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Aerobic granules dwelling vorticella and rotifers in an SBR fed with domestic wastewater

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ABSTRACT

Aerobic granules respectively dwelling abundant vorticella and rotifers in a sequencing batch reactor (SBR) fed with real domestic wastewater were cultured. Most vorticella anchored into the granules by stalks, while rotifers attached or adhered to the surface of granules. Vorticella and rotifers going through a process of growth, blooming and decline successively was mainly caused by sludge granulation and available food from detached fine biomass particles. The mean SVI of two type granules were 43.9 mL/g and 33.9 mL/g, respectively. Flower-like vorticella on the surface of granules led to the lower settling velocity and higher SVI compared to naked granules or granules with rotifers. A positive effect on the reduction of suspended solids (SSs) in the effluent can be linked to the ingested fine biomass particles by vorticella and rotifers. The direct immunofluorescence assay demonstrated the phenomenon of the FITC-labeled bacteria was ingested into the guts of rotifers.

1. Introduction

Aerobic granular sludge was considered to be a special case of biofilm which was composed of self-immobilized cells [1]. Aerobic granules in aerobic SBR present advantages compared to conventional activated sludge process such as high settling velocity, good solid–liquid separation, high biomass retention and an ability to withstand high loading rates [2–4]. These advantages indicate that aerobic granular technology has great potential for the treatment of various municipal and industrial wastewaters.

Vorticella is a genus of protozoa and inverted bell-shaped ciliates. Vorticella sometimes anchored in groups by individual stalk and could detach from the group when environmental conditions are unfavorable. Rotifers are swimming or creeping metazoans with a length of 40–500 μm and characterized by the possession of a ciliated area or a funnel-shaped structure at the anterior end that may look like rotating wheels [5]. Vorticella or rotifers were usually abundantly found in a stable activated sludge environment of municipal wastewater treatment plants [6,7]. Both of them were often used as indicators of wastewater treatment system performance [8,9]. Besides the function as predators of bacteria and fine suspended biomass particles they can also secrete a kind of mucous glue as a function of flocculants [10,11], therefore vorticella and rotifers have a significant impact on reducing effluent SS and turbidity.

For aerobic granulation, the reactors were always operated with very short settling time to cause the washout of fine and poor settling sludge flocs and particles [2]. It inevitably led to higher effluent SS. Vorticella and rotifers exactly can alleviate this problem. Very little information about the formation of aerobic granular dwelling vorticella or rotifers was discussed. According to the available literatures, Weber et al. reported the formation of stalk ciliates attached to aerobic granular sludge fed with malthouse, brewery and artificial wastewater [12]. Literatures about aerobic granules dwelling rotifers were even barely reported. The main objective of this work was to study the formation and characteristics of aerobic granular sludge dwelling vorticella or rotifers fed with real domestic wastewater.

2. Experimental

2.1. Reactor set-up and operation

A laboratory-scale aerobic SBR with a working volume of 11 L was used. The reactor had a height of 500 mm and an inner diameter of 200 mm. The inoculums were obtained from an activated sludge tank in a municipal wastewater treatment plant, while the raw wastewater was taken from a septic tank in a community. Due to the carbon to nitrogen (C/N) ratio of raw wastewater was too low, approximately 90 g of completely dissolved soluble starch and 60 L raw wastewater were added into a container, and then diluted to a total volume of 500 L for influent. Moreover, approximately 10 g of potassium dihydrogen phosphate was added to

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Table 1
Compositions of raw wastewater and the adjusted wastewater (mg/L).

Parameters	Raw wastewater	Adjusted wastewater
COD_{Cr}	400–650	250–450
NH_4^+–N	100–250	20–30
PO_4	5–12	6–11

maintain the PO_4 concentration every time. Some parameters of raw wastewater and the adjusted wastewater are presented in Table 1. In addition, the indoor temperature has been in the range of 20–35 °C.

The SBR was operated with eight 3-h cycles per day. Each cycle consisted of a 10-min of filling, a 120-min of aeration, a 5-min before day 33 and a 1-min after day 33 sludge settling, 15-min of effluent decanting was applied, the residual time was idling. Programmable logic controllers (PLCs) were used for the operation of the pumps and valves. The volume of effluent decanting was 7 L each cycle (Exchange ratio = 7/11). A gas velocity of about 1.2 cm/s was supplied during the aeration and the oxygen concentration varied from 3 mg/L to 5 mg/L before depletion of substrates.

2.2. Analytical methods

2.2.1. Analysis of granular sludge and water quality

The SVI, MLSS, SS, COD_{Cr} and NH_4^+–N were measured according to standard methods [13].

2.2.2. Morphology characteristics of the granular sludge

The complete granulation process from seeding sludge to mature granules was investigated and documented by a light microscopy. The mixed liquid was sampled every two days out of the reactor. Samples for Scanning Electron Microscopy (SEM) were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH = 7.3) for up to 12 h at 4 °C and post-fixed in 1% OsO_4 at room temperature for 90 min. Further SEM sample preparations and microscopic analysis were performed as described previously [14].

