Stability and performance of algal-bacterial granular sludge in shaking photo-sequencing batch reactors with special focus on phosphorus accumulation

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Abstract

The granular stability, nutrients removal and phosphorus (P) accumulation of algal-bacterial aerobic granular sludge(AGS) was examined by using shaking photoreactors (at a fixed light/dark cycle of 12h/12h). During the 25 days' operation, algal-bacterial AGS possessed good granular integrity (8.4±0.6%), and excellent removals of dissolved organic carbon (94.8±1.6%) and total nitrogen (71.1±3.3%). More extracellular proteins (153.7±2.3 mg/g) were excreted from the granules with a high proteins/polysaccharides ratio(~7.4) on day 25, especially the tightly bound proteins mainly responsible for granular stability. Decrease in P content, especially non-apatite inorganic P relating to Fe-PO_4 precipitates, was detected in the granules to some extent, although 54.8±17.1% of total P removal was achieved during the light-on cycles. Still, high P bioavailability (92.0%) was kept in the algal-bacterial AGS throughout the test period. Further optimization of light-on/light-off cycle and hydraulic/sludge retention time is demanding for better and stable P accumulation in the algal-bacterial granules with high bioavailability.

Keywords: Algal-bacterial aerobic granular sludge; Shaking photoreactor; Granular stability; Nutrients removal; Phosphorus accumulation

1. Introduction

Algal-bacterial consortia is regarded as a competitive alternative to conventional activated sludge (AS) process due to its unique features such as less energy demand for degradation of organic matter, enhanced nutrients removal, and higher potential for resources recovery (Quijano et al., 2017; Sforza et al., 2018). In a conventional wastewater treatment plant (WWTP) or AS process-based WWTP, up to 60% of the energy consumption is contributed by aeration units which provide oxygen (O_2) necessary for the growth of aerobic bacteria; while in the algal-bacterial consortia, algae can produce sufficient O_2 that is required by aerobic bacteria to efficiently remove nutrients from wastewater (Boelee et al., 2014). Thus, wastewater treatment systems using algal-bacterial consortia attract much attention.

On the other hand, algae harvesting and separation from the treated water is challenging the promising algal-bacterial consortia systems due to the small size of microalgae cells (3-30 μm), poor settleability and low density (Zhou et al., 2017; Hu et al., 2017). Recently, there's a growing interest in the use of innovative solutions that combine microalgae technology with aerobic granular sludge (AGS) process, aiming to solve this problem. In comparison to the AS process-based wastewater treatment, algal-bacterial granular consortia with a compact granular structure and good biomass settleability reflects a prospective option for more nutrients uptake or removal from wastewater (Ahmad et al., 2017; Liu et al., 2018; Zhang et al., 2018). In addition, the application of algal-bacterial AGS for potentially less energy consumption during wastewater treatment, especially in aeration units, has become a research hotspot. Tiron et al. (2015) claimed that the activated algae granular system could efficiently remove organic matter and nutrients from low-strength wastewater with O_2 being provided only by algae through photosynthesis. Most recently, Abouhend et al. (2018) attempted to treat wastewater with oxygenic photogranules in stirred-tank reactors under no aeration condition, achieving efficient removals of chemical oxygen demand (COD) and nitrogen (N). Although much efforts have been devoted to the algal-bacterial granular system, especially on energy saving via less aeration or no aeration, previous works mainly focused on organics or nutrients removal efficiencies under the test conditions.

As it is known, phosphorus (P) recovery from P-rich resources is very important due to the limited global reserves of high-quality phosphate rock, especially in Japan. In recent years, many researches concentrated on P recovery from sewage sludge produced from WWTPs due to its high P content (Ye et al., 2016). In addition, algal biomass produced from wastewater treatment can serve as P fertilizer in agriculture (Mulbry et al., 2005). A recent research (Zhao et al., 2018) reported that high P content (2.8-3.0%) with extremely high P bioavailability (up to 98%) could be achieved in the algal-bacterial AGS treating low influent carbon wastewater, which is easily used for P resource recovery. Besides, loss of granular stability during the long-term operation is a major concern for practical application of this promising biotechnology. However, very little information is available regarding P content and its bioavailability as well as stability of algal-bacterial granules in no air bubbling systems which are usually applied for cultivation of bacterial AGS or algal-bacterial AGS.

This preliminary trail aimed to investigate the characteristics and performance of the mature algal-bacterial AGS in shaking photoreactors instead of air bubbling systems usually used for bacterial AGS or algal-bacterial AGS process. Besides, P accumulation and its bioavailability in the algal-bacterial granules were detected. Results from this work are expected to provide basic information for the design of this cost-effective system to treat wastewater and the subsequent resources recovery from the algal-bacterial AGS biomass.

2. Materials and methods

2.1. Synthetic wastewater and seed algal-bacterial granules

Synthetic wastewater used in this work was the same as the influent to R_1 in a previous study (Zhao et al., 2018), which was mainly characterized as 400 mg COD/L, 50 mg NH_4^+-N/L, and 10 mg PO_4^{3-}-P/L to mimic the domestic wastewater in China.

