



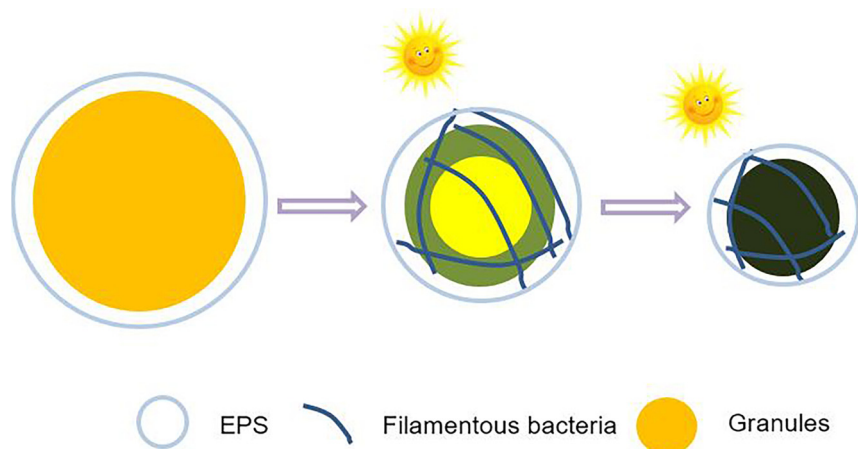
## Natural sunlight induced rapid formation of water-born algal-bacterial granules in an aerobic bacterial granular photo-sequencing batch reactor

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### GRAPHICAL ABSTRACT



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### ABSTRACT

Wastewater treatment by means of algal-bacterial granules has become a hot topic worldwide recently. Rapid granulation of algal-bacterial granules was achieved in an aerobic bacterial granular sequencing batch reactor (SBR) under natural sunlight exposure. Occurrence of abundant filamentous bacteria bridging the water-born algae, and overproduction of extracellular polymeric substances (EPS) (especially polysaccharides (PS), tryptophan & protein-like, and humic acid-like substances) were observed on the first 3 days, while the algae grew into the inner side of the granules and mature granules were obtained on day 7. The growth of the water-born algae slightly decreased the settleability, mean sizes of the granules, but stimulated the bioactivity significantly. Whereas, the biomass retention decreased before day 3, and got stable soon with the maturation period with symbiotic growth of algal-bacterial biomass. Illumina results revealed that the introduction of algae reduced the richness and diversity of bacterial community. Besides, few changes in structure and some compositions shifts in bacterial communities were found, while the predominant algae shifted from *Diatomea* to green algae *Chlorophyceae*. The possible mechanism for natural sunlight induced granulation of algal-bacterial granules was thus proposed based on the interactions between algae and bacteria.

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## 1. Introduction

Microalgae have raised attention recently due to their advantages of photosynthetic function, synergy with carbon dioxide fixation, fast growth and potential oil production [1]. Algae growth occasionally occurs in wastewater treatment and receives the interest of researches due to their capacity for nutrients uptake as well as oxygen production simultaneously [2]. Besides, the application of microalgae for potential biofuel production has been the hotspot for years [3,4]. However, the free algae behave poorly in settling ability, thus long settling time or huge space is required [5]. The harvesting of biomass remains to be a challenge due to the small size, poor settleability and low density of the microalgae [3,5].

Algal-bacterial consortia or aggregates have attracted the attention of researchers since bacteria could significantly enhance the settling velocity of microalgae, thus providing an alternative co-culture of microalgae in wastewater [6]. Compared to the algal-bacterial symbiotic consortia by combing microalgae with sludge flocs, the innovative application of aerobic granules tends to be more attractive [2,7,8]. Aerobic bacterial granular sludge has been proposed as an efficient and innovative technology for wastewater treatment, which possesses high settling velocity, rich biomass retention, ability for simultaneous organics and nutrients removal, and resistance to shock loadings and toxic substances [9]. Huang et al. [8], Li et al. [10], Ahmad et al. [2], and Zhang et al. [11] have cultivated algal-bacterial granular consortia with or without addition of microalgae. The granulation of algae with bacterial granules possesses the advantages of both aerobic bacterial granules and the microalgae described above [2,5,7,8,11,12].

