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Development of algae-bacteria granular consortia in photo-sequencing batch reactor

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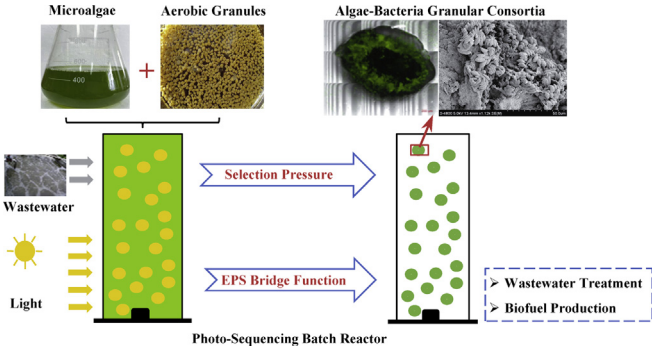
HIGHLIGHTS

- Aerobic granules can be used as the immobilized material of eukaryotic algae.
- EPS bridge function is dominant developed mechanism of the granular consortia.
- Cyanobacteria will not displace the dominance of eukaryotic algae in consortia.
- Competition for aerobic zone occur among aerobe, photoheterotrophs and algae.

GRAPHICAL ABSTRACT



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ABSTRACT

The development and properties of algae-bacteria granular consortia, which cultivated with the algae (*Chlorella* and *Scenedesmus*) and aerobic granules, was investigated in this experiment. The results indicated that the granular consortia could be successfully developed by selection pressure control, and the algal biomass and extracellular polymeric substances (EPS) concentration in the consortia showed notable correlation with the operating parameters of reactor. The maximum specific removal rates of total nitrogen and phosphate were obtained from the granular consortia with the highest algal biomass, yet the correlation between the fatty acid methyl esters yield and the algal biomass in the consortia was not markedly observed. The seed algae maintained dominance in the phototroph community, whereas the cyanobacteria only occupied a small proportion (5.2–6.5%). Although the bacterial communities with different operational strategies showed significant difference, the dominated bacteria (*Comamonadaceae*, 18.79–36.25%) in the mature granular consortia were similar.

1. Introduction

In aquatic environments, algae and bacteria play crucial roles in nutrient cycling and contaminants removal by biosynthesis and biological metabolic processes. A combined system consisting of algae and bacteria have been extensively studied for applications in wastewater treatment (Muñoz and Guieysse, 2006; Ramanan et al., 2016). Previous studies demonstrated that algal-bacterial consortia for wastewater treatment can achieve better nutrient removal efficiency than single bacterial or algal system did, and reduce the biomass harvesting cost compared to algal system (Muñoz et al., 2005; Su et al., 2011). Meanwhile, the excess biomass of the algal-bacterial combined system is also considered to

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be a potential source of biofuel or fertilizer production (Ramanan et al., 2016). Algae immobilization in several materials (e.g. alginate and chitosan) that showed better settlement capability has been reported by some research, yet material operating costs and residual polymeric materials in the process of resources recovery has limited the large-scale application of this technology (De-Bashan and Bashan, 2010). Thus, more research on bacteria-algae interactions is needed before the combined system can be widely implemented for wastewater treatment and biomass resources recovery.

Aerobic granules are compact aggregates of microorganisms, which could be used for organics degradation and nutrient removal, and can be classified as a type of self-immobilized microbial consortium without physic-chemical materials addition (Gao et al., 2011). Image observation and microbial sequencing analysis for the structure characteristics of aerobic granules revealed that except for bacteria, aerobic granules contain an abundance of various microorganisms, such as fungus and ciliate protozoa (Liu et al., 2015). In some recent research, without inoculum addition, the algae growth was also observed on the surface of aerobic granules in the photo-bioreactor by natural sunlight exposure (Huang et al., 2015; Li et al., 2015). Thereby, these results implied that algae and bacteria can form immobilized granular consortia only through biological processes. However, to date, there is no research aimed at culturing the granular consortia using aerobic granules and the selected algae. In addition, aerobic sludge granulation process is dependent on many influential factors and operational conditions, such as reactor configuration, aerobic starvation, hydraulic shear force and so on (Zhu et al., 2008; Gao et al., 2011). These environmental conditions also have a close relationship with growth rate and cell composition of algae (Schenk et al., 2008). Thus, in terms of algae-bacteria granular consortia, the effect of these operating parameters on the start-up of the systems also needs to be further evaluated.

In the present study, the development of algae-bacteria symbiotic granules in the photo-sequencing batch reactors (PSBR) with different operational strategies was investigated. *Chlorella* and *Scenedesmus*, which have been successfully and extensively used for wastewater treatment and biofuel production, were selected as target algae (Unnithan et al., 2013; Kesaano and Sims, 2014), and mature aerobic granules were used as biological carrier material of the target algae. The objectives of this experiment were (1) to develop aerobic algae-bacteria granular consortia for wastewater treatment, and (2) to compare the effect of aeration intensity, aerobic starvation and reactor configuration on the culturing process and properties of the granular consortia.

