) 45 mg/L, $CaCl_2 \cdot 2H_2O$ 30 mg/L, $MgSO_4 \cdot 7H_2O$ 25 mg/L, $FeSO_4 \cdot 7H_2O$ 20 mg/L, and trace elements which were same with those used. This wastewater quality gave the reactor an organic loading rate (OLR) of 6 g/L d with a cycle time of 4 h. Concentrations of Ca^{2+}, Mg^{2+} and Fe^{2+} in this study were totally same with those used by Liu and Tay [17], Qin et al. [14], and Liu et al. [8]. Ca^{2+} and Mg^{2+} concentrations in tap water used for the preparation of synthetic wastewater were 11 and 8 mg/L, respectively, leading to 19 mg/L total Ca^{2+} and 11 mg/L total Mg^{2+}

concentrations in the synthetic wastewater. pH of the synthetic wastewater was not adjusted with an average value around 8.4 due to the alkaline nature of sodium acetate. All chemicals used were as analytical grades.

4750 ml of raw activated sludge from a local municipal wastewater treatment plant was acclimated with synthetic wastewater used in this study in the sequencing batch reactor (SBR) at the similar conditions described above for reactor operation except that settling time was set at 30 min. After three-day acclimation, the settling time was changed to 15 min and the reactor was started up for granulation. Over the operation time, settling time was shortened gradually to 3 min, which was kept constant for long term operation.

2.3. Sorting of granules

After the reactor reached stable state in terms of biomass concentrating and the average granule size, aerobic granules were taken from the reactor on days 90, 120 and 150, respectively. The granules were washed three times with 1× phosphate buffered saline (PBS) before the wet-sieving was employed for granule sorting by size. Granules with a diameter range of 106–212, 212–355, 355–425, 425–500, 500–600, 600–710, 710–850, 850–1000, 1000–2000, and 2000–2800 μm were sorted using metal sieves, with a corresponding pore size in diameter.

2.4. Analytical methods

Chemical oxygen demand (COD), sludge volume index (SVI), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS), and the specific oxygen utilization rate (SOUR) were analyzed by standard methods [18].

COD concentration and MLVSS was set at around 300 and 100 mg/L, respectively, for SOUR measurement, which was based on the report by Liu et al. [36]. Dissolved oxygen concentration for SOUR was measured with DO-electrode (YSI 5000). Ash content of reactor sludge was obtained by calculation based on the equation below:

$$\text{Ash content} = \frac{\text{MLSS} - \text{MLVSS}}{\text{MLSS}} \times 100\%$$

Ash content of sorted granules with different sizes was measured by drying at 105 °C for 24 h and followed ignition at 550 °C for half hour. Ash content was then calculated by granule dry weight at 550 °C/granule dry weight at 105 °C.

Granules were sampled and granule size was measured immediately by a laser particle size analysis system with a stirring speed of 100 rpm (Malvern MasterSizer Series 2600) when size was smaller than 2000 μm, or an image analysis system (Image-Pro Plus, V4.0, Media Cybernetics, Maryland, USA) with an Olympus SZX9 microscope (Olympus, Token, Japan) when size was bigger than 2000 μm [38]. Microscopic observation was carried out to make sure that the stirring speed used in the size analysis does not damage granules.

Microbial composition of granules was observed qualitatively with scanning electron microscope (SEM) (Stereosan 420, Leica Cambridge Instruments) after fixing with 2.5% glutaraldehyde for 2 h, dehydration via successive passages through 30%, 50%, 75%, 85%, 90%, 95% and 100% ethanol, followed by critical drying in a critical point dryer (HCP-2) (Hitachi Ltd., Tokyo, Japan). To observe the granule surface and the granule interior, respectively, a few of granules were halved by a sterile surgical blade before fixation. Mineralogical analyses of granules were carried out by X-ray diffraction (XRD) with Bruker D8 Advance X-ray diffractometer equipped with a Cu Kα radiation source (Bruker AXS GmbH, Karlsruhe, Germany) after freeze drying followed by pulverization.