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Advanced Treatment of Salty Eutrophication Water Using Algal-Bacterial Granular Sludge: With Focus on Nitrogen Removal, Phosphorus Removal, and Lipid Accumulation

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A new algal-bacterial granular sludge treatment method was used to treat salty eutrophication water. The results indicated that the treatment removed more than 98% of the total inorganic nitrogen and the total phosphorus after a 15 d cultivation period using 2% salinity simulated eutrophication water. For the 4% salinity simulated water, the total phosphorus was not able to be removed and was even higher in the effluent; and the total inorganic nitrogen was only removed 17%. Thus, the algal-bacterial granules were efficient for removing nitrogen and phosphorus in 2% salinity eutrophication water but were not effective for 4% salinity water. High levels of filamentous algae proliferation growing on the surface of the granules was primarily responsible for the good performance in 2% salinity water. However, the lipid accumulation was greatly enhanced (reactor R2 at a 27.6% increase and reactor R4 at a 107% increase) for both granule types due to the algal growth. Thus, treatment of the salty eutrophication water can also greatly increase the added-value of the algal-bacterial granules.

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INTRODUCTION

Anthropogenic eutrophication in salty aquatic ecosystems is a worldwide problem that occurs in coastal areas or industrial districts (Xiong *et al.* 2016; McIver *et al.* 2018; Corrales-González *et al.* 2019; Katsuki *et al.* 2019). With the overproduction of salts and organic/inorganic nutrients from anthropogenic inputs into rivers, ditches, lagoons, and lakes, the eutrophication of saltwater bodies can be caused by low levels of residual nitrogen (N) and phosphorus (P) (Derolez *et al.* 2019; Longley *et al.* 2019; Yao *et al.* 2019; Zheng *et al.* 2019). The residues of N and P result in the proliferation of some opportunistic algal species, which can lead to the crisis of the ecosystem and extensive death of aquatic animals (Bhagowati and Ahamad 2018; McIver *et al.* 2018; Vinçon-Leite and Casenave 2019). In China, many urban zones in the Binhai New Area of Tianjin have a serious problem with the eutrophication of the saltwater bodies (Xiong *et al.* 2016). The ditches and small lakes in these areas often have a green color, as well as a foul smell and dead animals found in the water, which reduces the environmental health of nearby areas.

Large quantities of nutrients (N and P) in eutrophication water come from wastewater treatment plants (Hu *et al.* 2017; Bhagowati and Ahamad 2018). The typical threshold for N and P levels that cause eutrophication are 1.2 mg/L and 0.1 mg/L, respectively (Chambers *et al.* 2012; Hu *et al.* 2017; Bhagowati and Ahamad 2018). However, the discharging limits for N and P from wastewater treatment plants are 10 mg/L to 15 mg/L and 0.5 mg/L to 1 mg/L (Hu *et al.* 2017). Thus, the eutrophication process can be induced from these and other sources. The residual N and P are difficult to eliminate *via* traditional biological methods. The very limited organic nutrients in the water cannot provide sufficient carbon sources for bacterial growth (Hamza *et al.* 2019), and a high salinity level may cause the death of bacteria cells (Jorfi *et al.* 2017). Hence, the thorough removal of N and P is critical for the remediation of salty eutrophication water.

Algal-bacterial granular sludge is an innovative biotechnology that combines algae with aerobic granular sludge (AGS). This technology is efficient in removing N and P due to the assimilation of a large quantity of algae in the granules (Meng *et al.* 2019b; Zhao *et al.* 2019). In addition, it overcomes the difficulty of biomass harvest for algae because the AGS shows excellent settling ability. The algal-bacterial granules have a high biodiesel content, as many algal species are rich in lipids (Ishika *et al.* 2017; Liu *et al.* 2017). Meng *et al.* (2019a) successfully cultivated algal-bacterial granules in high salinity wastewater (1% to 3%). Meng *et al.* (2019a) reported that the algal-bacterial granules, as well as the algae inside, could withstand a high salinity environment and still was efficient in the removal of N and P in salty wastewater.