2. Material and methods

2.1. Experimental setup and reactor operation

An experiment was performed in six cylindrical glass PSBRs (AR-R1, AR-R2, AR-R3, AS-R1, AS-R2 and AS-R3) with a working volume of 2 L. The PSBRs were placed in an illumination incubator, which was set at a constant temperature (25 °C) and half-day photoperiod (12 h light/12 h dark, 6000 ± 200 lx). The pH of the six PSBRs was maintained at 7.4 ± 0.2 using real-time pH control systems. Algae-bacteria granular consortia in the six PSBRs under aerobic condition were cultured by selection pressure control. The volumetric exchange ratio of the six PSBRs was set to 40%, and was then increased gradually from 40% to 80% during the granular consortia cultured stage. According to the objectives, this experiment was divided into three comparative groups, namely group I, group II and group III (Fig. A1). In group I, the aeration intensity in three PSBRs (diameter 8.5 cm, height 35.5 cm) was fixed at 2 L/min (AR-R1), 4 L/min (AR-R2) and 6 L/min (AR-R3), respectively, which results in a superficial upflow air velocity of 0.58 cm/s, 1.17 cm/s and 1.76 cm/s for AR-R1, AR-R2 and AR-R3. Two cycles were operated in the light period, and each cycle time of the three PSBRs was set to 6 h (1 min of influent feeding, 355 min of aeration, 2 min of settling and 2 min of effluent withdrawal); In group II, the three PSBRs (diameter 12.5 cm, height 16.5 cm) were operated in two cycles in the light period, and each with a time of 2 h, 4 h, and 6 h, with 1 min of influent feeding, 2 min of settling and 2 min of effluent withdrawal maintained in AS-R1, AS-R2 and AS-R3, respectively, resulting in the aeration time of 115 min in AS-R1, 235 min in AS-R2 and 355 min in AS-R3 in each cycle. The aeration intensity of these three PSBRs was kept at 4 L/min (superficial upflow air velocity of 0.54 cm/s) by air pump and gas rotameter; In group III, the effect of reactor configuration on the symbiotic process and properties of the algal-bacterial granules was investigated based on the operation of AR-R1, AR-R2 and AS-R3. Height/diameter (H/D) ratio and illuminated surface/volume (S/V) ratio of AR-R1 and AR-R2 was 4.1 and 0.5, while those of AS-R3 were 1.3 and 0.38, respectively.

Chlorella (FACHB-31) and *Scenedesmus* (FACHB-416) were obtained from Freshwater Algae Culture Collection of the Institute of Hydrobiology, the Chinese Academy of Sciences. The inoculative cell density of *Chlorella* and *Scenedesmus* was approximately 10^9 cells for each PSBR, respectively. Mature aerobic granules taken from an aerobic granular reactor, which the experimental conditions were similar to those of the AR-R2 without illumination condition, were used as the seed granules for the six PSBRs at a respective initial sludge concentration of 2000 mg/L in mixed liquor suspended solids (SS) and mixed liquor volatile suspended solids (VSS) of 1700 mg/L. A synthetic domestic wastewater prepared with tap water was used with the following compositions: chemical oxygen demand (glucose) was 300 mg/L, ammonia nitrogen (NH_4Cl) was 35 mg/L, phosphate (KH_2PO_4) was 11 mg/L, and trace element solution was 5 mL/L. The composition of the trace element solution was $MgSO_4$ 5 g/L, $CaCl_2$ 2 g/L, $FeCl_3$ 60 mg/L, $CuSO_4$ 50 mg/L, $MnSO_4{\cdot}H_2O$ 50 mg/L, KCl 18 mg/L, $AlCl_3$ 15 mg/L, $ZnSO_4{\cdot}7H_2O$ 30 mg/L.

2.2. Analytical methods

Ammonia nitrogen, phosphate, nitrite, nitrate, total nitrogen, sludge volume index (SVI), SS and VSS were measured following the standard methods (APHA, 1998). The concentration of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b) and total chlorophyll (total Chl) in the granules was detected according to Ritchie (2008). The proportion of cyanobacteria in the phototroph community was determined with a PHYTO-PAM phytoplankton analyzer (Heinz Walz, Effeltrich). EPS in the granules were extracted and detected using the method described by Adav and Lee (2008). The fatty acid methyl esters (FAMEs) from the different granules were prepared by in situ transesterification (Mondala et al., 2009). The size distribution of the granules was measured by a laser particle size analysis system (Malvern Mastersizer 2000). The morphology, microstructure and fluorescence image were observed using digital camera (IXUS8601S, Canon), scanning electronic microscope (S-4800, Hitachi) and Laser Scanning Confocal Microscope (LSM710, Zeiss), respectively. Algae-bacteria granular consortia in the six PSBRs for the bacterial and algal communities analysis was sampled at the end of the experiment (36th day). The detailed analytical method of chlorophyll, cyanobacteria concentration, FAMEs and microbial community is summarized in the Appendices.

Statistical analysis was conducted using SPSS Version 16.0 (SPSS Inc.) to compare the characteristics of the algae-bacteria granular consortia in the six PSBRs, and the comparisons were considered