Prior to the experiments, mature algal-bacterial AGS was cultivated in the laboratory-scale sequencing batch reactor (SBR) which has been operated stably for more than 3 months. The dominant algae species, *Leptolyngbya*, was the same as that in Zhao et al. (2018). The initial concentration of mixed liquor volatile suspended solids (MLVSS) was around 3.87±0.03 g/L in each photoreactor. The mean diameter of the granules was 2.32±0.52 mm with a sludge volume index at 5 minutes (min) (SVI_5) about 38.1±0.4 mL/g.

2.2. Photoreactors' operation and analysis

Six 250 mL glass flasks (FB-501-250, Fisher Scientific, USA) with a working volume of 200 mL each were shaken constantly in a multi shaker (MMS-310, EYELA, Japan) at 150 rpm and room temperature (25±2 °C), resulting in suspended granular biomass during shaking. These reactors were operated in parallel under the same conditions. Two artificial solar lights (NLSS15CBM-AC, Nikki Trading Corp., Japan) were applied to the reactors with the light intensity on the surface of the outer wall being 88-122 μmol/(m^2·s) when the artificial solar light was on (light-on/light-off=12 h/12 h). All the experiments were carried out without air bubbling (used for bacterial AGS or algal-bacterial AGS cultivation and treatment processes), during which oxygen could be provided by the algae through photosynthesis when the light was on. Each cycle (12 hours) consisted of 1 min filling, 715 min shaking, 2 min settling, and 2 min effluent discharge. The volumetric exchange ratio was kept at 50%, resulting in a hydraulic retention time (HRT) of about 24 h. Sludge retention time (SRT) was controlled at approximately 30 days through timely and quantitative sludge discharge (once every two days). The whole test lasted for 25 days till the stable system performance being achieved.

All the analytical methods were described elsewhere (Zhao et al., 2018), including the quantification and characterization of granular biomass (ML(V)SS, SVI, chlorophyll *a*, granular size and distribution, granular strength, P fractionation in biomass), water quality (total nitrogen (TN), ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), phosphorus (PO_4^{3-}-P), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), dissolved total carbon (DTC)), and extracellular polymeric substances (EPS) extraction and determination.

2.3. Statistical analysis

All analyses were carried out in triplicate with the results being expressed as the mean ± standard deviation. Pearson's correlation analysis was performed by using Origin 2017 to clarify the correlation between P fractions and metal ions in the granules. The significant difference was assumed at $p < 0.05$.

3. Results and discussion

3.1. Properties of algal-bacterial AGS in shaking photoreactors

3.1.1. Changes in morphology and biomass retention

At the beginning, the mature algal-bacterial AGS was green, reflecting an irregular, compact and dense structure. After day 20, filamentous bacteria were observed on the outer surface of the granules. Gradually, the number of large-sized granules with superficial filamentous bacteria increased in all the reactors. Moreover, most parts of the granules turned to dark green with a larger particle diameter (3.77±1.09 mm) compared to the seed granules (2.32±0.52 mm). Restated, although filamentous bacteria co-existed in the reactors till the end of experiment, no obvious disintegration was observed in the algal-bacterial granules which maintained their compact and integrated structure throughout the whole shaking test.

The reactors had an initial MLSS of 4.99±0.06 g/L (MLVSS/MLSS=77.6±1.1%), and an obvious decrease in MLSS was detected after 25 days of operation (3.77±0.12 g/L, MLVSS/MLSS=77.6±2.0%), most probably attributable to a short SRT (about 30 days) applied in this study. As it is known, chlorophyll *a* concentration can be used to represent the growth of algal biomass (Lee et al., 2015), which was detected to increase from the initial 7.99±0.13 to 13.40±0.01 mg/g-MLVSS in the granules from the reactors on day 25. This observation signals the fast growth of algae in the algal-bacterial granules under the shaking condition.

3.1.2. Granular settleability and stability

Effective biomass separation through the formation of fast settling biomass is one of the major advantages of AGS technology. Under the designed shaking condition, an obvious increase in SVI_5 (from 38.1±0.4 to 77.5±1.8 mL/g) was noticed, suggesting somewhat worsen settleability of algal-bacterial granules. This observation was partially in agreement with the finding by Abouhend et al. (2018) who attributed the increase in SVI_{30} of oxygenic photogranules to the overgrowth of filamentous green algae in the stirred-tank reactors without aeration. The presence of filamentous bacteria and the low hydrodynamic shear force provided by the shaker might be the key factors for the resultant slightly lower granular settleability than the seed algal-bacteria granules. Thus some decrease in granular settling velocity (from 29.1±3.9 to 21.4±6.2 m/h) was observed in this study. Still, the SVI_5 of algal-bacterial AGS on day 25 was better than or comparable to the conventional AGS (55.3-82.8 mL/g) or algae granules (~125 mL/g) in the air bubbling systems (Cai et al., 2019; Wang et al., 2019).