For eutrophication water, the bacteria have difficulty surviving, and the removal rate of N and P is low due to the limited nutrient levels. In contrast, some salt-tolerant algae continue to grow and assimilate N and P with the help of photosynthesis (Meng *et al.* 2019a). Thus, the algal-bacterial granules could have a strong potential for the removal of N and P when treating salty eutrophication water, due to its large algae content. The increased growth rate of the algae in the algal-bacterial granules could increase the biodiesel content during the eutrophication water treatment process (Blaas and Kroeze 2014; Vo Hoang Nhat *et al.* 2018; Meng *et al.* 2019b). Thus, the application of algal-bacterial granular sludge is promising for eutrophication water treatments. There have been no previous studies performed using algal-bacterial granules for the treatment of salty eutrophication water, and the relative information is sparse.

In the present study, an algal-bacterial granular sludge was used for the treatment of simulated salty eutrophication water. The morphology and characteristics of the algal-bacterial granules were observed to better understand the mechanisms of the treatment process. The amount of nutrient removal (N and P) was monitored to evaluate the performance of the granules. The lipid production was monitored to determine the added value of the granules in the cultivation. This study provides meaningful reference for future salty eutrophication water treatment, with a new application of algal-bacterial granular sludge technology.

EXPERIMENTAL

Reactor Design and Operation

Two identical sequencing batch reactors (SBR) (D × H = 6 cm × 100 cm) made of acrylic plastic were used for the experiments (Fig. S1). The effective working volume of

each reactor was 2.0 L. Four 7 W white LED tubes (Opple, T5, Shanghai, China) were evenly distributed around each reactor at a 3 cm distance from the outer wall to provide light illuminance (180 µmol m^{-2} s^{-1}). A large incubator was used to cover the reactors from external light sources. The operation ran for 12 h per cycle, including: 2 min of feeding, 2 min of non-aeration, 706 min of aeration, 5 min of settling, 3 min of decanting, and 2 min of idling. The room temperature was maintained at 23 ± 2 °C. The light was consistently provided for all of the cycles each day. An outlet port was set in the middle of each SBR. The volume exchange ratio was 50% and the hydraulic retention time (HRT) was 24 h. Aeration was provided (3 L/min) from an air pump through an air stone at the bottom. The dissolved oxygen (DO) concentration was kept above 7 mg/L in the reactors.

Seed Sludge and Simulated Eutrophication Water

The seed algal-bacterial granular sludge was prepared according to Meng *et al.* (2019a). Granules were cultivated separately in 2% salinity and 4% salinity wastewater. After a 130 d cultivation period, the mature algal-bacterial granules were used as seed sludge for the treatment of the salty eutrophication water. Seed granules were put into two identical SBRs, with the initial mixed liquor suspended solids (MLSS) of the two SBRs set as 7.0 g/L.

A wastewater treatment plant is an important source for nutrients (N and P) to be added into eutrophication water. Thus, the synthesized salty eutrophication water was created according to the simulated wastewater methodology used by Meng *et al.* (2019a). The nutrient components in the wastewater were modified to a very low concentration in order to achieve a relative excess content of N and P (Chambers *et al.* 2012; Hu *et al.* 2017). The synthesized salty eutrophication water contained 0.5 mg/L of PO_4-P (KH_2PO_4), 7.5 mg/L of NH_4-N (($NH_4)_2SO_4$), 30 mg/L of COD (glucose 50% and sodium acetate 50%), 30 mg/L of $NaHCO_3$, 3 mg/L of $CaCl_2$, 2.5 mg/L of $MgSO_4 \cdot 7H_2O$, and 2 mg/L of $FeSO_4 \cdot 7H_2O$. However, the salinity levels of some rivers in the Binhai New Area of Tianjin were reported to be below 2.3% salinity (Xiong *et al.* 2016). In addition, lakes or lagoons in some costal places could have a higher salinity level closer to seawater (3.5% salinity) due to tidal intrusion. Therefore, two salinity levels, low (2%) and high (4%), were set for this study. The NaCl concentrations in the influent of the two reactors were kept at 20 g/L (2% salinity) and 40 g/L (4% salinity), respectively. Namely, the two reactors were set as R2 and R4 according to their salinity level. The seed granules cultivated from the 2% salinity wastewater were put in R2, and the granules from the 4% salinity wastewater were put in R4. All the chemicals (AR grade) were purchased from Aladdin Industrial Corporation Ltd., Shanghai, China.

