Rapid establishment and stable performance of a new algal-bacterial granule system from conventional bacterial aerobic granular sludge and preliminary analysis of mechanisms involved

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Abstract

This work proposed a promising method to rapidly establish stable algal-bacterial aerobic granular sludge (AGS) system from conventional bacterial AGS. It was realized in this study within 18 days by inoculating 4.3% (w/w, dry mass basis) of mature algal-bacterial AGS into a sequencing batch reactor with conventional bacterial AGS treating synthetic domestic wastewater. The newly established algal-bacterial AGS reactor (R_{A-B}) exhibited better performance in nutrients removal, averagely 66.21±5.18% of total nitrogen (TN) and 63.96±6.00% of total phosphorus (TP) in comparison to 53.36±2.37% and 48.87±7.79% by the conventional bacterial AGS reactor (R_B), respectively. Better AGS settleability indicated by sludge volume index (SVI_{30}) was also noticed in R_{A-B} (45.48±0.92 ml/g versus 71.30±3.03 ml/g in R_B) on day 25. Most importantly, under the same operation strategy, the algal-bacterial AGS in R_{A-B} well maintained its granular stability and became dense and compact, while the bacterial AGS (R_B) collapsed to a large extent during the final stage of 25 days' operation. Algae content was found to be a good indicator of the maturation of newly formed algal-bacterial AGS. In this study, preliminary analysis was also conducted on the mechanisms involved in the rapid formation of algal-bacterial AGS and the changes of its biological communities.

Keywords: Algal-bacterial aerobic granular sludge; Bacterial aerobic granular sludge; Inoculation; Nutrients removal; Stability

1. Introduction

Aerobic granular sludge (AGS) is more promising and sustainable for biological wastewater treatment in comparison to the widely applied conventional activated sludge (CAS) process [1]. AGS can realize simultaneous removals of carbon(C), nitrogen(N), phosphorus(P), and other pollutants in a single sludge system [2]. AGS is originally formed from CAS, while the former possesses incomparable advantages over the latter, including dense and compact structure, better organics and ammonia removals, excellent sludge settleability, high potential for nutrients recovery, high resilience to toxicants, etc. [3-6].

During the past two decades, much attention has been paid to the granulation mechanisms of AGS which is usually obtained from sequencing batch reactors (SBRs). Up to the present, only a few reports addressed one of the most important and difficult issues for AGS biotechnology, i.e. the startup time for granulation of CAS. Generally, a relatively long period around one month is necessary to achieve granulation from CAS [7-9]. By using SBR, Beun et al. [10] accelerated the granulation process in 3 weeks at a chemical oxygen demand (COD) loading of 5 kgCOD/m^3·d and a minimal settling velocity of 12 m/h. A shorter granulation period about 14-15 days was reported by Su and Yu [11] and Benzhai et al. [12]. In fact, how to speed up the granulation process and keep granules' stability are crucial when taking the transformation of the widely applied CAS-based WWTPs into the novel AGS-based WWTPs. Most recently, Zhang et al. [13] summarized the efficient operation strategies to accelerate the granulation process of CAS, in which seeding

pellets/crushed or intact granules, and inoculation of self-aggregation strain(s) seem to be more efficient. For instance, Long et al. [14] inoculated mature AGS(25%, w/w) into CAS and obtained stably rapid granulation in 18 days. Pijuan et al. [15] also achieved a granulation time of 18 days by seeding 50% (dry basis, w/w) of crushed granules into flocculent sludge(CAS). Restated, granulation startup and granular stability are still the major bottlenecks for this biotechnology.