Besides, the stability of granular sludge is a crucial factor to ensure the effluent quality from the algal-bacterial granule system. In this study, the granular stability was expressed in terms of integrity coefficient (%), and a lower value denotes a better stability (Muda et al., 2010). It was found that the integrity coefficient of the granules only slightly increased from the initial 7.4±0.3 to 8.4±0.6% on day 25, implying that the algal-bacterial AGS possesses a strong enough structure to maintain its granular stability even under the constantly shaking condition.

3.2. Performance on carbon and nutrients removal

3.2.1. DOC removal

The changes in effluent carbon concentration from the reactors were recorded during the 25 days' operation. The effluent DOC concentration from the reactors was always < 14 mg/L from day 2 onwards, achieving an average DOC removal efficiency of 94.8±1.6% and 94.4±1.4% during the light-on and light-off cycles of the whole test period. This observation indicates that the algal-bacterial AGS system could also have good performance on organics removal under shaking condition, and the light illumination exhibited no obvious influence on organics removal by the algal-bacterial AGS. A similar organic removal efficiency was also achieved from the AGS systems with bacterial AGS (averagely 96.0%) or algal-bacterial AGS (averagely 96.2%) under air bubbling (Cai et al., 2018; Zhao et al., 2018). It is worth noting that an enhanced DIC reduction was detected during the light cycle from day 11 onwards, which might be attributable to the photosynthesis of algae. That is, light illumination can influence DIC removal process to some extent.

3.2.2. Nutrients removal

In this study, the algal-bacterial AGS exhibited excellent performance on NH_4^+-N removal (> 99%) during the light-on and light-off cycles from the very beginning of this test, indicating that stable nitrification could be quickly established in this shaking system. TN removal efficiencies in the reactors were almost at the same levels, 71.1±3.3% and 69.2±4.1% during the light-on and light-off cycles, respectively from day 3 to the end of experiment. Compared to a previous report by using the same algal-bacterial AGS in the air bubbling system (Zhao et al. 2018), a higher TN removal efficiency was detected in this study. This suggests that algal-bacterial AGS possesses great potential for N removal even under shaking condition, which could be further optimized with the relationship between DO change in bulk liquor and light illumination duration being considered. Interestingly, the TN removal efficiency in the photoreactors demonstrated an evidently increasing tendency along with the operation. This observation might be attributable to the bigger granule size with the operation, creating a more beneficial environment for denitrification (Li et al., 2017).

The effluent P concentration from the reactors during the light-on and light-off cycles, however, showed an obviously different trend during the operation. When fed with 10 mg/L of P, the reactors produced the effluents with P concentrations about 4.5±1.7 mg/L (varied from 2.8 to 8.5 mg/L) and 13.5±4.1 mg/L (ranged between 9.1-20.9 mg/L), respectively during the light-on and light-off cycles from day 3 to the end of experiments. Biotic assimilation and abiotic precipitation are reported as the main P removal means from wastewater (de Godos et al., 2009). Algae can absorb and accumulate large quantities of P through luxury uptake (Solovchenko et al., 2016). In the case of algal-bacterial AGS system, co-precipitation of phosphate with other co-existing metals and algae assimilation are the major contributors to P removal (Zhao et al., 2018). As seen, almost no P removal was detected during the light-off cycles in the shaking photoreactors, suggesting algae growth and assimilation might be responsible for the enhanced P removal with light illumination in this study. Further research is necessary for the optimization of light cycle for the photoreactors to improve P uptake and effluent quality.

3.2.3. Cycle tests on day 20

In order to have a better understanding of the algal-bacterial AGS system under shaking condition, typical cycle tests (including DOC, TP, DO and N profiles) were also conducted and compared during the light-on and light-off cycles on day 20.

DO concentration decreased from the initial 2.7 and 2.6 mg/L to 1.37 and 0.83 mg/L at the 60th min during the light-on and light-off cycles, respectively. The observation indicates that the activity of aerobic bacteria (nitrifiers and heterotrophs) could be maintained in the shaking photoreactors. From the 60th min to the 720th min, the DO values continuously increased, and reached around 8.2 and 3.5 mg/L at the end of light-on and light-off cycles. As for the DOC concentration, a similar decreasing trend was observed during the light-on and light-off cycles, which sharply declined to 37.5 and 32.1 mg/L respectively at the 60th min, and then gradually decreased to 5.8 and 7.7 mg/L at the end of each cycle. On the other hand, the decrease of NH_4^+-N concentration in the reactors was slightly faster during the light-on cycle than that during the light-off cycle, indicating that besides algae assimilation, nitrification with simultaneous denitrification processes may occur under shaking and illumination conditions.

As for P, TP concentration was firstly observed to release and then uptake during the subsequent period, suggesting that P was not only taken up by algae but also removed by phosphorus accumulating organisms (PAOs) in this study. Interestingly, a higher P release (22.6 mg/L at the

120th min) during the light-off cycle was detected, in comparison to 16.7 mg/L of P release during the light-on cycle (at the 60th min). In addition, the effluent P concentrations were averagely about 2.5 and 10.0 mg/L at the end of light-on and light-off cycles, respectively. Almost little contribution of algal-bacterial AGS to P removal was observed during the light-off period, most probably attributable to the designed light-on/light-off cycle (12h/12h) which might not be suitable for the metabolism of PAOs. More in-depth research is still ongoing with respect to the optimization of light-on/light-off cycle and hydraulic/sludge retention time.