Analytical Methods

The total phosphorus (TP), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonia nitrogen (NH_4-N), sludge volume index (SVI_{30}), and mixed liquor (volatile) suspended solids (ML(V)SS) were measured by the standard methods (APHA 2012). The total inorganic nitrogen (TIN) was calculated with Eq. 1,

$$[TIN] = [NO_3\text{-}N] + [NO_2\text{-}N] + [NH_4\text{-}N] \qquad (1)$$

where TIN is the total inorganic nitrogen, NO_3-N is the nitrate nitrogen amount, NO_2-N is the nitrite nitrogen amount, and NH_4-N is the ammonia nitrogen amount. A dissolved oxygen (DO) meter (HQ40d, Hach, Loveland, CO, USA) was used to detect the DO

concentration. The pH in the bulk liquid was measured with a pH meter (AS-711, AS ONE, Osaka, Japan) and kept between 7.0 pH and 8.5 pH. The chlorophyll *a* content in the granules was measured according to Zhang *et al.* (2018). The total lipid content was detected according to Meng *et al.* (2019b), and the light intensity was detected by a photometer (TES-1339, TES, Taipei, China). The morphology of the granules was observed with an optical microscope (CX40, Sunny, Yuyao, China) with a digital camera (700D, Canon, Tokyo, Japan) equipped on it. A scanning electron microscope (SEM) (FEI-Quanta 200, Thermo Fisher Scientific, Waltham, USA) was also used for the analysis of the morphology of the granules. The biological microbial community was detected according to Zhang *et al.* (2017) by a biotech testing company ALLWEGENE Inc. (Beijing, China). Statistical significance was calculated using one-way analysis of variance in Microsoft Office Excel 2010. The difference of results was announced statistically significant when the *p* value < 0.05.

RESULTS AND DISCUSSION

Effects of Simulated Salty Eutrophication Water on the Characteristics of Algal-bacterial Granular Sludge

The cultivation process in simulated salty eutrophication water could greatly influence the morphology and characteristics of algal-bacterial granules. However, the use of simulated eutrophication water with a low or a high salinity level had a different effect on the morphology and characteristics of the algal-bacterial granules. After a 15 d cultivation period in low (2%) salinity eutrophication water, the MLSS in R2 showed an increase from 7.0 g/L to 7.9 g/L, as shown in Table 1. In contrast, the MLSS in R4 (4% salinity) greatly decreased from 7.0 g/L to 2.8 g/L. The difference of MLSS between the two reactors on 15 d was significant ($p = 7.0 \times 10^{-6} < 0.05$). The different change in biomass content could be due to the large difference in algae growth and stability of the two granular systems. The stability of the granules closely related to the filamentous algae growing on the surface of the granules in the two reactors. It was observed that some filamentous algae began to appear and tightly bind to the surface of the granules in R2 after 5 d, as shown in Fig. 1. The algae became more abundant and covered the whole granule after 15 d. However, for the high (4%) salinity eutrophication water, a low amount of green algae was found on the surface of the granules in R4 after 15 d.
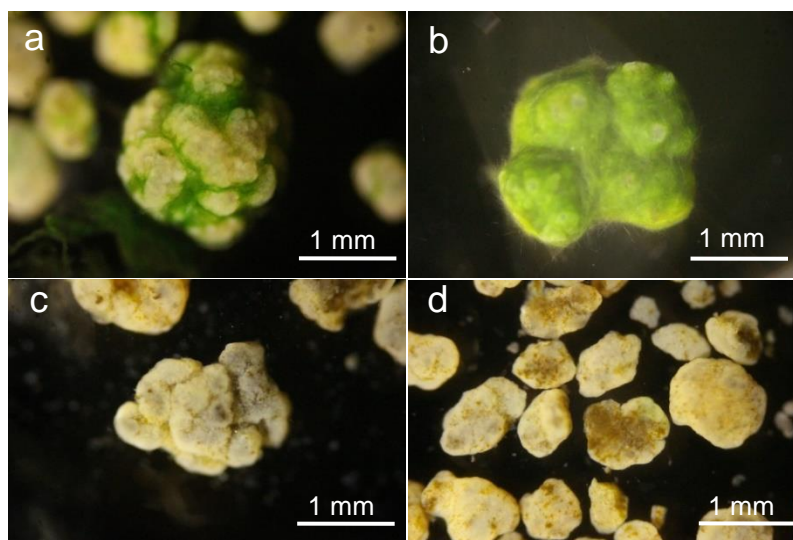
Fig. 1. Digital images of the algal-bacterial granules in R2 at 5 d (a) and at 15 d (b), and in R4 at 0 d (c) and at 15 d (d), respectively

