Fast cultivation and harvesting of oil-producing microalgae *Ankistrodesmus falcatus var. acicularis* fed with anaerobic digestion liquor *via* biogranulation in addition to nutrients removal

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Abstract

This study examined the feasibility of cultivation and harvesting of oil-producing microalgae (i.e. *Ankistrodesmus falcatus* var. *acicularis*) via biogranulation in two identical sequencing batch reactors (SBRs) fed with synthetic anaerobic digestion liquor. Easily settled algae granules with compact structure appeared around day 90 and mature granules were obtained after 150 days' operation. The microalgae settleability was remarkably improved, signaling by the substantial decrease of sludge volume index (SVI_{30}) from initially >3000 to 53.44±3.31 ml/g, with settling velocity correspondingly increased from nearly 0 to 18.47±0.23 m/h. Although the percentage of the target microalgae (*Ankistrodesmus falcatus* var. *acicularis*) decreased along with the granulation process, the biomass concentration (2-4 g/L) and biomass

productivity (130-270 mg/L/d) using biogranulation were 10-20 times and 16-34 times that by the traditional suspension method. Compared to the seed microalgae cells, more extracellular polymeric substances (EPS) (162.54±3.60 mg/g-volatile suspended solids (VSS)) with a higher proteins/polysaccharides ratio (7.62) were excreted from the mature algae granules. Moreover, the mature microalgae granules showed comparable nutrients removal, averagely 96% and 86% of dissolved organic carbon (DOC) and NH_4^+-N from the digestion liquor, respectively, reflecting its great potential for simultaneous microalgae cultivation, harvesting and wastewater treatment.

Keywords: Microalgae harvesting; Biogranulation; Anaerobic digestion liquor; *Ankistrodesmus falcatus* var. *acicularis*; Settleability; Nutrients removal

1. Introduction

The depletion of natural resources especially fossil fuels has stimulated a global concern on material recycling and waste minimization. Special attention has been paid to renewable energy resources including geothermal, wind, solar, hydropower, and bioenergy (Demirbas and Demirbas, 2011). Bioenergy is regarded as one of the most potential energy alternatives, and biofuels have been promoted as energy substitute in many countries. As it is known, various kinds of lipid-rich materials can be used for biofuel production, including naturally derived crops, refined/used/waste vegetable oils, animal fats, algae and sewage sludge (Tasić et al., 2016). As a new

renewable resource for biofuel production, recently microalgae have been paid more attention by the researchers worldwide.

Microalgae can be cultivated without competing with food consumption and disruption of the environment, which can also produce oil for biofuel utilization up to 7-31 times greater than soy oil and palm oil per unit (Demirbas, 2011). However, biofuels from microalgae haven't been commercialized on a large scale yet, mainly due to the high cost and energy input for the production of algae biomass (Borowitzka, 2013). Microalgae strains and their cultivation medium selection are identified as the important contributors to the reduction of overall production costs (Kadir et al., 2018). Among the various microalgae, *Ankistrodesmus* sp. is more promising due to its high contents of lipids, carbohydrates and proteins in the cells (Do Nascimento et al., 2013; George et al., 2014). Cultivation of microalgae using wastewater has been intensively studied during the past few years towards microalgal production of useful chemicals (including biofuels) as well as for wastewater treatment simultaneously (Álvarez-Díaz et al., 2017; Cabanelas et al., 2013). From the earlier studies, nutrients in wastewater including nitrogen (N) and phosphorus (P) can be utilized for microalgal growth with potential reduction in cultivation cost for microalgae (Christenson and Sims, 2011; Kadir et al., 2018). Moreover, anaerobic digestion (AD) process has been widely applied in the world as an effective energy and resources recovery approach from organic wastes including waste activated sludge (WAS), livestock manure or other organic solid wastes, and high-strength

organic wastewaters. However, AD process can also release secondary pollutants to the environment if its liquid effluent (digestate, or digestion liquor) being discharged without proper treatment. Various components including mineral materials, non- or slowly biodegradable organics, and some intermediate products like volatile fatty acids (VFAs) are still remained in the digestion liquor after AD process (Barker et al., 1999; Ji and Chen, 2010; Cai et al., 2016). If microalgae can be cultivated with wastewaters like digestion liquor, the microalgae-based technology can be more beneficial for the establishment of a sustainable society due to its low cultivation cost and great potential for pollution amelioration.

Owing to its substantial costs and energy input, microalgae harvesting is another obstacle to develop microalgae biotechnology into a commercial scale (Grima et al., 2003; González-Fernández and Ballesteros, 2013; Pragya et al., 2013). The tiny cell size (0-30 μm), low settleability (settling velocity < 0.0036 m/h) and much diluted culture (microalga biomass concentration < 0.6 g L^{-1}) of microalgae have brought about the economic inefficiency in energy production and wastewater treatment (Granados et al., 2012). It is claimed that the current microalgae harvesting step accounts for 20-30% of the operation cost for microalgae cultivation, mainly through coagulation/flocculation, centrifugation, flotation, etc. (Chen et al., 2011). Among these technologies, centrifugation is regarded as the most time-efficient method for microalgae harvesting, which has been widely applied for the separation of microalgal biomass (Grima et al., 2003; Barros et al., 2015). However, the main drawback of

centrifugation is energy intensive, greater than 3000 kWh/t as reported by Schenk et al. (2008). Hence, the microalgae type, value, and properties in addition to its sedimentation velocity, cell viability, recyclability of culture medium, cell density and size should be considered together when making decision on the suitable harvesting method for a large-scale application (Barros et al., 2015). In this context, based on the hitherto reports, till today none of the commonly used harvesting technologies have been proven to be economical and efficient at large scale (Mathimani and Mallick, 2018; Shi et al., 2019). Therefore, seeking a proper harvesting technology is still crucial for a sustainable and cost-effective biofuel production.

On the other hand, biogranulation can be realized in the symbiosis system of algae and bacteria when the bacterial granulation system is exposed to natural sunlight, achieving excellent biomass settleability (with sludge volume index in 30 min or SVI_{30}=37-40 ml/g after maturation), and relatively dense and compact granule structure (Huang et al., 2015). Specific microalgae strains (*Chlorella* and *Scenedesmus*) can be introduced into bacterial aerobic granules, finally forming algal-bacterial aerobic granules with the seed algae dominated (Liu et al., 2017). Moreover, algae granules can be successfully achieved from activated algae (Tiron et al., 2017) or natural microalgae/bacteria consortium isolated from the algal-bacterial aerobic granular sludge (AGS) system (Cai et al., 2019). However, little information is available for the formation of algae granules from single-cell oil-producing microalgae, i.e. *Ankistrodesmus falcatus* var. *acicularis* applied in this study, through

biogranulation to date, so as to realize the fast microalgae harvesting. Furthermore, no report can be found on simultaneous microalgae biogranulation of *Ankistrodesmus* sp. and nutrients removal from anaerobic digestion liquor.

In this study, the oil-producing microalgae strain *Ankistrodesmus falcatus* var. *acicularis* previously cultivated in the sterilized suspended medium was firstly inoculated into two identical sequencing batch reactors (SBRs) under the designed operation strategies for granule formation, aiming to obtain mature granules with good settleability for fast harvesting. Nutrients (C, N and P) removal from the synthetic digestion liquor by the SBRs was also evaluated. In addition to the changes of biological communities, the content and composition of extracellular polymeric substances (EPS) were quantified in order to make clear the functions of EPS and its relationship with the mechanisms involved in the granulation process. Results from this work are expected to provide scientific data for functional microalgae cultivation and fast harvesting through biogranulation with low energy and time consumption as well as simultaneously treating digestion liquor after ammonia recovery with stripping method.