3.3. P fractionation in algal-bacterial granules

The P fractions in granules are presented in Fig. 1, including total phosphorus (TP), organic phosphorus (OP), inorganic phosphorus (IP), non-apatite inorganic phosphorus (NAIP) and apatite phosphorus (AP). The quantitative relationship among these P fractions can be expressed as follows: TP = OP + IP, and IP = NAIP + AP. According to Huang et al. (2015), NAIP is mainly composed of the fraction associated with Al, Fe and Mn, which together with OP is regarded as bioavailable P that can be potentially released and utilized by microorganisms and plants. AP is the Ca-bound P that is hard to be utilized by microorganisms and plants. It is clearly seen that the content of each P fraction in the seed algal-bacterial AGS was much higher than those in the granules on day 15 and day 25 (Fig. 1a). The TP content in the granules decreased obviously, from the initial 30.4±0.4 to 23.8±0.3 on day 15 and to 21.5±0.4 mg/g-MLSS on day 25, respectively. Correspondingly, the OP and NAIP contents decreased from the initial 5.8±0.3 (occupying 19% of TP) and 22.5±0.3 mg/g-MLSS (74% of TP) to 3.1±0.2 and 16.7±0.1 mg/g-MLSS on day 25, accounting for 14% and 78% of TP in the granules, respectively. However, the AP content maintained at around 1.6±0.1 mg/g-MLSS in the granules during the whole process, signaling its relative stability in quantity under the test condition. This observation also indicates that the decrease of TP content in the granules is mainly contributed by OP and NAIP under the shaking condition. Specifically, Fe content in the granules reflected a similar decrease trend as NAIP, and a strongly positive correlation (R = 0.999) was found between the amount of Fe accumulated in the granules and its NAIP content. This result suggests that it might be difficult to maintain the stable Fe-PO_4 precipitates in the algal-bacterial granules under the designed operation condition, which needs further investigation. However, the proportion of potentially bioavailable P ((OP+NAIP)/TP) was relatively stable, which varied from the initial 93.2% to 92.0% in the granules on day 25. As a result, the test condition has limited effect on the bioavailable P in the algal-bacterial granules, which can be potentially recovered.

3.4. EPS secretion from algal-bacterial granules

EPS play an essential role in the formation and stability of granule (Guo et al., 2016). As shown in Fig. 1b. The total EPS content increased significantly from the initial 100.0±1.5 mg/g-MLVSS to 174.5±4.8 mg/g-MLVSS on day 25, in which proteins (PN) was much more abundant than polysaccharides (PS). During the operation, PN increased from the initial 86.2±1.9 to 153.7±2.3 mg/g-MLVSS on day 25, and the PN/PS ratio rose from 6.3 to 7.4, correspondingly. The increment in PN excretion in this study indicates that PN might function as protective agents for bacteria and maintain the stability of algal-bacterial granules under the shaking condition (Zhang et al., 2018).

Previous works indicate that EPS may have a different content distribution in the layered structure of AGS (Adav et al., 2008), which can be mainly divided into two different fractions, i.e. loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) (Li and Yang, 2007). Fig. 1b also shows the changes of LB-EPS and TB-EPS contents with their major components (PN and PS) during the 25 days' operation. In general, the PN and PS contents in the LB-EPS and TB-EPS increased with a similar trend as those in the total EPS in the granules. The PN/PS ratio slightly decreased from the initial 5.6 to 5.5 in LB-EPS, whereas it increased from 6.6 to 9.0 in TB-EPS on day 25. This observation suggests that PN in the TB-EPS is more sensitive than those in the LB-

EPS of algal-bacterial granules under the test condition. The above results also imply that PN play a crucial role in the maintenance of the stable inner structure of algal-bacterial AGS.

4. Conclusions

The stability and performance of mature algal-bacterial AGS was examined by using shaking photoreactors instead of air bubbling systems. The algal-bacterial AGS possessed good granular integrity (8.4±0.6%), excellent DOC removal (94.8±1.6%) and high TN removal (71.1±3.3%) during 25 days' operation. The stability of Fe-PO_4 precipitates in the granules was affected to some extent under the test condition, during which a high P bioavailability (92.0%) was still maintained. Further research is directed to the optimization of light-on/light-off cycle and hydraulic/sludge retention time in order to realize the minimum energy consumption for wastewater treatment by using this novel algal-bacterial AGS system.

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Appendix. A. Supplementary data

E-supplementary data associated with this article can be found in the online version.