Table 1. Characteristics of Granules in the Reactors

Parameters	R2$_{0 d}$	R2$_{15 d}$	R4$_{0 d}$	R4$_{15 d}$
MLSS (g/L)	7.0 ± 0.1	7.9 ± 0.2	7.0 ± 0.2	2.8 ± 0.2
MLVSS/MLSS	0.88 ± 0.01	0.90 ± 0.00	0.88 ± 0.01	0.91 ± 0.01
SVI$_{30}$ (mL/g)	10.9 ± 1.3	19.6 ± 1.7	7.3 ± 1.1	14.2 ± 1.4
Chlorophyll a (mg/g (SS))	3.2 ± 0.1	8.5 ± 0.1	3.8 ± 0.0	5.9 ± 0.1

High filamentous algae levels were important for the improvement of the stability of the algal-bacterial granules (Ahmad *et al.* 2017). As the nutrient levels in the simulated eutrophication water were insufficient for heterotrophic microorganism growth; the granular systems remained in endogenous respiration and were unstable, with the granules easily broken up. However, the filamentous algae in R2 improved the stability of the granules and protected them from losing their biomass into the effluent. On the contrary, the granules in R4 had limited filamentous algae on the surface. Therefore, the system showed an unstable status in the 15 d cultivation period with a large number of the granules breaking up and losing a majority of its biomass.

The total chlorophyll a content was determined to indicate the total algae content in the granules for both of the reactors. After a 15 d cultivation period, the total chlorophyll a in R2 increased from 3.2 mg/g (SS) to 8.5 mg/g (SS), while the chlorophyll a in R4 only increased from 3.8 mg/g (SS) to 5.9 mg/g (SS). This showed that the algal content in R2 and R4 both greatly increased after the cultivation. However, the low salinity level water reactor (R2) finally had a much higher algal content than R4 ($p = 6.0 \times 10^{-6} < 0.05$). The algae could have a negative effect on the settling ability of the granules. The SVI$_{30}$ for R2 and R4 showed an increase from 10.9 mL/g and 7.2 mL/g at 0 d to 19.6 mL/g and 14.2 mL/g at 15 d, respectively. The final settling ability was a little lower for granules in R2 ($p = 0.013 < 0.05$). However, the settling ability in the two reactors were still excellent due to maintenance of the SVI$_{30}$ values at low levels. Moreover, a small increase in the MLVSS/MLSS ratio was found in both the reactors ($p = 0.090 > 0.05$), which indicated the algae content might also improve the organic

components in the granules. It is known that many algal species are rich in lipid content (Ishika *et al.* 2017; Meng *et al.* 2019a). Thus, the cultivated granules in R2 and R4 probably had a higher lipid production than the initial algal-bacterial granules.