2. Materials and methods

2.1 Synthetic digestion liquor and seed microalgae

The synthetic digestion liquor was prepared according to a previous study (Cai et al., 2016), which was mainly composed of 500 mg chemical oxygen demand

(COD)/L, 50 mg PO_4^{3-}-P/L, and 100 mg NH_4^+-N/L to mimic the fermentation liquor after ammonia recovery by stripping. The trace elements solution contained (in mg/L) H_3BO_3 (50), $ZnCl_2$ (50), $CuCl_2$ (30), $MnSO_4 \cdot H_2O$ (50), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (50), $AlCl_3$ (50), $CoCl_2 \cdot 6H_2O$ (50), and $NiCl_2$ (50) (Adav et al., 2008). The influent pH to the reactors was adjusted with sodium bicarbonate to be within 7.0-8.3.

The microalgae strain, *Ankistrodesmus falcatus* var. *acicularis* (NIES-2195) was supplied by the Microbial Culture Collection at the National Institute for Environmental Studies (NIES), Japan. All the stock and experimental algae were maintained at room temperature (25 ± 2ºC) in the artificial CT medium containing the following ingredients (per liter): $Ca(NO_3)_2 \cdot 4H_2O$ (150 mg), KNO_3 (10 mg), β–Na_2glycerophosphate$\cdot 5H_2O$ (50 mg), $MgSO_4 \cdot 7H_2O$ (40 mg), Vitamin B_{12} (0.1 μg), Biotin (0.1μg), Thiamine HCl (10 μg), $Na_2EDTA \cdot 2H_2O$ (3 mg), $FeCl_3 \cdot 6H_2O$ (0.59 mg), $MnCl_2 \cdot 4H_2O$ (108 μg), $ZnSO_4 \cdot 7H_2O$ (66 μg), $CoCl_2 \cdot 6H_2O$ (12 μg) and $Na_2MoO_4 \cdot 2H_2O$ (7.5 μg) (Waterbury and Stanier, 1981).

2.2 SBRs set-up and their operation strategies

Two identical laboratory-scale cylindrical column SBRs with a working volume of 0.9 L each (5.7 cm in diameter and 36 cm in effective height) were operated in parallel with their average results being reported. The SBRs were operated at room temperature (25 ± 2 ºC) and illuminance of 146-157 μmol/m^2·s for 24 hours at the top and 88-97 μmol/m^2·s at the bottom by two artificial solar lights fixed above the SBRs.

The light illuminance was measured by a pocket digital lux meter (ANA-F11, Tokyo Photo-electric Co., Ltd., Japan). Before being inoculated to the SBRs for biomass accumulation, microalgae were centrifuged at 3000 rpm for 5 min after being washed with 0.9% sodium chloride to exclude the influence of CT medium.

Before the SBRs being operated in a 6 h cycle, pre-cultivation for biomass accumulation and settleability enhancement was performed for 10 days in both SBRs. During this pre-cultivation period, the influent was manually filled and discharged (after centrifugation). And non-aeration and aeration periods were alternatively operated every 3 hours. An air pump (AK-30, KOSHIN, JAPAN) was used for air supply at an air uplift velocity of 0.98 cm/s. After SBRs being switched to automatic operation, the cycle duration was set as 6 h, consisting of 2 min filling, 90 min non-aeration, 146 min aeration, 120 min settling and 2 min discharging at the very beginning. During the operation, the settling time was stepwise reduced from initial 120 min to 10 min, 5 min and 2 min, respectively. Meanwhile, the aeration time was increased accordingly with the reduction of settling time. The volumetric exchange ratio was kept at 50%, equivalent to a hydraulic retention time (HRT) of 12 h. After the initial granules appeared in the reactors on day 90, the algal biomass in the reactors was discharged daily to control the sludge retention time (SRT) at around 15 days.

2.3 Analytical methods

Mixed liquor (volatile) suspended solids (ML(V)SS) were measured to represent the biomass of microalgae or algal granules according to standard methods (APHA, 2012). Chlorophyll *a* (Chl-*a*) content was used to estimate the algal biomass according to the modified method proposed by Ngearnpat et al. (2018). Briefly, 10 ml sample of microalgae or algal granules after crushed completely with a glass rob was centrifuged at 3000 rpm for 5 min. Then 10 ml of 90% methanol solution containing 0.2% $MgCl_2$ was used to suspend the settled fraction after centrifugation, and the mixture was heated at 70°C for 30 min in the dark for extraction. The extract was centrifuged at 3000 rpm for 10 min after cooling down. The Chl-*a* concentration was determined at the wavelengths of 750, 665, 645 and 630 nm by a spectrophotometer (UV 1800, Shimadzu, Japan), and then calculated using Eq. (1):

$$\text{Chl-}a\ (mg/L) = (11.6\times(A_{665}-A_{750}) - 1.31\times(A_{645}-A_{750}) - 0.14\times(A_{630}-A_{750}))\times\frac{Vm}{Vf} \quad (1)$$

where *Vm* (ml) and *Vf* (ml） are the volume of the extraction methanol and sample, respectively.

Sludge volume index (SVI) (APHA, 2012) and settling velocity (Ghangrekar et al., 2005) were used to indicate the settleability of microalgae or algae granules. Integrity coefficient (%) is defined as the ratio of solids in the supernatant after being shaken on a platform shaker at 200 rpm for 5 min to the total solid weight of the determined sample (Ghangrekar et al., 2005), which was used to estimate the strength of algal granules in this study. Dissolved oxygen (DO) concentration in the reactors

was measured with a DO meter (HQ40d, Hach, USA), and pH was monitored using a compact pH-meter (Horiba, Japan).

The size of granules was measured using the wet sieve separation method as previously described (Cai et al., 2018), and the morphology of microalgae or algal granules was observed by Leica M205 C Microscope (Leica Micro-systems, Switzerland).

As for the evaluation of nutrients removal, the suspension samples were filtrated through 0.45 μm filter and the filtrates were used for determination. Ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and total phosphorus (TP) were analyzed according to standard methods (APHA, 2012). Dissolved organic carbon (DOC) was quantified by a TOC analyzer (TOC-VCSN, Shimadzu, Japan) equipped with an auto-sampler (ASI-V, Shimadzu, Japan).

The extraction of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) was performed using the heating method (Morgan et al., 1990). Polysaccharides (PS) and proteins (PN) contents were determined by the phenol-sulfuric acid method (DuBois et al., 1956) and Lowry-Folin method (Lowry et al., 1951) with glucose and bovine serum albumin (BSA) as the standards.

As for heat value, the samples were firstly dried at 105°C for 24 h to constant weight, and then determined using Auto-Calculating Bomb Calorimeter (CA-4AJ, Shimadzu, Japan)

Samples for microbial community analysis were harvested on day 0, day 25, day 105 and day 155, respectively, and the methods for DNA extraction and PCR were described elsewhere (Zhang et al., 2020). The specific primers for bacteria and algae to amplify the hypervariable region of 16S rRNA and 18S rRNA were also the same as Zhang et al. (2020). After quantification using QuantiFluorTM-ST (Promega, USA), the PCR products of all samples were taken for high-throughput sequencing on Illumina platform (Illumina PE250, USA). MOTHUR (version: 1.31.2) was used for analyzing the biodiversity of bacteria and eukaryote in the samples.

2.4 Calculations

DOC, NH_4^+-N, and TP removal efficiencies were calculated according to Eq. (2).

$$\text{Removal rate (\%)} = 100\times\ (\rho_{inf} - \rho_{eff}) / \rho_{inf} \quad (2)$$

in which ρ_{inf} (mg/L) and ρ_{eff} (mg/L) are the influent and effluent DOC, NH_4^+-N or TP concentrations, respectively.

