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Characteristics and performance of aerobic algae-bacteria granular consortia in a photo-sequencing batch reactor

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ABSTRACT

The characteristics and performance of algae-bacteria granular consortia which cultivated with aerobic granules and targeted algae (*Chlorella* and *Scenedesmus*), and the essential difference between granular consortia and aerobic granules were investigated in this experiment. The result indicated that algae-bacteria granular consortia could be successfully developed, and the algae present in the granular consortia were mainly *Chlorella* and *Scenedesmus*. Although the change of chlorophyll composition revealed the occurrence of light limitation for algal growth, the granular consortia could maintain stable granular structure, and even showed better settling property than aerobic granules. Total nitrogen and phosphate in the algal-bacterial granular system showed better removal efficiencies (50.2% and 35.7%) than those in the aerobic granular system (32.8% and 25.6%) within one cycle (6 h). The biodiesel yield of aerobic granules could be significantly improved by algal coupled process, yet methyl linolenate and methyl palmitoleate were the dominant composition of biodiesel obtained from granular consortia and aerobic granules, respectively. Meanwhile, the difference of dominant bacterial communities in the both granules was found at the order level and family level, and alpha diversity indexes revealed the granular consortia had a higher microbial diversity.

1. Introduction

Aerobic granular sludge is a type of self-aggregate formed by microorganisms under aerobic condition without carrier materials addition. Compared to flocculent sludge, the special structure of granular sludge makes it to have better settling property, and this is beneficial to biodiversity because of the simultaneous presence of aerobic and anoxic zones, and greatly reduce area requirement [1–3]. In recent years, some research showed that aerobic granular sludge not only contained bacteria, fungus and protozoa [4], but also able provide the growth region for photosynthetic organism under illumination condition [5,6]. It is well known that algae are the key composition of photosynthetic organism. Besides, algae are also considered to be the functional microorganism for wastewater treatment and the potential raw material of biodiesel or fertilizer production [7], but algal harvesting as the major production costs commonly associated with the mass application of algal technology [8,9]. Although immobilization was considered to be a feasible method to simplify the separation of algae from water for biodiesel production [10], the operating cost was the most serious drawback of this technology for its application in reality. Therefore, according to the theory of microorganism self-aggregate, a novel microbial granular technology based on algae and bacteria is practicable. However, to our knowledge, although a combined system consisting of algae and flocculent sludge have been extensively studied [11–13], the information about the characteristics of the granular consortia using granular sludge and targeted algae in the photo-bioreactor is still sparse. Thus, in the present study, aerobic algae-bacteria granular consortia were developed in a photo-sequencing batch reactor (PSBR). Meanwhile, the characteristics and performance of the mature granular consortia was investigated, and was compared with those of aerobic granular sludge under the similar operating conditions.

2. Material and methods

2.1. Experimental operation

This experiment was divided into two phases, namely culturing phase and mature phase. In the culturing phase, a cylindrical glass

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PSBR with a working volume of 2 L was used to culture the granular consortia in an illumination incubator as shown in the Appendices, which was set at a constant temperature (26 ± 1℃) and half-day photoperiod (12 h light/12 h dark, 6000 ± 200 lx). Air was introduced through a diffuser at reactor bottom using an air pump, pH value and air aeration intensity in the PSBR were maintained at 7.6 ± 0.1 and 4 L/min by real-time pH control system and gas rotameter, respectively. Two cycles were operated in the light period, and each cycle time of the PSBR was set to 6 h (about 1 min of influent feeding, 355 min of aeration, 2 min of settling and 2 min of effluent withdrawal), while the PSBR was in idle state during the dark period. Considering the application of mature granular sludge and sedimentary property of algae, the volume exchange ratio of the PSBR was set as selection pressure to control the coupled process [14]. The volumetric exchange ratio of the PSBR was increased gradually from 40% to 70% during the culturing phase based on the change of total chlorophyll (total Chl) concentration. In the mature phase, the volume exchange ratio of the algae-bacteria granular system was set to 85%, and the other parameters of the PSBR were maintained as mentioned above. Meanwhile, an aerobic granular system that provides the seed granules for this experiment was operated in parallel in this phase, and the experimental conditions of this system were kept consistent with the algae-bacteria granular system, except the illumination condition (0 lx). The mixed liquor suspended solids (MLSS) in the both systems were remained at a similar level (about 2800 mg/L).

Chlorella (FACHB-31) and *Scenedesmus* (FACHB-416) were selected as target algae. The respective inoculative cell density of *Chlorella* and *Scenedesmus* was approximately 10^8 cells for the aerobic algae-bacteria granular system. Mature granular sludge was obtained from an aerobic granular system treating artificial municipal wastewater in our laboratory. Artificial municipal wastewater prepared with tap water was used throughout this experiment, and the main composition of the wastewater was as follows: chemical oxygen demand was 300 mg/L (glucose), ammonia nitrogen was 35 mg/L (NH_4Cl), and phosphate was 10 mg/L (KH_2PO_4).