Overall Performance of Reactors for N and P Removal

Total phosphorus and TIN removal levels in the two reactors are shown in Fig. 2. At 2 d, the TP concentrations from the effluent in R2 increased rapidly to 1.5 mg/L at 3 d, much higher than the influent TP content (0.5 mg/L). This could be due to the inorganic nutrients, including N and P, being released from the extracellular polymeric substance (EPS) or from bacteria cells into the water in the endogenous respiration (Zhang *et al.* 2016; Zhou *et al.* 2019). But the TP concentration began to decline afterwards and was nearly at 0 mg/L at 12 d, achieving a removal efficiency of greater than 98%. By comparison, the TP concentration in the effluent from R4 increased more slowly in the beginning and slowly declined after 6 d. However, the final concentration after 14 d was approximately 0.7 mg/L in R4, still much higher than the influent. This indicated that the algal-bacterial granules in the 4% salinity eutrophication water were inefficient at the removal of phosphorus. A similar trend was also observed for the effluent TIN variation in the two reactors and the R4 still had a low TIN removal rate, approximately 17% at 14 d. However, the TIN concentration in R2 declined more quickly to 0 mg/L at 7 d with a removal efficiency of greater than 98%.
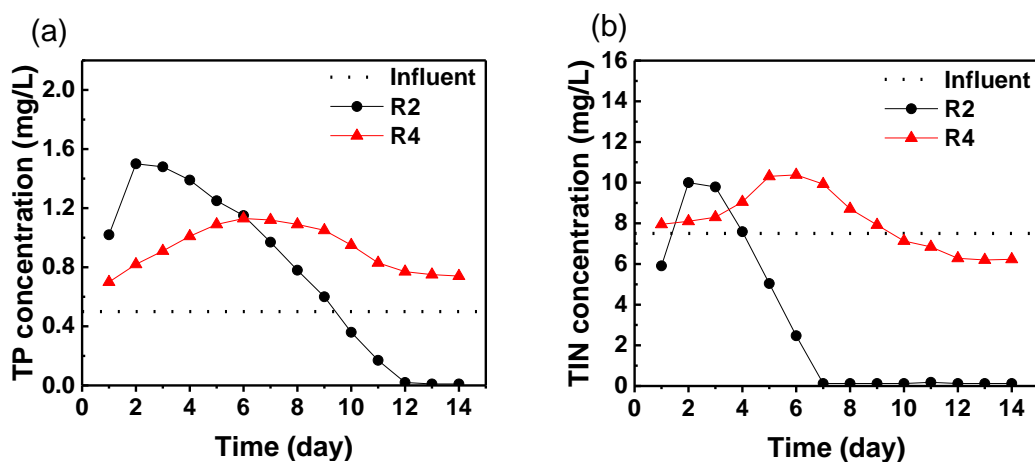

Fig. 2. Variations of TIN (a) and TP (b) removal during the operation

The algal-bacterial granules in R2 were able to efficiently remove the N and P nutrients after cultivation in 2% salinity eutrophication water. However, the granules in R4 did not seem fit to treat the 4% salinity eutrophication water. As the influent organic nutrients were quite low, the removal of the inorganic N and P could be mainly ascribed to the assimilation of the algae. The different performance between the two reactors might be due to the different algae content levels and their distribution between the granules.

The algal-bacterial granules in R2 had a greater algal content than R4, as shown in Table 1. In addition, the biological community structure analysis showed that the Cyanobacteria phylum dominated the prokaryote phyla present in the granules of R2, as shown in Fig. S2. The *Nitzschia genus* from the Bacillariophyta phylum dominated the eukaryote genera present in both R2 and R4. The Cyanobacteria phylum contains many

types of filamentous algae, which are known to readily proliferate in eutrophication water (Anahas and Muralitharan 2018; Manolidi *et al.* 2019). It was observed that a large number of filamentous algae grew on the surface of the granules in R2, as shown in Fig. 1 and S3, most of which were Cyanobacteria. The filamentous algae quickly covered whole granules on the surface after several days of cultivation. They could assimilate the released N and P from the EPS and bacteria cells for their growth and prevent the nutrients from going into the bulk liquid. Thus, the effluent N and P levels quickly dropped with the algae growth. For the granules in R4, most of the algae (*Nitzschia*) were distributed in the inner part of the granules for the entire cultivation period, and bacteria were primarily located in the outer layer, as shown in Fig S3. Thus, large amounts of N and P from the EPS and bacteria cells were easily released into the water environment, which resulted in low removal efficiency.