TP removal capacity was calculated according to Eq. (3) modified from Cai et al. (2016), which was used to describe P removal on the basis of MLSS.

$$\text{TP removal capacity (mg/g-MLSS}\cdot\text{ d)} = 4\times\ (TP_{inf} - TP_{eff})\times 0.5/\ (MLSS\times\ 0.9) \quad (3)$$

where TP_{inf} (mg P/L) and TP_{eff} (mg P/L) are the average influent and effluent TP concentrations during the operation period, respectively; MLSS (g/L) is the average MLSS concentration in SBR during this period, and 4, 0.5 and 0.9 are the number of cycles per day, exchange ratio and working volume (L) of SBR, respectively in this study.

3. Results and discussion

3.1 Morphological changes and formation of microalgae granules

A. falcatus var. *acicularis* is a kind of unicellular green microalgae and its cells are acicular to narrowly fusiform with the ends of taping to acute apices, 2-4 μm wide and 25-45 μm long. It represents one of the smallest taxa from microalgae category. The seed algae are independently single cells smaller than 50 μm under microscopic observation (Fig. 1a). After 40 days' operation, the microalgae were aggregated with white organisms to form activated algae flocs, exhibiting irregular and mainly opened flocs, and filamentous microorganisms and the inoculated algae were frequently observed (Figs. 1b and f). Along with the operation, an obvious decrease in the number of free microalgae was noticed. The mean diameter of activated algae flocs was about 480 ± 11.2 μm after 90 days' operation and irregular algae granules appeared with loose structure (Figs. 1c and g). Some vorticella and rotifers anchored into the granules or adhered to the surface of granules under microscope observation, which is in agreement with Cai et al. (2018). After 150 days' operation, regular algae granules with a compact structure and average size of 2.25 mm predominated the reactors (Figs. 1d and h).

According to the experimental records (Fig. S1), only a small percentage (6.10 ± 0.09%) of total biomass volume were granules < 1 mm, and the largest proportion (50.00 ± 1.12%) were granules > 2 mm. The remaining biomass (43.91%) were composed of larger granules > 3 mm (32.93 ± 1.12%), and medium-sized granules

ranged between 1 mm and 2 mm (10.98 ± 0.90%). The above size change revealed an evolution of microalgae from single cells to microalgae granules with round shape and compact structure (Fig. 1). Compared to the light green color of the single cells of *A. falcatus var. acicularis*, the mature microalgae granules reflected a dark-green color (Fig. 1h).

3.2 Changes in biomass concentration during granulation in the reactors

The initial MLSS of seed algae were around 0.32 g/L in each SBR (after pre-cultivation), which remarkably increased to 2.19 g/L within 10 days after being inoculated to the SBRs fed with the synthetic digestion liquor containing high nutrients concentration (Fig. 2a). A slight decrease (to 1.59 g/L) was detected on day 14 due to a shorter settling time applied (from 2 h to 10 min). After that, the biomass concentration steadily increased till day 95 (5.45 g/L), and then decreased with some fluctuations due to the start of SRT control at 15 days. After day 112, the biomass concentration increased again and peaked at 4.0 g/L on day 129 when 5 min settling time was applied. From day 130 on, when the settling time was decreased to 2 min, the biomass concentration decreased to around 2.0 g/L and kept stable. According to the experimental results, the biomass concentration (2-4 g/L) and productivity (130-270 mg/L/d) of *A. falcatus var. acicularis* in this study are respectively around 10-20 times and 16-34 times that cultivated with traditional suspension method under the optimum conditions (0.21g/L and 7.9 mg/L/d) (George et al., 2014). This observation

implies that biogranulation process for microalgae cultivation can achieve much higher biomass concentration and biomass productivity in the reactors, reflecting great potential for large reductions in reactor volume and land occupation, and energy saving.

The ratio of MLVSS to MLSS (MLVSS/MLSS) denotes the organic proportion of the total biomass. The MLVSS/MLSS ratio in this study was observed to decrease sharply from around 0.8 to lower than 0.6 during the first 20 days, then increase continuously during the granulation period till the appearance of mature granules (Fig. 2a). This ratio kept greater than 0.7 from day 60 to day 100; and a relatively stable MLVSS/MLSS ratio ranging between 0.58 to 0.67 was noticed after day 130, possibly attributable to the balance between algal-bacterial symbiosis growth and minerals accumulation. This observation agrees with Cai et al. (2018) who cultivated algae granules by using natural microalgae. As noticed, Chl-*a* content showed almost the same trend as the biomass concentration in the reactors. In addition, a higher Chl-*a* content was also detected during the granulation period. As Hu et al. (2017) pointed out that, the decrease of Chl-*a* content is associated with the combination effect of community change, nutrients deficiency, aging and the accumulation of EPS and chemical precipitates in the biomass. Thus, further characterization of the biomass or microalgae granules should be conducted.

3.3 *Changes of microalgae settleability during the granulation*

The pure *A. falcatus* var. *acicularis* cells are generally suspended in the culture medium, which are difficult to be separated. As seen, along with the formation of granules, more biomass can be easily separated from the liquor only after 5 min sedimentation. Also, the SVI_{30} value showed a decline trend during the granulation, from > 3000 ml/g of the seed algae cells to 205.48 ml/g on day 10, and finally to 53.44 ± 3.31 ml/g for the mature granules (Fig. 2b), revealing the increase of settling efficiency during operation. The SVI_{30} data of mature granules are slightly greater than those conventional bacterial AGS (~ 25 ml/g) and algal-bacterial AGS (~38 ml/g) (Huang et al., 2015), reflecting their different structure compactness. A higher proportion of algae in the granules obtained in this study might be the major reason for this settleability difference. While this observation to some extent is in agreement with the findings by Choi et al. (2010) and Su et al. (2012). However, compared to the seed microalgae cells in this study, the settleability of microalgae granules was remarkably improved, and almost all the biomass can be harvested within 2 min after maturation, which can also be reflected by the changes of settling velocity (Fig. 2b). Tiron et al. (2017) stated that the settling velocity of microalgae cells (*Chlorella* sp.) was lower than 0.54×10^{-2} m/h. In another previous study, microalgae settleability was claimed to efficiently achieve with a settling velocity of 0.36 m/h (Granados et al., 2012). The biomass settling velocity in this study reached averagely 18.47 ± 0.23 m/h after the algae cells being transformed to activated algae granules, which is also

faster than the algae granules achieved from natural microalgae flocs (Cai et al., 2019).

3.4 Overall performance on nutrients removal

Microalgae can utilize carbon, nitrogen, phosphorus and other nutrients for biomass accumulation simultaneously from wastewater. Nutrients removal efficiencies during the operation are illustrated in Fig. 3. The granules exhibited excellent organic removal efficiency: The effluent DOC was always less than 5 mg/L after day 60, achieving 95.70± 1.22% of DOC removal till the end of experiments (Fig. 3a).

P removal capability was 7.34 ± 0.53 mg-P/g-MLSS·d, which is higher than the bacterial AGS (6.15 mg-P/g-MLSS·d) treating the same synthetic digestion liquor (Cai et al., 2016). During day 66 to day 86, the SBRs showed better phosphorus utilization capacity and produced effluent with a lower TP concentration. This observation is closely related to the relatively high algae biomass concentration during this period (Fig. 2a) as no SRT control was applied, suggesting this microalgae strain has high potential for phosphorus uptake from wastewater. The TP content in biomass was also monitored during the operation. After mature granules formed, the granular TP content kept relatively stable, around 53.33 ± 3.48 mg/g-MLSS in the granules (Table 1).