On the other hand, algal-bacterial AGS system reflects more stable structure and better nutrients removal than the conventional bacterial AGS. As it is known, algae can uptake nutrients (N and P), sequester C, and produce oxygen that can be used for wastewater treatment [16, 17]. Previous studies found that algae could grow spontaneously and co-exist with CAS during the granulation process in open SBR systems [18, 19]. By using a photobioreactor, Zhang et al. [20] obtained mature algal-bacterial granules within 40 days from CAS. Almost at the same time, Liu et al. [21] successfully formulated algal-bacterial granules by using the target algae (*Chlorella* and *Scenedesmus*) in a photo-SBR. In addition, a relatively quick formation (7 days) of algal-bacterial granules from bacterial AGS was reported when exposed to natural sunlight [22]. However, some issues like poor settleability and small mean size of the formed algal-bacterial granules are pending. Up to now, still, limited information is available on the rapid formation of stable algal-bacterial AGS, and the mechanisms involved is unclear.

Therefore, this study attempted to achieve the rapid establishment of algal-bacterial AGS by inoculating mature algal-bacterial AGS into conventional bacterial

AGS. The granular characteristics and nutrients removal were compared between the newly established algal-bacterial AGS and the conventional bacterial AGS systems. Results from this study are expected to find out a feasible and simple means for the transformation of a conventional bacterial AGS system into an algal-bacterial one.

2. Materials and methods

2.1. Reactor set-up and operation

Two identical SBRs (R_B and R_{A-B}), each with a diameter of 70 mm and a height of 200 mm were used in this study. R_B was the control reactor with the mature conventional bacterial AGS, while R_{A-B} was inoculated with the mature algal-bacterial AGS. The two reactors had a working volume of 500 ml with an exchange ratio of 50% during the operation. A 6 h-cycle was applied to them, including 3 min of feeding, 90 min of no aeration, 262 min of aeration, 2 min of settling and 3 min of effluent discharge according to the preliminary experiment. A LED light (Japan Equipment Co., Ltd) was set right above R_{A-B} with light intensity around 5500 lux (Fig. S1). Air was introduced into each reactor by the same air pump (KOSHIN Co., Ltd), and the uplift air flow velocity was controlled at 0.86-0.87 cm/s each by an air flowmeter, respectively.

Both mature conventional bacterial AGS and algal-bacterial AGS were cultivated in the laboratory, which have been stably operated for more than 6 months. The size of the seed algal-bacterial AGS and bacterial AGS mainly ranged between 1.0-3.0 mm, and their performance in total N and P removals were averagely about 65% and

63% by the seed algal-bacterial AGS, and 55% and 50% by the bacterial AGS, respectively in their cultivation reactors. The dominant microalgae genus (20.5%) in the seed algal-bacterial AGS was *Leptolyngbya* in this study. Initially, both reactors (R_B and R_{A-B}) were stably operated at a mixed liquor suspended solids (MLSS, conventional bacterial AGS) of about 3.0 g/L, with an initial sludge volume index in 30 min (SVI_{30}) around 48 ml/g. R_B was covered by an opaque cloth to avoid exposure to the room light and kept at operation using the same strategy as R_{A-B} except the light illumination. After being inoculated with 20 ml of mature algal-bacterial AGS (MLSS of 3.2 g/L, at an inoculation ratio of 4.3% (w/w, dry mass basis)), R_{A-B} was then illuminated by the LED light for 24 h. Both reactors were operated at room temperature (25±2°C) and their solid retention time (SRT) was controlled at around 40 days.

2.2. Synthetic domestic wastewater

In this study, the concentrations of dissolved organic carbon (DOC), ammonia nitrogen (NH_4-N) and phosphorus (PO_4-P) in the synthetic domestic wastewater (Table S1) were 150, 50 and 10 mg/L, respectively. Other components like Ca^{2+}, Mg^{2+}, Fe^{2+} ions and trace metals solution were also added to the influent according to Huang et al. [18]. Influent wastewater pH was kept around 7.5 by adding sodium hydrogen carbonate ($NaHCO_3$).

2.3. Analytical methods

NH_4-N, nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), and PO_4-P, mixed liquor(volatile)suspended solids (ML(V)SS), and sludge volume index (SVI) were measured in accordance with the standard methods [23]. In this study, the concentration of total N(TN) was the sum of NH_4-N, NO_2-N and NO_3-N, and that of total P(TP) was PO_4-P, as no organic N or P was added into the influent. DOC was determined by a total organic carbon (TOC) analyzer (TOC-VCSN, Shimadzu, Japan) after the sample being filtrated through 0.22 μm membrane filter. All the other methods, including characterization of granular sludge, extraction and determination of extracellular polymeric substances (EPS), and quantification of chlorophyll a (Chl-a) in biomass, etc. were the same as described elsewhere [17].