2.2. Analytical methods

Ammonia nitrogen, phosphate, nitrite, nitrate, total nitrogen, chemical oxygen demand and suspended solids (SS) in the liquid samples, and sludge volume index were measured following the published methods [15]. Concentration of chlorophyll-*a* (Chl-*a*), chlorophyll-*b* (Chl-*b*) and total Chl in the granular consortia were detected according to Ritchie [16]. The suspension liquid of treated granular samples after disperse treatment by syringe aspiration/injection [17], were detected by nano series zeta potential analyzers (ZEN 3600, MALVERN) to determine the zeta potential. The size distribution of the granular samples was measured by a laser particle size analysis system (Malvern Mastersizer 2000). Organic and inorganic elements concentrations of the freeze-dried granular samples were detected by macro elemental analyzer (Vario MAX) and X-ray fluorescence spectrometer (Axios mAX). Extracellular polymeric substances (EPS) in the granules were extracted by heating-centrifugation extraction method, and phenol-sulfuric acid method and Lowry method were used to quantify polysaccharides (PS) and protein (PN) in extracted EPS, respectively. The images of morphology and microstructure were photographed using digital camera (IXUS8601S, Canon) and scanning electronic microscope (SEM) (S-4800, Hitachi), respectively. Algal fluorescence in the cross-sectional slice of the mature granular consortia was photographed using an inverted laser scanning confocal microscope (LSM710, Zeiss), and the slice preparation as following: the granular consortia sample was cut along the center axis of granules by slicing knife, then the treated sample was slightly washed three times with ultrapure water to remove the suspended algae, and the slice sample was placed on microscope slide for fluorescence detection. Fatty acid methyl esters (FAMEs) were prepared from the freeze-dried granular samples by in situ transesterification [18], and the concentration and composition of the FAMEs samples were analyzed by gas chromatography in combination with tandem mass spectrometry (Agilent 7890A – Agilent 5975C). Based on the datum of FAMEs composition, cetane number of biodiesel production was calculated using the calculation model described by Piloto-Rodríguez et al. [19]. Both granules for microbial community investigation were sampled at the end of this experiment (75th day), and the samples was detected using high throughput microbial analysis method. In addition, algae communities of the mature algae-bacteria granular consortia after disperse treatment were also detected using PHYTO-PAM phytoplankton analyzer (Heinz Walz, Effeltrich). The detailed analytical methods of FAMEs and microbial community were described in the Appendices.

3. Results

3.1. Development process

The total Chl concentration in the granular consortia was set as indicative parameter to evaluate the coupled process between the targeted algae and aerobic granular sludge. We defined the implementation of coupled process as no statistically significant difference in the change range of total Chl concentration in the granular consortia. In the culturing phase, as shown in Fig. 1, the Chl was clearly detected in the granules after 4 days of operation (about 0.45 mg/g SS), which means the algae start to grow in the granules. In order to promote the targeted

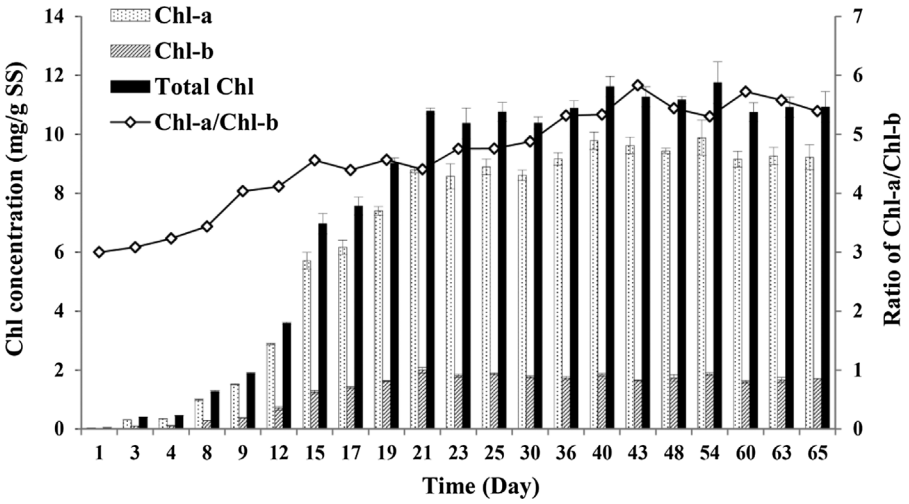

Fig. 1. Change in Chl concentration and Chl-*a*/Chl-*b* ratio of aerobic algae-bacteria granular consortia.