As for N removal, after 30 days' operation, the effluent NH_4^+-N concentration was <14.41± 2.71 mg/L, averagely achieving 85.59 ± 2.71% of NH_4^+-N removal till the end of experiments. This observation indicates that a stable nitrification could be quickly established in this reactor system. However, TN removal was about 54.66 ± 0.86 % after reaching a stable state, due to nitrite accumulation occurred under the test conditions. In this study, nitrite concentration (26.06 ± 1.49 mg/L) higher than nitrate concentration (4.50 ± 1.93 mg/L) was always detected in the effluent. This phenomenon is to some extent in agreement with the finding by Liu et al. (2017) who inoculated two microalgae strains (*Chlorella* and *Scenedemus*) into aerobic granules.

In order to better understand carbon, phosphorus and nitrogen uptake by the mature algae granules, typical cycle tests were also conducted on day 150 (Fig. S2). The DOC concentration decreased sharply from 43.83 ± 1.17 mg/L to 11.59 ± 0.12 mg/L within 120 min, revealing that organic carbon can be utilized efficiently during the non-aeration period, possibly attributable to the co-existing bacteria. DO level was detected to decrease from 3.32 mg/L to 0.32 mg/L during the non-aeration phase, suggesting the possible existence of some heterotrophs in the reactors, and then increased to 6.36 ± 0.10 mg/L during the aeration period. A higher P uptake was detected in the aeration phase than in the non-aeration phase, implying that P was consumed by algae as well as by phosphorus accumulating organisms (PAOs). With the decrease of NH_4^+ -N concentration, NO_2^- -N accumulated gradually with no

obvious change of NO_3^--N during the aeration phase. That is, partial nitrification may occur in the reactors.

3.5 Variation of EPS extracted from algae biomass

EPS are metabolic products accumulated on the surface of algal and bacterial cells, which contain a variety of organic substances such as exopolysaccharides (PS) and exoproteins (PN) (Wang et al., 2006). EPS are considered to play an important role in the granulation and flocculation of activated sludge. EPS can be mainly divided into two fractions, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), which are identified by their different distribution in the layered structure of AGS (Adav et al., 2008; Li and Yang, 2007). LB-EPS and TB-EPS contents and their major components (PN and PS) were also quantified during the granulation in this study (Fig. 4). It can be clearly seen that the amount of total EPS increased remarkably from 63.06 ± 3.42 mg/g-MLVSS of seed algae (day 0) to 162.54 ± 3.60 mg/g-MLVSS of mature algae granules (day 150). During the granulation period, however, a slight decrease of total EPS on day 90 (123.78 ± 5.30 mg/g-VSS) was detected when compared to day 40 (131.93 ± 1.37 mg/g-VSS), mainly contributed by the decrease in PS content (LB-PS and TB-PS, from 29.12 ± 0.39 mg/g-VSS to 13.21 ± 0.13mg/g-VSS) in the aggregates (Fig. 4). Wang et al. (2006) claimed that EPS can serve as nutrients and energy source in the starvation phase, in which PS are easier to be utilized than PN. In this study, the biomass concentration was maintained at a

relatively high level around day 90 (Fig. 2a) due to no SRT control was applied, providing more possibility for microorganisms' starvation and EPS consumption. The good correlation between EPS and MLSS and/or SVI indicates that EPS could accelerate the accumulation of biomass, thus promoting algae granulation. Both LB-EPS and TB-EPS showed a similar increase trend with the total EPS content, while TB-EPS were observed to be more sensitive to algae granulation in this study. In addition, a continuous increase in PN/PS ratio was noticed during the granulation process, from 0.81 to 6.05 as per LB-EPS, from 2.19 to 8.54 for TB-EPS, and from 1.40 to 7.42 for the total EPS, reflecting that a higher PN/PS ratio is beneficial for algae granulation. The above results imply that a higher content of EPS is advantageous for algae granulation, and TB-EPS may play an important role in the maintenance of stable algae granules. Further research is still necessary on the functions of the various kinds of PN and PS in EPS.

3.6 Changes in biological community during granulation process

The changes in biological community structure from seed algae to algal granules were explored through high-throughput sequencing technology. The dominant eukaryote at genus level and bacteria at phylum level are shown in Fig. 5. As expected, the seed algae *Ankistrodesmus* genus kept predominant in the eukaryote community of granular consortia. No obvious accumulation of other harmful algae that would greatly affect the harvesting and algal composition was observed. The

proportion of *Ankistrodesmus* genus was found to gradually decline from 99.99% (day 0) to 96.61% (day 25), 58.92% (day 105), and 17.77% (day 155), while the eukaryote species quantity and diversity increased as seen from the increased operational taxonomic units (OTUs) and the decreased Simpson index (Table S1). Unclassified eukaryote and chlorella emerged after day 25, occupying about 2.20% and 1.19%, respectively. It was noticed that the percentage of unclassified eukaryote increased to 40.99% on day 105 after the formation of algae granules, most probably attributable to some planktons such as vorticella and rotifer as they were frequently observed under microscope. Li et al. (2013) also reported that vorticella and rotifer appeared during aerobic granulation, which showed some positive effect on reduction in effluent suspended solids. Beun et al. (1999) claimed that protozoa enhanced the granulation process at the beginning of AGS system. Further research should also be concentrated on the identification of unclassified eukaryote and their effects on granule formation. The genus *Chlorella* from family *Chlorellaceae* occupied about 15.85% in the mature granules. As Nuramkhaan et al. (2019) pointed out, *Chlorella* sp. has excellent auto-aggregation capability with a higher EPS excretion, which may accelerate the formation and stable operation of algal-bacterial AGS. Results from this study indicate that *Chlorella* may also play an important role in the formation of algae granules from single cell suspension of *Ankistrodesmus*.

The predominant bacteria cover Proteobacteria, Firmicutes, Cyanobacteria and Bacteroidetes, accounting for 92.80%, 99.25%, 97.45% and 97.76% on day 0, day 25,

day 105 and day 155, respectively. No significant difference was found in the proportion of dominant bacteria between the seed algae and the mature granules except the phylum Actinobacteria which almost disappeared after granules formed. Nitrite oxidizing bacteria (NOB) belonging to phylum Nitrospirae shortly appeared in the reactors, accounting for 0.41% in the initial granules, while vanished in the mature granules. This observation suggests that this kind of bacteria were inhibited under the symbiotic growth of algae and bacteria, in agreement with the finding from Huang et al. (2015). The disappearance of NOB can also be reflected by the accumulation of NO_2^--N in the effluent (Fig. 3b and Fig. S2). In the mature granules, the proportion of phylum Proteobacteria was about 48.74%, the largest one in bacterial community, and the percentage of order Betaproteobacteriales from this phylum was found to largely increase from 6.20% in the seed algae suspension to 19.49 %. Two genera, *Thauera* and *Zoogloea* from the family Rhodocyclaceae belonging to the order Betaproteobacteriales appeared after granulation, about 2.28% and 4.84%. These two genera are reported to closely associate with EPS excretion (Allen et al., 2004; Shao et al., 2009). The secondly higher percentage phylum was Bacteriodetes, about 34.46%, were reported to have capability of complex biopolymers degradation (Weon et al., 2005), providing the possibility for the treatment of wastewater containing higher organic compounds by using this algae-based technology. Moreover, the phylum Cyanobacteria increased from 3.04 % to 13.34%, in which class Oxyphotobacteria occupied 13.33%. According to the OTUs and Simpson index

results, the bacterial community diversity was greatly improved during the granulation process (Table S1). Restated, further works are still necessary on the optimization of algae cultivation conditions to enhance the target microalgae proportion in the formed granules and the functional identification of algal and bacterial species involved in the formation of granules.