However, the formation of the algal-bacterial granules varies with the operational conditions including the seeding sludge and microalgae [5,7,8], hydraulic selection pressure [2,7,11] and other parameters like the light/dark regimes [1,6,12,13]. Though much efforts have been put to realize formation of algal-bacterial granules [2,7,8,10,11], most of them focused on the formation of granular consortia with microalgae inoculation, lamp light addition, and the activated flocs as the seeding sludge. However, none of the present work has attempted to obtain rapid granulation of algal-bacterial granular consortia by seeding with aerobic bacterial granules, though similar enhancement effects have been verified during the aerobic bacterial granulation process by Long et al [14]. The spontaneous formation of algal-bacterial granules under natural sunlight, including the granulation process and the inner mechanisms involved also remains unknown, even though the aerobic bacterial granules can be referenced to some degrees [2,15]. Besides, the growth of water-born algae could also alter the microbial communities in the algal-bacterial symbiotic consortia in terms of both the bacteria and microalgae. Whereas there is not sufficient information on the elucidations of the bacterial and algal communities in the symbiotic system.

Therefore, the present study aimed at the fast formation of algal-bacterial granular consortia with aerobic bacterial granules as the seeding sludge under the summer natural sunlight exposure without microalgae inoculation. The morphological characteristics, physicochemical attributes, as well as the extracellular polymeric substances (EPS) were explored to investigate the possible mechanisms involved. Illumina pyrosequencing technology was employed for demonstrating the microorganism communities during this process. The results of present work might aid the comprehensive understanding of the spontaneous formation of water-born algal-bacterial granular consortia under natural sunlight.

## 2. Materials and methods

### 2.1. Aerobic granular SBR configuration

An aerobic granular sequencing batch reactor (SBR) was configured with an inner diameter of 100 mm, and a height of 500 mm, making a

working volume of 3.6 L. Synthetic wastewater was pumped into the reactor at the end of each cycle with an exchange ratio of 50% (1.8 L each cycle). A mechanical stirrer was set at a constant speed of 200 rpm except the time for feeding, settling, and discharge periods. Air was introduced from the bottom of the reactor with a fine aerator at constant aeration rate of 300 mL/min by an aerator meter. The SBR was run under anaerobic/oxic/anoxic (AOA) mode for simultaneous nitrification, denitrification and phosphorus removal. The 6 h-cycle consisted of 2 min of feeding, 120 min of anaerobic phase, 90 min of oxic phase, 144 min of anoxic phase, 2 min of settling, and 2 min of discharge periods.

Prior to the present work, aerobic granules cultivated by He et al. [16] were inoculated into the reactor with an initial mixed liquor suspended solid (MLSS) of about 7.8 g/L. The newly configured aerobic granular SBR had been operated stably for enough time before use. Then the aerobic granular SBR was placed near the window (facing south) without any cover in the Laboratory of Municipal Engineering, Wuhan University, Wuhan, China (30°32'47"N, 114°21'20"E). The reactor was irradiated around 12 h per day (6:30–18:30) by natural sunlight in July. Water temperature during the operation period was not controlled and monitored. The strong summer light irradiation led to the highest temperature of about 38–42 °C during the day, while it was about 23–25 °C during the night.

### 2.2. Synthetic wastewater compositions

Synthetic wastewater mimicking the low-strength domestic wastewater in southern China was used in the present work for preventing the huge fluctuations of real wastewater [17]. The detailed compositions of the wastewater were listed in Table 1. Generally, the concentrations of chemical oxygen demand (COD), ammonia ( $\text{NH}_4^+$ -N), and TP were 200, 20 and 5 mg/L, provided by sodium acetate, ammonium chloride, potassium phosphate sulfate, respectively. 10 mg/L calcium and magnesium ions were added to help maintain the shape of aerobic granules, as well as 1 mL/L of trace solutions (Table 1). The pH of the wastewater was adjusted to 7.5 using hydrochloric acid (HCl) or sodium hydroxide (NaOH) solutions.

### 2.3. Analytical procedures

The analytical items included water, sludge, and the microorganisms. Influent and effluent water from the reactor were monitored including the COD, nitrogen, and phosphorus removal, which were all conducted according to the standard methods [18]. A PHS-25 m and YSI5000 m were used for detecting the pH and DO values. The attributes of sludge as well as the algal-sludge consortia including settling volume index at 5 min ( $\text{SVI}_5$ ), MLSS, MLVSS, specific oxygen uptake rate (SOUR) were determined to previous methods by He et al. [17]. The morphology of the sludge and the consortia was observed using the scanning electron microscope (SEM, VEGA3, TESCAN). Samples taken from the reactor were pretreated and analyzed as previous research by

**Table 1**  
Compositions of synthetic wastewater used in the present work.

Main compositions	Concentration (mg/L)	Trace compositions	Concentration (mg/L)
$\text{CH}_3\text{COONa}$	256	$\text{FeCl}_3$	0.9
$\text{NH}_4\text{Cl}$	76.5	$\text{H}_3\text{BO}_3$	0.15
$\text{KH}_2\text{PO}_4$	14.6	KI	0.18
$\text{CaCl}_2$	10	$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.03
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	10	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.06
		$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.12
		$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.15
		$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.06
		EDTA	10

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