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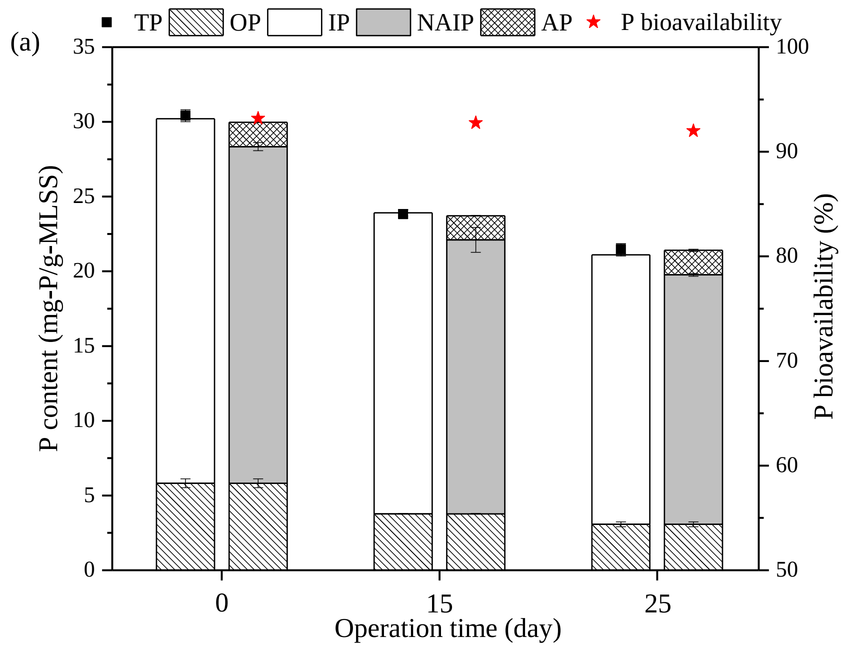


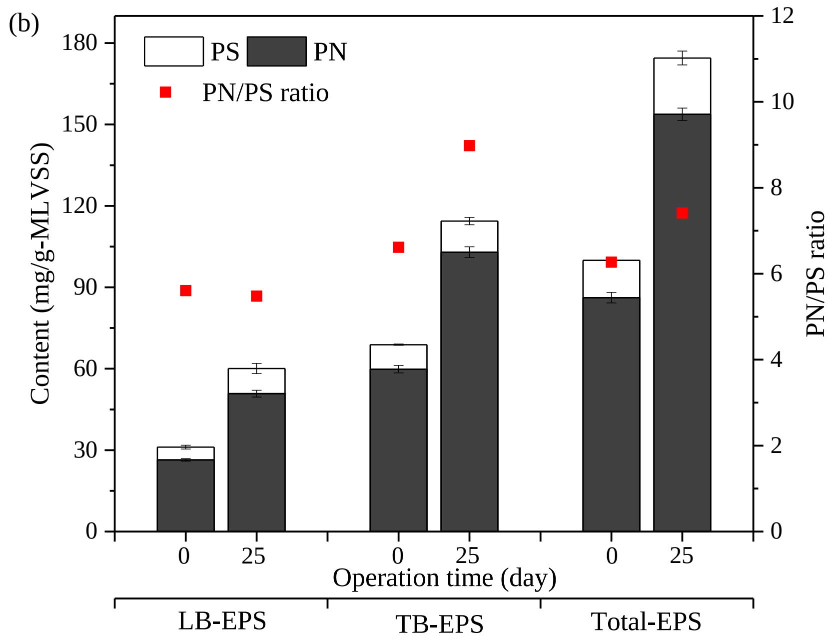


Fig. 1. Changes in P content and its fractions (a), and loosely bound EPS (LB-EPS), tightly bound EPS (TB-EPS), and total EPS contents and their major components (proteins (PN) and polysaccharides (PS)) in the algal-bacterial granules.

Highlights

- Highly stable algal-bacterial granules were maintained after shaking 25 days
- Algal-bacterial granules removed about 95% dissolved organic C and 71% total N
- 55% of total P removal was achieved during light-on cycles in shaking photoreactors
- 92% of total P was still bioavailable in the algal-bacterial AGS after the test
- Tightly bound extracellular proteins played a crucial role in this study

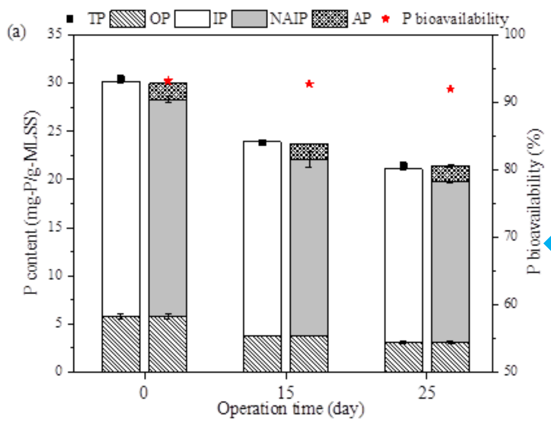
Graphical abstract

Good granular integrity (~8.4%)

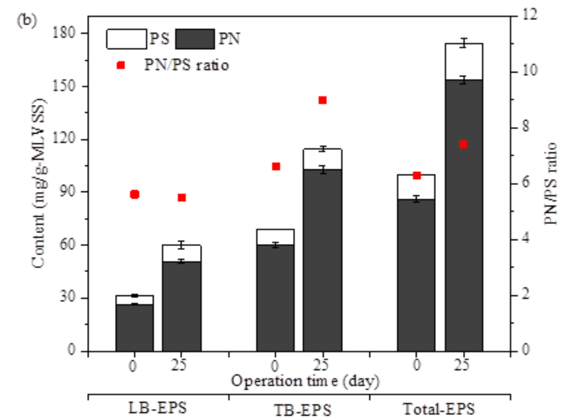
Excellent DOC removal (94.8±1.6%)