3.7 Preliminary analysis on energy consumption for microalgae harvesting in this study

The preliminary analysis on energy consumption for microalgae harvesting was conducted among this novel biogranulation and conventional harvesting methods according to the energy and materials consumption data in this experiment and previous studies (Brentner et al., 2011; Shi et al., 2019) with the algae harvesting step being only considered (Table 2). The electricity requirement was calculated based on per kg of algae output, and the energy consumption in this study was assumed to be mainly from artificial light, air pump, influent and effluent pumps. Daily energy consumption of each equipment was calculated as the total operation time per day multiplied by energy consumption rate mentioned in the device specification sheet.

As shown in Table 2, centrifugation process consumes 10.8 MJ/kg-algae output, which is much higher than other technologies. Probably this is the major reason why centrifugation is generally applied for harvesting algae cells rich in more valuable compounds other than those for biofuels (Brentner et al., 2011). Electrocoagulation

requires pretty lower electricity, but its downstream process is usually affected by the metal (like Fe) retained in the algae biomass (Richardson et al., 2014; Richardson and Johnson, 2015). As for the chitosan flocculation method, the algae harvesting efficiency is proportional to the initial biomass concentration and the highest 95% can be achieved under optimum chitosan addition at a relatively low energy consumption (1.98×10^{-1} MJ/kg-algae output). The main barriers for its large-scale application are the cost for chemicals addition and the residual co-product added into the culture medium (Beach et al., 2012; Billuri et al., 2015). Ultrasonic technology can concentrate algae cells and continuously separate lipids and proteins by applying a standing acoustic wave, and no chemicals and materials are consumed at a relatively low energy consumption. However, the average harvesting efficiency (64%) by this technology is relatively low compared to other technologies. As for biogranulation, microalgae granules can be harvested by gravity sedimentation easily within 2 min without energy consumption, and only wastewater (digestion liquor in this study) is fed with no other materials addition. In addition, this biogranulation technology can maintain much higher biomass concentration at 2-4 g/L with a much higher biomass productivity (130-270 mg/L/d) in the reactors, which can definitely compete against all the other algae harvesting technologies in large scale applications.

On the other hand, the heating value of microalgae was detected to decrease from the initial 22.79 MJ/kg (day 0) to 13.33 MJ/kg-MLSS on day 150 (Table 1), which is still much higher than 9.45 MJ/kg-cultivated or harvested algae by other technologies

(Shi et al., 2019). When taking its much higher biomass productivity into consideration, biogranulation process using digestion liquor can realize quick cultivation and harvesting, which is more promising than other microalgae cultivation and harvesting methods. Still, it should be further addressed whether the biogranulation process influence the lipid production from *A. falcatus* var. *acicularis* and more in-depth research works should be conducted on the optimization of granulation conditions (light-on/light-off cycle, aeration time, etc.), targeting higher oil production capacity.

4. Conclusions

Ankistrodesmus falcatus var. acicularis can be harvested within 2 minutes by simple gravity sedimentation after microalgae granule formation, which was stably achieved around 150 days' operation of SBRs. The granulation system can maintain much higher algae biomass concentration (2-4 g/L) with much higher biomass productivity (130-270 mg/L/d) compared to the traditional suspension cultivation method under optimum conditions. The microalgae granules showed great potentials for nutrients removal from digestion liquor with excellent and stable DOC (95.7± 1.22%) and NH_4^+-N (85.59 ± 2.71%) removals. Future research is directed to the optimization of granulation process for a stable lipid content in microalgae granular biomass and function identification of algal and bacterial species in the granulation process.

Supplementary materials

Supplementary data of this work can be found in online version of the paper.

Declarations of Interest

None

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References

Adav, S. S., Lee, D.-J., Tay, J.-H., 2008. Extracellular polymeric substances and structural stability of aerobic granule. *Water Res.* 42 (6-7), 1644-1650.

Allen, M. S., Welch, K. T., Prebyl, B. S., Baker, D. C., Meyers, A. J., & Sayler, G. S., 2004. Analysis and glycosyl composition of the exopolysaccharide isolated from the floc-forming wastewater bacterium *Thauera* sp. MZ1T. *Environ. Microbiol. 6*(8), 780-790.

Álvarez-Díaz, P. D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Pérez, M. C., Perales, J. A., 2017. Freshwater microalgae selection for simultaneous wastewater nutrient removal and lipid production. *Algal Res. 24*, 477-485.

APHA, 2012. Standard Methods for the Examination of Water and Wastewater. American Public Health Association/American Water Work Association/Water environment Federation, Washington, D.C., USA.

Barker, D. J., Mannucchi, G. A., Salvi, S. M., Stuckey, D. C., 1999. Characterisation of soluble residual chemical oxygen demand (COD) in anaerobic wastewater treatment effluents. *Water Res. 33*(11), 2499-2510.

Barros, A. I., Gonçalves, A. L., Simões, M., Pires, J. C., 2015. Harvesting techniques applied to microalgae: a review. *Renew. Sust. Energ. Rev. 41*, 1489-1500.

Beach, E. S., Eckelman, M. J., Cui, Z., Brentner, L., Zimmerman, J. B., 2012. Preferential technological and life cycle environmental performance of chitosan flocculation for harvesting of the green algae *Neochloris oleoabundans*. *Bioresour. Technol*. *121*, 445-449.

Beun, J. J., Hendriks, A., Van Loosdrecht, M. C. M., Morgenroth, E., Wilderer, P. A., Heijnen, J. J., 1999. Aerobic granulation in a sequencing batch reactor. *Water Res*. *33*(10), 2283-2290.

Billuri, M., Bonner, J. S., Fuller, C. B., Islam, M. S., 2015. Impact of natural cationic polymers on charge and clarification of microalgae suspension. *Environ. Eng. Sci*. *32*(3), 212-221.

Borowitzka, M. A., 2013. Techno-economic modeling for biofuels from microalgae. In Borowitzka M., Moheimani N. (eds), *Algae for Biofuels and Energy,* part of book series of *Developments in Applied Phycology*, vol. 5 (pp. 255-264). Springer, Dordrecht.

Brennan, L., Owende, P., 2010. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renew. Sust. Energ. Rev*. *14*(2), 557-577.

Brentner, L. B., Eckelman, M. J., Zimmerman, J. B., 2011. Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel. *Environ. Sci. Technol*. *45*(16), 7060-7067.

Cabanelas, I. T. D., Arbib, Z., Chinalia, F. A., Souza, C. O., Perales, J. A., Almeida, P. F., Druzian, J.I., Nascimento, I. A., 2013. From waste to energy: Microalgae production in wastewater and glycerol. *Appl. Energ*. *109*, 283-290.

Cai, W., Huang, W., Li, H., Sun, B., Xiao, H., Zhang, Z., Lei, Z., 2016. Acetate favors more phosphorus accumulation into aerobic granular sludge than propionate during the treatment of synthetic fermentation liquor. *Bioresour. Technol*. *214*, 596-603.

Cai, W., Jin, M., Zhao, Z., Lei, Z., Zhang, Z., Adachi, Y., Lee, D.-J., 2018. Influence of ferrous iron dosing strategy on aerobic granulation of activated sludge and bioavailability of phosphorus accumulated in granules. *Bioresour. Technol. Rep*. *2*, 7-14.

Cai, W., Zhao, Z., Li, D., Lei, Z., Zhang, Z., Lee, D.-J., 2019. Algae granulation for nutrients uptake and algae harvesting during wastewater treatment. *Chemosphere 214*, 55-59.

Chen, C.-Y., Yeh, K.-L., Aisyah, R., Lee, D.-J., Chang, J.-S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresour. Technol*. *102*(1), 71-81.