Effects of Simulated Salty Eutrophication Water on Lipid Production

The changes in lipid content *via* the granules of R2 and R4 are described in Fig. 3. Lipid content in granules from R2 had a large increase from 50.7 mg/g (SS) to 64.7 mg/g (SS) after a 15 d cultivation period. A large improvement of 27.6% was observed for the lipid content. For the granules in R4, the lipid content had a more dramatic increase from 54.1 mg/g (SS) to 112.0 mg/g (SS). A higher improvement rate of 107.0% was obtained, with the lipid content more significantly motivated by 4% salinity ($p = 1.1 \times 10^{-5} < 0.05$). Salty eutrophication water with low (2%) and high salinities (4%) both enhanced the lipid content in the algal-bacterial granules. The increase in lipid content could be mainly ascribed to the algae content increase in both of the reactors, as shown in Table 1. However, the different lipid content levels in the two reactors might be due to the fact that the algae species and the salinities were different.
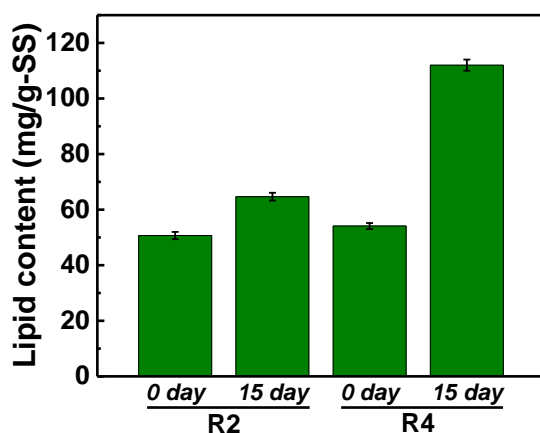

Fig. 3. Lipid content in the granules from the two reactors

As mentioned above, a large amount of filamentous algae from the Cyanobacteria phylum grew on the surface of the algal-bacterial granules in R2. However, the main algae genus in R4 was still *Nitzschia*, since limited filamentous algae was found in the granules. In addition, salinity was an important motivator for lipid production in *Nitzschia*. The *Nitzschia* was reported to possess a higher lipid content (27.2%) when salinity levels reached 3%, which was more abundant than when under no salt conditions

(9.9%) (Cheng *et al.* 2014). The lipid content in fifteen common algae species from the Cyanobacteria phylum were found to only vary from 5.9% to 18.9% (Anahas and Muralitharan 2018), which was much less than *Nitzschia* under 3% salinity conditions. Therefore, the algal-bacterial granules in R4 had more lipid production than R2, which was probably due to the lipid content in *Nitzschia* being enhanced by the higher salinity level.

Currently, the waste biomass from the wastewater treatment plants in China is mainly disposed by sanitary landfill due to its low cost, but it may induce environmental problems (Yang *et al.* 2015). Other disposal methods are also used, including land application, incineration, and producing building materials (Yang *et al.* 2015). Meanwhile, energy reuses of the waste biomass have been explored, which include anaerobic digestion to biogas or extracting the lipid (Tyagi and Lo 2013; Bora *et al.* 2020; Luo *et al.* 2020). Microalgae are known to have relatively higher lipid content, and also they have the advantage of high growth rate, as well as a high ability to fix carbon dioxide (Shi *et al.* 2018). The lipid in microalgae was reported to be converted into jet fuel, indicating the big potential for industry use (Ju *et al.* 2018). Therefore, the lipid enhancement by algae increase in the algal-bacterial granules in the two reactors might be helpful for reusing the waste biomass as feedstock to biodiesel extraction.

Summary of Algal-bacterial Granules for Salty Eutrophication Water Remediation at Different Salinity Levels

The algal-bacterial granular sludge was shown to be effective for the removal of N and P under the treatment conditions of 2% salinity eutrophication water in the present study. Meanwhile, the lipid content also increased by approximately 27.6% during the cultivation process. This was quite beneficial for extracting biodiesel from the waste algal-bacterial granules. With both of these benefits, the algal-bacterial granules are quite useful for certain salinity eutrophication water treatment processes (2% salinity). However, the algal-bacterial granules in R4 were ineffective at the removal of N and P during the treatment, although the lipid content dramatically improved by 107% when treating 4% salinity eutrophication water. Thus, the use of algal-bacterial granules for the treatment of 4% eutrophication water is not recommended. But it can still be considered when the main purpose is to extract the enhanced lipids from the waste algal-bacterial granules.

CONCLUSIONS

1. Algal-bacterial granular sludge was successfully used for the treatment of simulated salty eutrophication water with 2% salinity in the present study. A high removal rate of N and P nutrients (greater than 98%) was observed, and the filamentous algae from the Cyanobacteria phylum played an important role in the removal process. A lipid enhancement rate of 27.6% was also obtained.

2. The algal-bacterial granules in the 4% salinity eutrophication water were inefficient at the removal of N and P nutrients. However, they led to a dramatic increase in lipid production (107%), which showed a strong potential for biodiesel extraction processes.