High TN removal (71.1±3.3%)

High TP (54.8±17.1%) removal (light-on)



High P bioavailability (92%)



More extracellular proteins (153.7±2.3mg/g)

Algal-bacterial AGS after 25 days' shaking

(instead of air bubbling used for bacterial AGS or algal-bacterial AGS cultivation)

Supplementary Materials

Stability and performance of algal-bacterial granular sludge in shaking photo-sequencing batch reactors with special focus on phosphorus accumulation

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Materials and Methods

Determination of granular strength

Integrity coefficient (%) was used to estimate the strength of algal-bacterial granules by a shock method as described by Ghangrekar et al. (2005), which was 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 weight of the granular sludge used for this test.

Reference

1. 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, 1123-1133.

Results and discussion

Table S1

Comparison in characteristics of algal-bacterial AGS in the shaking photoreactors between day 0 and day 25.

Parameter	Day 0	Day 25
MLSS (g/L)	4.99±0.06	3.77±0.12
MLVSS/MLSS (%)	77.6±1.1	77.6±2.0
SVI_5 (mL/g)	38.1±0.4	77.5±1.8
Mean diameter (mm)	2.32±0.52	3.77±1.09
Integrity coefficient (%)	7.4±0.3	8.4±0.6
Settling velocity (m/h)	29.1±3.9	21.4±6.2
Chlorophyll *a* (mg/g-MLVSS)	7.99±0.13	13.40±0.01

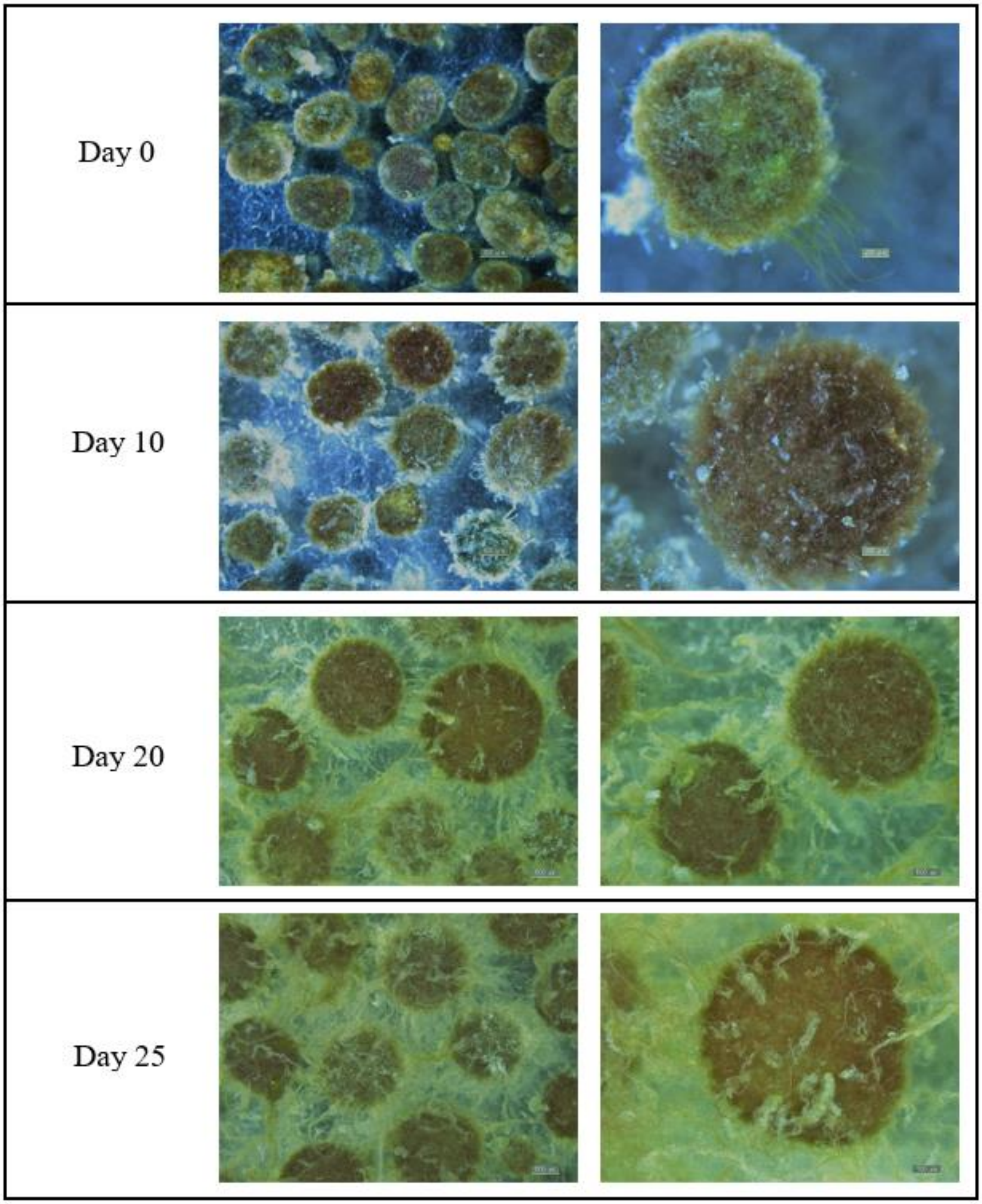

Fig. S1. Morphological changes of the algal-bacterial AGS in the two reactors on day 0, 10, 20, and 25, respectively.