Choi, O., Das, A., Yu, C. P., Hu, Z., 2010. Nitrifying bacterial growth inhibition in the presence of algae and cyanobacteria. *Biotechnol. Bioeng*. *107*(6), 1004-1011.

Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnol. Adv*. *29*(6), 686-702.

Demirbas, A., Demirbas, M. F., 2011. Importance of algae oil as a source of biodiesel. *Energ. Convers. Manage*. *52*(1), 163-170.

Demirbas, M. F., 2011. Biofuels from algae for sustainable development. *Appl. Energ*. 88(10), 3473-3480.

Do Nascimento, M., de los Angeles Dublan, M., Ortiz-Marquez, J. C. F., Curatti, L., 2013. High lipid productivity of an *Ankistrodesmus–Rhizobium* artificial consortium. *Bioresour. Technol*. *146*, 400-407.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem*. *28*(3), 350-356.

George, B., Pancha, I., Desai, C., Chokshi, K., Paliwal, C., Ghosh, T., Mishra, S., 2014. Effects of different media composition, light intensity and photoperiod on morphology and physiology of freshwater microalgae *Ankistrodesmus falcatus*–A potential strain for bio-fuel production. *Bioresour. Technol*. *171*, 367-374.

Ghangrekar, M. M., Asolekar, S. R., Joshi, S. G., 2005. Characteristics of sludge developed under different loading conditions during UASB reactor start-up and granulation. *Water Res*. 39(6), 1123-1133.

González-Fernández, C., Ballesteros, M., 2013. Microalgae autoflocculation: an alternative to high-energy consuming harvesting methods. *J. Appl. Phycol*. *25*(4), 991-999.

Granados, M. R., Acién, F. G., Gomez, C., Fernandez-Sevilla, J. M., Grima, E. M., 2012. Evaluation of flocculants for the recovery of freshwater microalgae. *Bioresour. Technol*. *118*, 102-110.

Grima, E. M., Belarbi, E. H., Fernández, F. A., Medina, A. R., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol. Adv*. *20*(7-8), 491-515.

Hu, Y., Hao, X., van Loosdrecht, M., Chen, H., 2017. Enrichment of highly settleable microalgal consortia in mixed cultures for effluent polishing and low-cost biomass production. *Water Res*. *125*, 11-22.

Huang, W., Li, B., Zhang, C., Zhang, Z., Lei, Z., Lu, B., Zhou, B., 2015. Effect of algae growth on aerobic granulation and nutrients removal from synthetic wastewater by using sequencing batch reactors. *Bioresour. Technol*. *179*, 187-192.

Ji, Z., Chen, Y., 2010. Using sludge fermentation liquid to improve wastewater short-cut nitrification-denitrification and denitrifying phosphorus removal via nitrite. *Environ. Sci. Technol*. *44*(23), 8957-8963.

Kadir, W. N. A., Lam, M. K., Uemura, Y., Lim, J. W., Lee, K. T., 2018. Harvesting and pre-treatment of microalgae cultivated in wastewater for biodiesel production: a review. *Energ. Convers. Manage*. *171*, 1416-1429.

Li, J., Ma, L., Wei, S., Horn, H., 2013. Aerobic granules dwelling vorticella and rotifers in an SBR fed with domestic wastewater. *Sep. Purif. Technol*. *110*, 127-131.

Li, X. Y., Yang, S. F., 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Res*. *41*(5), 1022-1030.

Liu, L., Fan, H., Liu, Y., Liu, C., Huang, X., 2017. Development of algae-bacteria granular consortia in photo-sequencing batch reactor. *Bioresour Technol*. *232*, 64-71.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem*. *193*, 265-275.

Mathimani, T., Mallick, N., 2018. A comprehensive review on harvesting of microalgae for biodiesel–Key challenges and future directions. *Renew. Sust. Energ. Rev*. *91*, 1103-1120.

Morgan, J. W., Forster, C. F., Evison, L., 1990. A comparative study of the nature of biopolymers extracted from anaerobic and activated sludges. *Water Res*. *24*(6), 743-750.

Ngearnpat, N., Klayluk, B., Kumla, A., Ngamta, S., Issakul, K., 2018. Phytoplankton composition and water quality of Kwan Phayao Reservoir, Thailand, during rainy and cold dry seasons. *J. Food Heal. Bioenviron. Sci*. *11*(2), 46-55.

Nuramkhaan, M., Zhang, Y., Dong, X., Huang, W., Lei, Z., Shimizu, K., Zhang, Z., Utsumi, M., Lee, D.-J., 2019. Isolation of microalgal strain from algal-bacterial aerobic granular sludge and examination on its contribution to granulation

process during wastewater treatment in respect of nutrients removal, auto-aggregation capability and EPS excretion. *Bioresour. Technol. Rep*. *8*, 100330.

Pragya, N., Pandey, K. K., Sahoo, P. K., 2013. A review on harvesting, oil extraction and biofuels production technologies from microalgae. *Renew. Sust. Energ. Rev*. *24*, 159-171.

Richardson, J. W., Johnson, M. D., 2015. Financial feasibility analysis of NAABB developed technologies. *Algal Res*. *10*, 16-24.

Richardson, J. W., Johnson, M. D., Lacey, R., Oyler, J., Capareda, S., 2014. Harvesting and extraction technology contributions to algae biofuels economic viability. *Algal Res*. *5*, 70-78

Schenk, P. M., Thomas-Hall, S. R., Stephens, E., Marx, U. C., Mussgnug, J. H., Posten, C., Kruse O., Hankamer, B., 2008. Second generation biofuels: High-efficiency microalgae for biodiesel production. *Bioenerg. Res*. *1*(1), 20-43.

Shao, Y., Chung, B. S., Lee, S. S., Park, W., Lee, S. S., & Jeon, C. O., 2009. *Zoogloea caeni* sp. nov., a floc-forming bacterium isolated from activated sludge. *Int. J. Syst. Evol. Micr*. *59*(3), 526-530.

Shi, R., Handler, R. M., Shonnard, D. R., 2019. Life cycle assessment of novel technologies for algae harvesting and oil extraction in the renewable diesel pathway. *Algal Res*. *37*, 248-259.

Su, Y., Mennerich, A., Urban, B., 2012. Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. *Bioresour. Technol*. *105*, 67-73.

Tasić, M. B., Pinto, L. F. R., Klein, B. C., Veljković, V. B., Maciel Filho, R., 2016. *Botryococcus braunii* for biodiesel production. *Renew. Sust. Energ. Rev*. *64*, 260-270.

Tiron, O., Bumbac, C., Manea, E., Stefanescu, M., Lazar, M. N., 2017. Overcoming microalgae harvesting barrier by activated algae granules. *Sci. Rep*. *7*(1), 4646.

Wang, Z., Liu, L., Yao, J., Cai, W., 2006. Effects of extracellular polymeric substances on aerobic granulation in sequencing batch reactors. *Chemosphere* *63*(10), 1728-1735.

Waterbury, J. B., Stanier, R. Y., 1981. Isolation and Growth of Cyanobacteria from Marine and Hypersaline Environments. In: Starr M.P., Stolp H., Trüper H.G., Balows A., Schlegel H.G. (eds), *The Prokaryotes* (pp.221-223). Springer Berlin Heidelberg.

Weon, H. Y., Kim, B. Y., Kwon, S. W., Park, I. C., Cha, I. B., Tindall, B. J., ... & Go, S. J., 2005. *Leadbetterella byssophila* gen. nov., sp. nov., isolated from cotton-waste composts for the cultivation of oyster mushroom. *Int. J. Syst. Evol. Micr*. *55*(6), 2297-2302.