2.2.3. Microfauna counting

A drop of mixed liquor was taken from the SBR, and the number of rotifers and vorticella visible was counted under a light microscopy (CX31, Olympus). The counting was repeated three times and the average was taken to get the density that indicated the vorticella or rotifers number per volume (individuals per milliliter).

2.2.4. Free-settling velocity test

The free-settling velocity testing of granules was conducted in a glass cylinder with a height of 300 mm. Each measurement of the settling velocity was performed three times and the average was taken to get the settling velocity.

2.2.5. Observation of a rotifer ingesting sludge particles

The direct immunofluorescence (DIF) assay [15] was used to confirm the rotifer can feed on the biomass particles. Fine sludge particles treated with fluorescein isothiocyanate (FITC) and the bacteria attached to the fine sludge particles were labeled. FITC was dissolved in dimethyl sulfoxide (DMSO) solvent. The mixed liquor taken from SBR were settling in a beaker and fine sludge particles with poor settling were collected from the supernatant by a dropper afterwards. The sludge particles were dipped in this FITC solvent and incubated in a moist and closed container at room temperature for 5 h, and then washed by dipping sequentially into three jars of phosphate buffered saline (PBS). A live rotifer was placed among the fine sludge particles with FITC-labeled bacteria and observed the process of ingestion of the FITC-labeled bacteria into the gut. In order to make the microscopic observation easily, the rotifer was killed and finally the corpse was photographed through a fluorescence microscopy (DMI3000B, Leica) after washed by PBS.

3. Results and discussion

3.1. Formation and apparent properties of granules

According to the apparent properties varied in microstructure of granules, the granulation process was divided into six phases: initial phase (phase a: 1–10 d), naked granules (phase b: 11–39 d), granules dwelling vorticella (phase c: 40–70 d), granules dwelling vorticella and rotifers (phase d: 71–76 d), granules dwelling rotifers (phase e: 77–102 d) and granules dwelling rotifers and vorticella (phase f: 103–110 d). Naked granules mean vorticella and rotifers barely attached on the surface of granules. Compared to naked granules, the term granules dwelling vorticella indicate granules where vorticella have been the dominant microfauna settled on granules, while granules dwelling rotifers indicated rotifers were dominant. Granules dwelling vorticella and rotifers indicated dominant vorticella was transferred to coexistence of vorticella and rotifers. Granules dwelling rotifers and vorticella indicated dominant rotifers was transferred to coexistence of rotifers and vorticella.

The first granules were naked and observed around 7 days of SBR setup (Fig. 1b). The naked granules were the dominant aggregate form with a mean diameter of 200 μm in day 33 (Fig. 1c). Granules dwelling vorticella have been detected not earlier than day 40. The image taken on day 55 shows swarming vorticella settled on the surface of granules (Fig. 1d) and free swimming vorticella was barely found in this phase. On day 71, rotifers occurred on granules dwelling vorticella for the first time while the quantity of vorticella decreased. The image taken on day 74 shows both rotifers and vorticella settled simultaneously on the surface of granules (Fig. 1e). Vorticella almost disappeared around day 76. The image from day 92 shows large amounts of rotifers attached on the surface of granules. At that time the mean granule size increased to about 300 μm (Fig. 1f).

On a macroscopic level, two types of granules dwelling vorticella and rotifers were similarly spherical and smooth (Fig. 2a and b). However, the images taken with light microscopy and SEM show bulky growth of tufted vorticella rooted in the surface of the granules (Fig. 2c and e), rotifers wriggled around the granules or adhered to the granules by the toe of each posterior end (Fig. 2d). Pretreatment for SEM did obviously not affect the presence of vorticella on the granule surface (Fig. 2e). Compared to that the rotifers were not found in SEM image (Fig. 2f), one reason might be the rotifers were washed away from the surface of granules during the pretreatment for SEM. Furthermore, the surface of granules dwelling rotifers was relatively rough (Fig. 2f).

3.2. Performance of sludge settling

The average of the MLSS, SVI and SS changed over the six phases (Fig. 3). The mean MLSS of sludge fluctuated during the first two phases and then showed an upward trend. The mean MLSS increased fast during the phase of granules dwelling rotifers resulted from the effluent SS was kept low and wasted less, and finally reached about 4500 mg/L. The mean SVI of six phases showed a downward trend with the aerobic granulation. The mean SVI of seed sludge and six phases were 103.9, 77.3, 63.4, 43.9, 45.4, 33.9, and 38.3 mL/g respectively (Fig. 3).

The mean settling velocity of naked granules, granules dwelling vorticella and granules dwelling rotifers were 30.6, 20.1 and 38.8 m/h, respectively. The layer of flower-like vorticella was