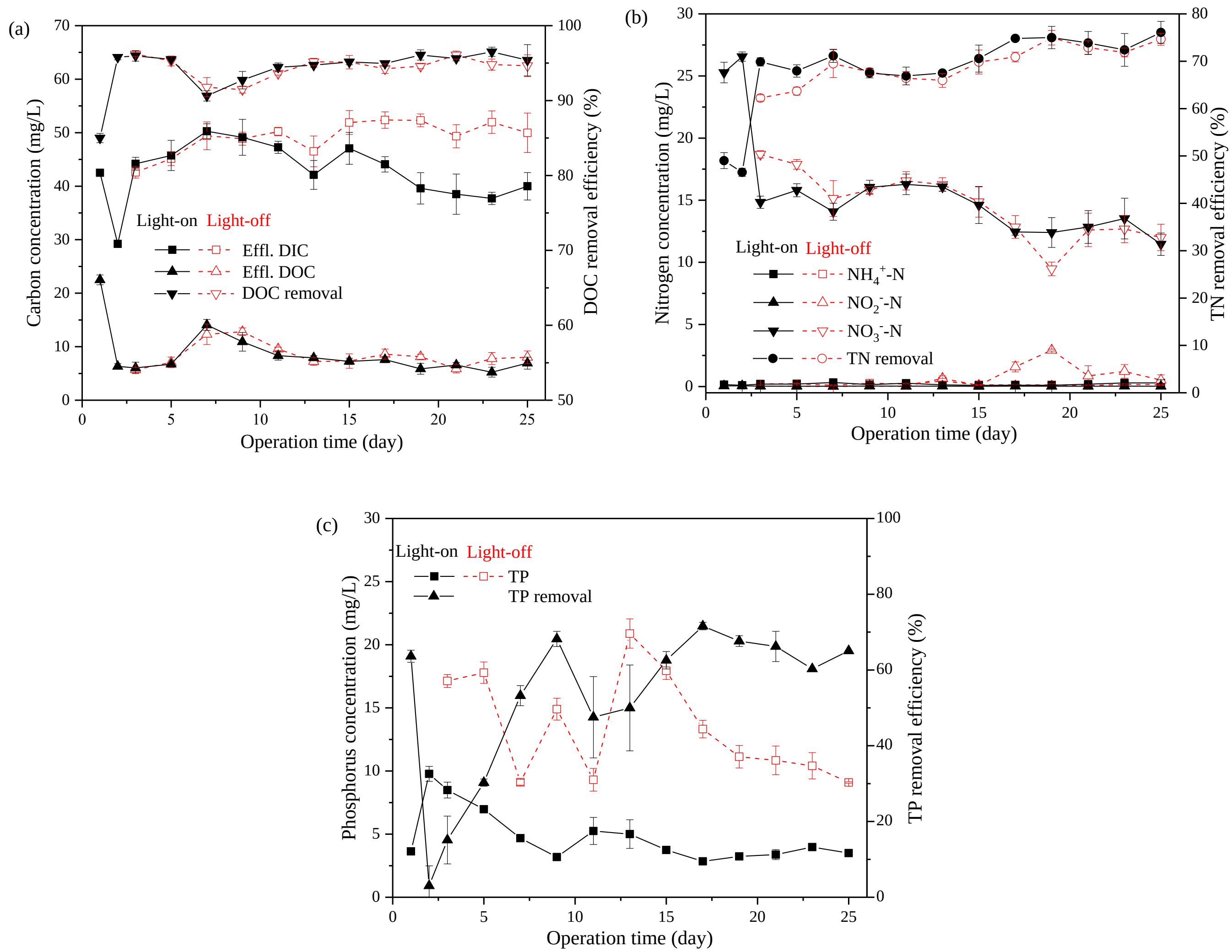

Fig. S2. Changes in nutrients profiles during the operation: carbon (a), nitrogen (b), and phosphorus (c).