Zhang, Y., Dong, X., Liu, S., Lei, Z., Shimizu, K., Zhang, Z., Adachi, Y., Lee, D. J., 2020. Rapid establishment and stable performance of a new algal-bacterial

granule system from conventional bacterial aerobic granular sludge and preliminary analysis of mechanisms involved. *J. Water Process Eng*. *34*, 101073.

Zheng, Y.-M., Yu, H.-Q., Sheng, G.-P., 2005. Physical and chemical characteristics of granular activated sludge from a sequencing batch airlift reactor. *Process Biochem*. *40*(2), 645-650.

Tables

Table 1

Main characteristics of seed algae and mature algae granules in the reactors before and after granulation.

Parameters	Day 0	Day 150
Mean size	25-45 μm	2.25 mm
SVI_{30} （ml/g）	3125 ml/g	53.44 ± 3.31
Settling velocity (m/h)	~ 0	18.47 ± 0.23
Integrity coefficient (%)	--	6.18 ± 0.01
Total phosphorus content (mg/g-TSS)	3.32 ± 0.18	53.33 ± 3.48
Total nitrogen content (mg/g-TSS)	11.19 ± 0.98	24.99 ± 1.65
Heating value	22.79 MJ/kg	13.33 MJ/kg

All data are the average values of triplicate tests.

Table 2

Comparative analysis of performance, chemicals consumption and energy inputs among algae harvesting technologies

Harvesting technologies	Centrifugation[a]	Membrane filtration [b]	Electrocoagulation[b]	Chitosan flocculation[b]	Ultrasonic[b]	Biogranulation (this study)
Electricity required (MJ/kg-algae output)	10.8	7.45×10^{-1}	9.55×10^{-2}	1.98×10^{-1}	1.29×10^{-1}	0 (Only gravity sedimentation used for harvesting step)
Chemicals/ materials consumption	No	Porous Ni membrane	Fe	Chitosan	No	No
Harvesting efficiency	~95%	~100%	~98%	~95%	~64%	~100%
Time consumption (min/ton-algae)	10 ~ 30 (based on centrifuge capacity)	21.4	146.77	20 ~ 30	2.18	$\leq$ 2

[a] Brentner et al. (2011); [b] Shi et al. (2019)

Figures

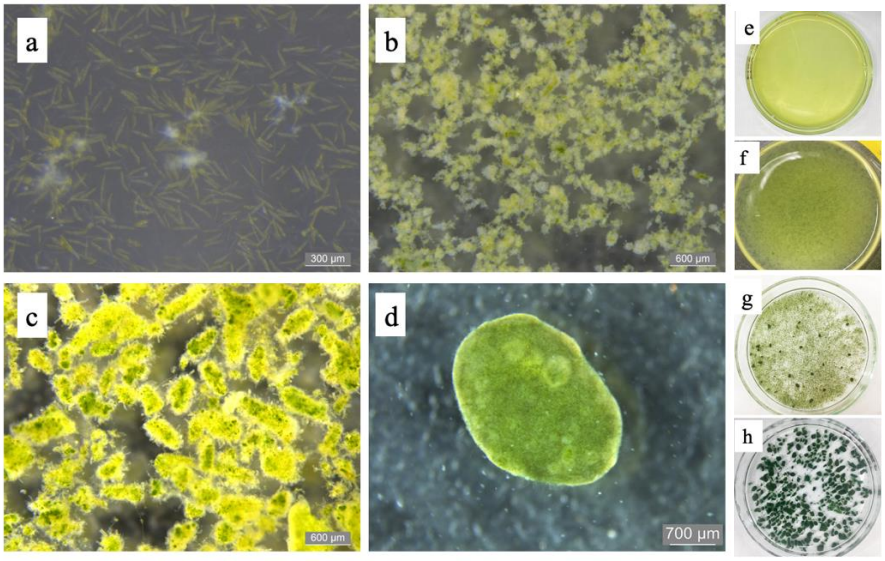
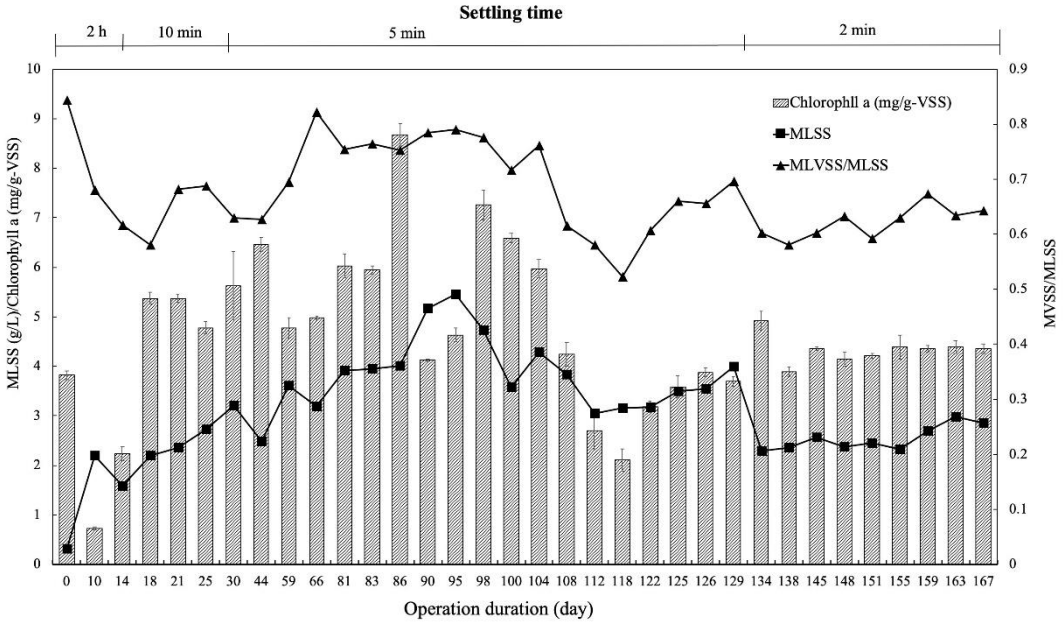


Figure 1. Transition from single cells of microalgae to granular structure. Light microscopy images of algae and algae aggregates during granulation: (a, e) seed algae, (b, f) activated algae flocs on day 44, (c, g) developed activated algae aggregates on day 90, and (d, h) mature activated algae granules on day 150.

(a)



(b)