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SUPPLEMENTAL INFORMATION

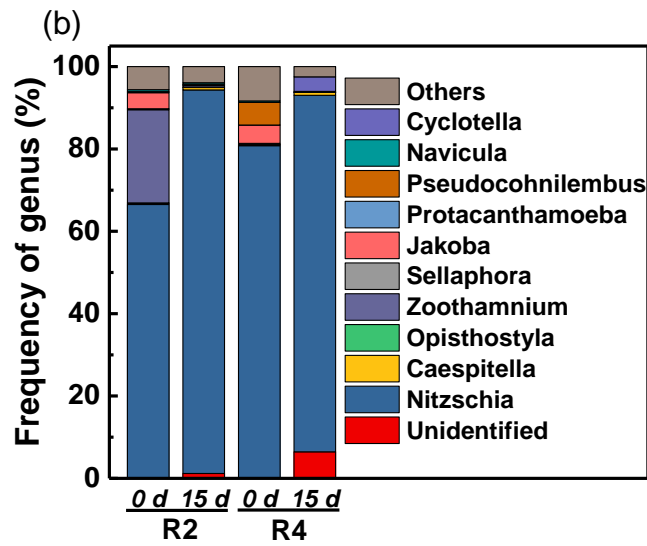**Fig. S1.** Photo-sequencing batch reactors
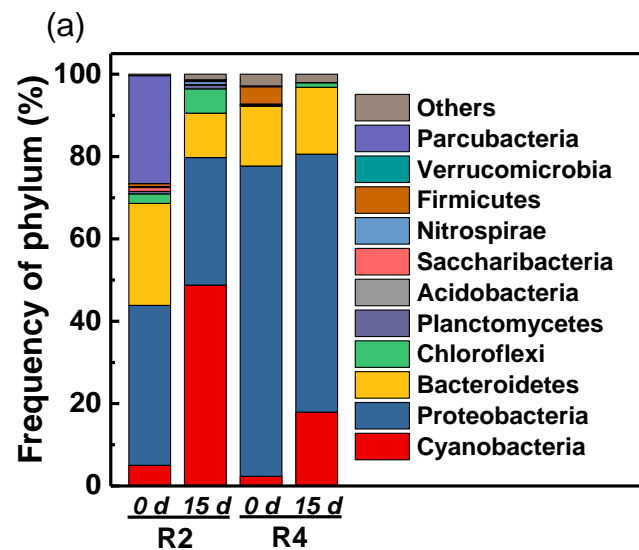
Fig. S2. Abundance of dominant prokaryotes at the phylum level (a) and eukaryotes at the genus level (b) in the granules of the two reactors at 0 d and at 15 d.
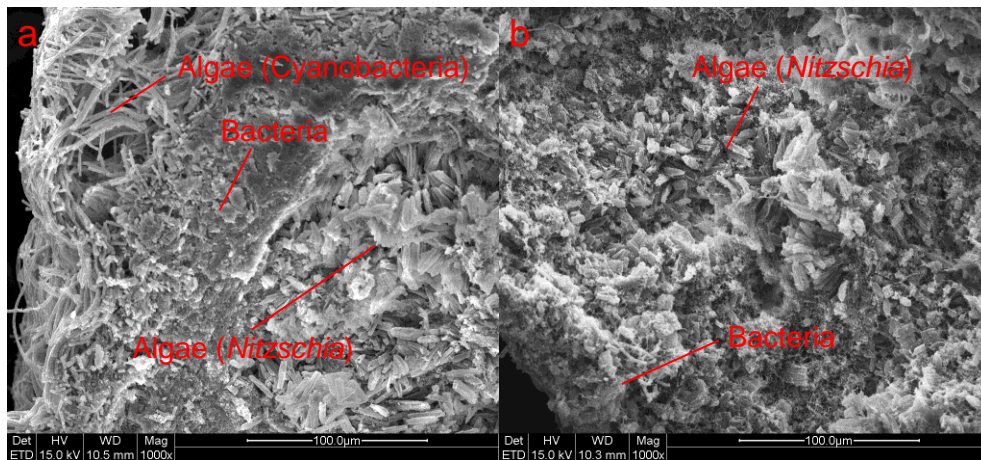

Fig. S3. SEM images for the cross section of granules from R2 (a) and R4 (b) at 15 d.