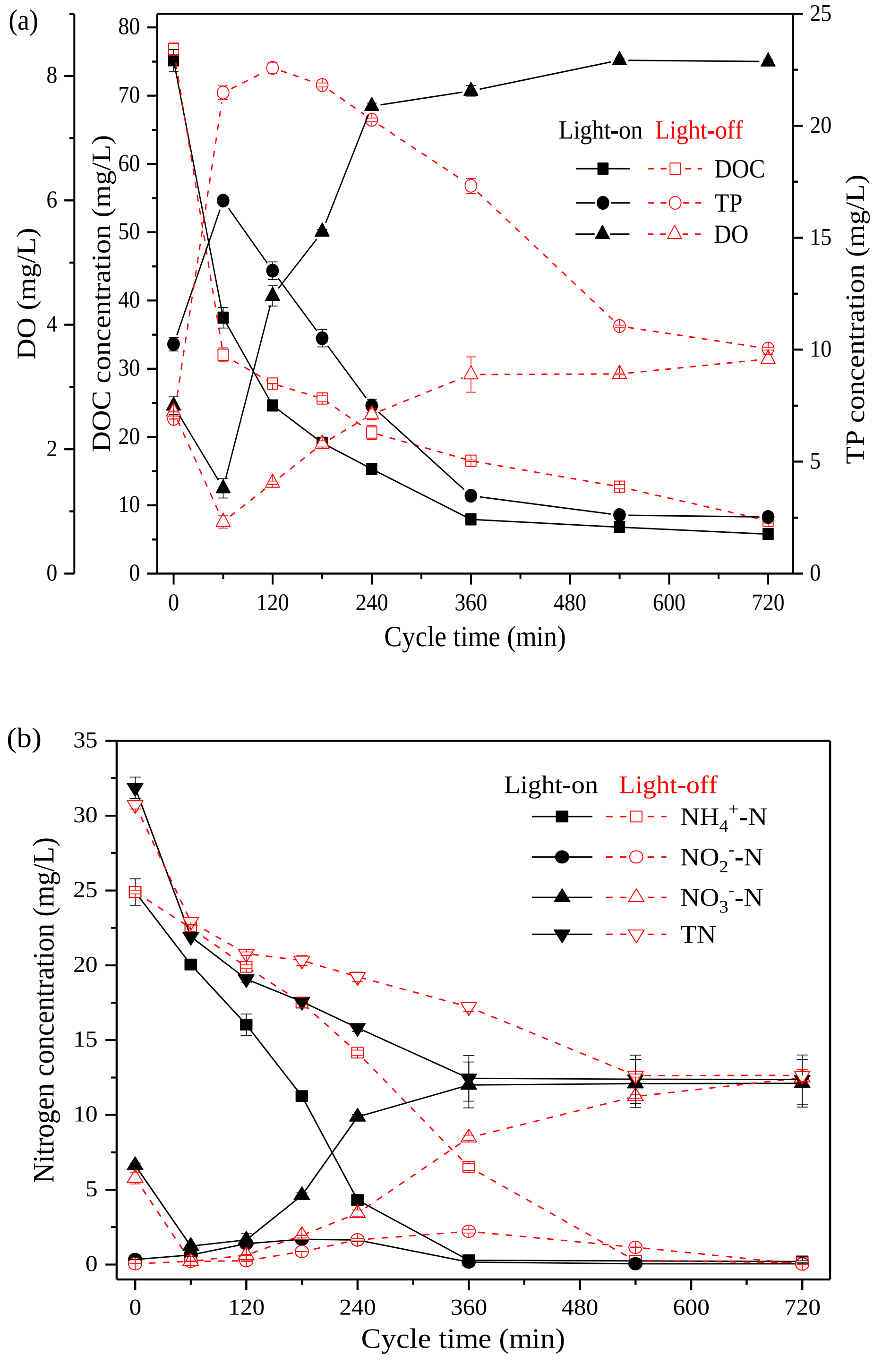

Fig. S3. Variations of DO, DOC, and TP (a), and N species (b) in the typical light-on and light-off cycles on day 20.

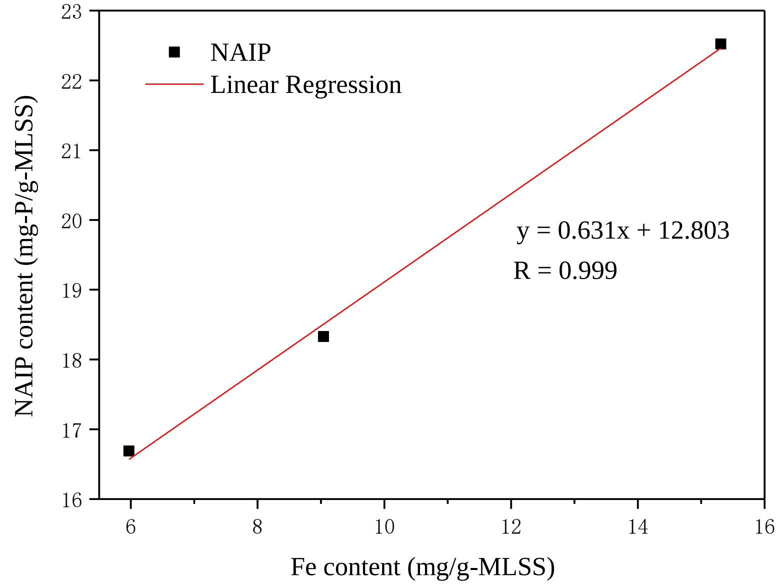

Fig. S4. The correlationship of Fe content and NAIP content in the granules.