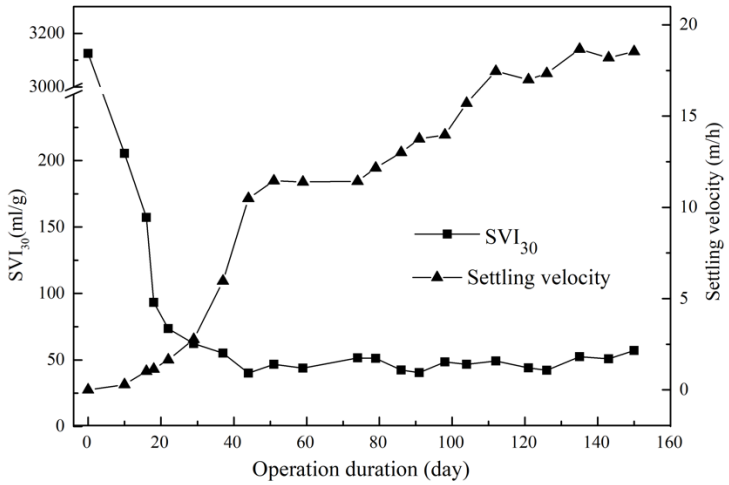
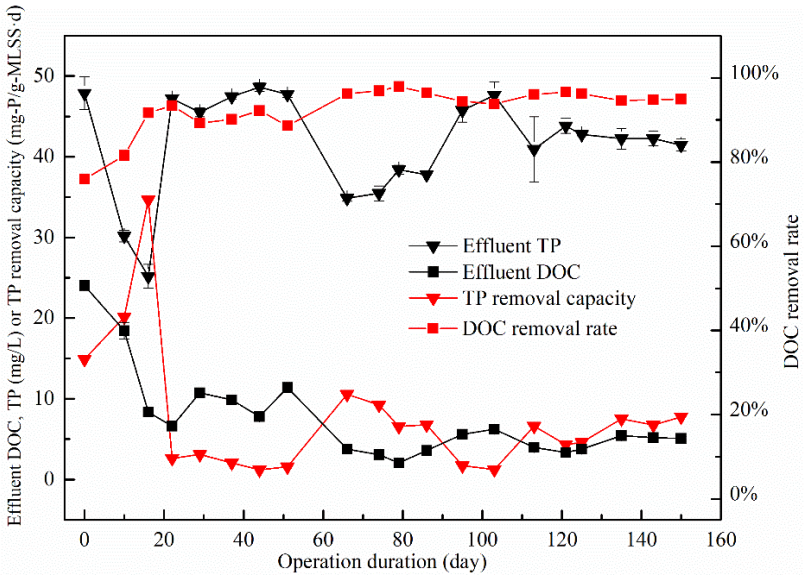


Figure 2. Variations of MLSS, MLVSS/MLSS, chlorophyll *a* (a), and SVI_{30} and settling velocity during algae granule formation.

(a)



(b)

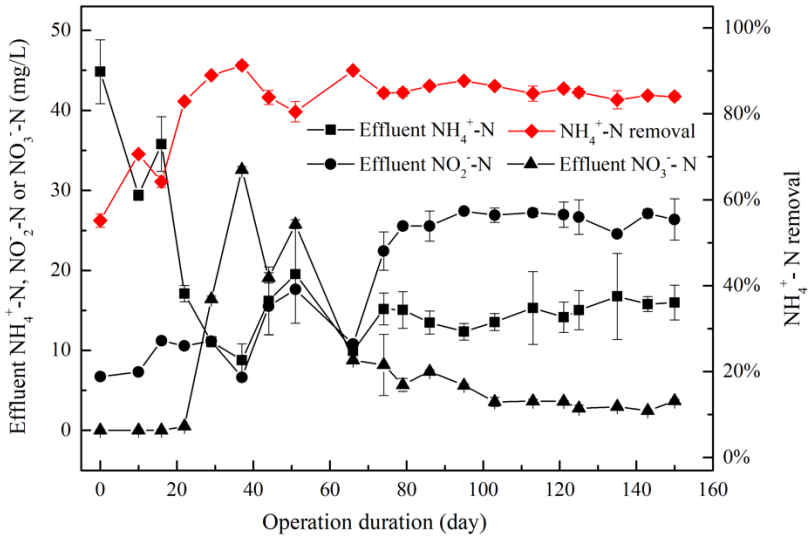


Figure 3. Nutrients variation and removal during operation. (a) Effluent TP and DOC concentrations, TP removal capacity and DOC removal rate; (b) Effluent ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N) and nitrite nitrogen (NO_2^--N) concentrations and NH_4-N removal rate.

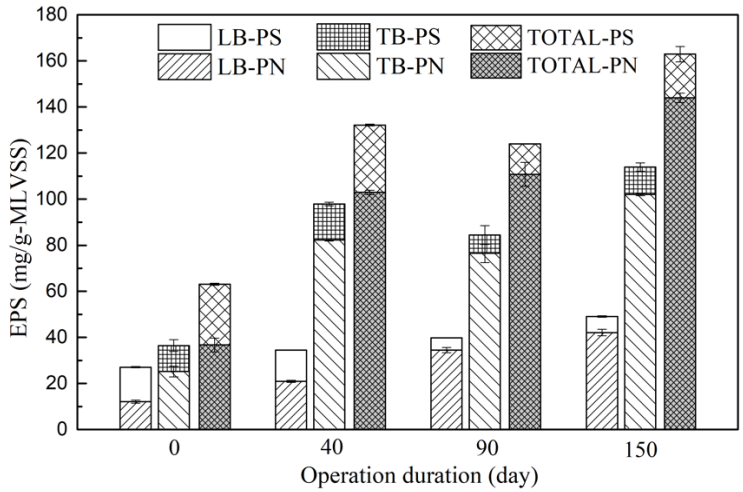
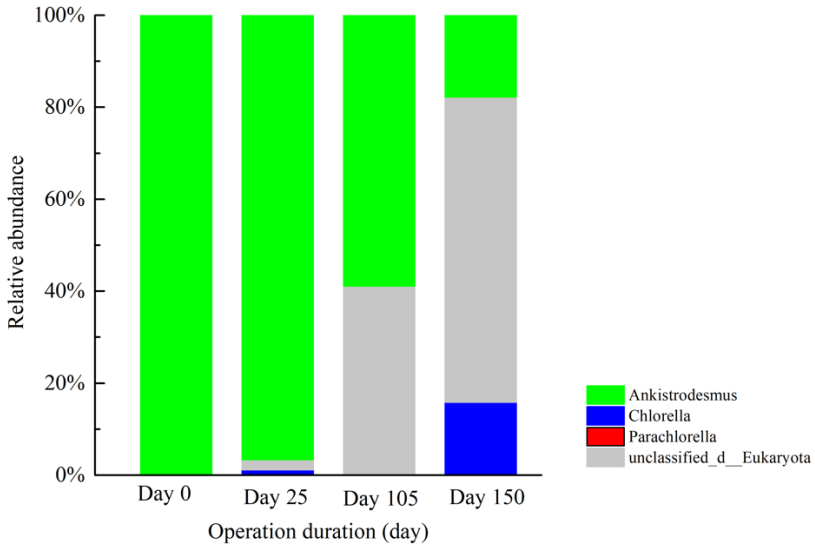


Figure 4. Changes of loosely bound EPS (LB- EPS), tightly bound EPS (TB-EPS), and total EPS contents, and their major components (proteins (PN) and polysaccharides (PS)) in the algae biomass or granules.

(a)



(b)

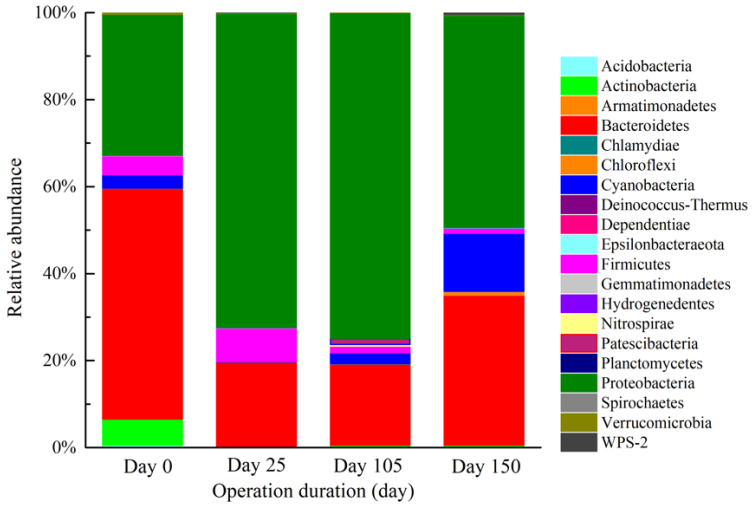


Figure 5. Abundance of dominant eukaryote at genus level (a) and bacteria at phylum level (b) in the algae biomass or granules.