As for the microbial community analysis, the granules were sampled from the two reactors (R_B and R_{A-B}) on day 18, respectively. Total DNA was extracted from each granule sample using the PowerMax ®Soil Kit (QIAGEN GmbH, Germany) following the manufacturer's protocol. The extracted DNA was kept at -20°C until further process. Genomic DNA was detected by 1% agarose gel electrophoresis. The specific primers set with barcode 338F 5′-ACTCCTACGGGAGGCAGCA-3′ and 806R 5′-GGACTACHVGGGTWTCTAAT-3′ for bacteria, and P23SrV-1F 5′-GGACAGAAAGACCCTATGAA-3′ and P23SrV-1R 5′-TCAGCCTGTTATCCCTAGAG-3′ for algae were used for amplification of the hypervariable region of 16S rRNA and 18S rRNA, respectively. PCR was performed in ABI GeneAmp® 9700 (Applied Biosystems, USA) under the following conditions: 95°C for 3 min, followed by 35 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s

and a final extension step at 72°C for 10 min. After purification using the QIAquick PCR Purification Kit (Qiagen, Germany) and quantification using QuantiFluor™-ST (Promega, USA), the PCR products of all samples were taken for high-throughput sequencing on an Illumina platform (Illumina PE250, USA). MOTHUR (version: 1.31.2) was used for analyzing the biodiversity of bacteria and eukaryote in the granules. The data were analyzed on the free online platform of Majorbio Cloud Platform (www.majorbio.com).

2.4. Statistical analysis

This comparative experiment was performed in duplicate with similar phenomenon observed. All the determinations were conducted in triplicate, and their average values were reported in this work. One-way analysis of variance (ANOVA) was applied to compare the difference between the two AGS reactors by using IBM SPSS Statistics 22.0. Statistical difference was assumed if $p < 0.05$.

3. Results and discussion

3.1. Performance of the two reactors

3.1.1. Organics removal

Both reactors exhibited excellent and stable DOC removal performance, about 90% with no significant difference ($p = 0.084 > 0.05$). R_B was detected to have a little bit higher DOC removal potential than $R_{A\text{-}B,}$ indicating that algal-bacterial AGS may have less organic carbon source requirement than the conventional bacterial AGS.

This observation agrees with the statement by Zhao et al. [17] who found that algal-bacterial AGS has great potential for the treatment of low carbon source wastewaters.

3.1.2. Nitrogen and phosphorus removals

Both reactors reflected similar and outstanding performance on NH_4-N removal, > 99% ($p = 0.715 >> 0.05$) during the 25 days' operation (Fig. 1a). For NO_2-N, although significant difference in effluent NO_2-N concentration ($p < 0.05$) was discerned in the two reactors, their concentrations were very low, averagely about 0.18±0.06 mg/L in R_{A-B} and 0.06 ±0.03 mg/L in R_B, respectively. NO_3-N was detected to be the major N species in the effluents from the two reactors. Clearly seen from Fig. 1a, the effluent NO_3-N from R_{A-B} was significantly lower than that from R_B during the whole operation ($p < 0.05$) and both reactors showed a relatively stable TN removal performance. As shown in Table S2 and Fig. 3a, compared to R_B with a fluctuated biomass concentration indicated by MLSS (2.5 ~ 4.3 g/L) or MLVSS (1.8 ~ 3.4 g/L), R_{A-B} reflected a steady increase trend in MLSS (from 2.5 to 5.8 g/L) and MLVSS (from 2.0 to 4.6 g/L) during the granulation period. The steady increase in biomass concentration in R_{A-B} might contribute a lot to its higher TN (mainly total inorganic nitrogen, TIN) removal.

Figure 1(b) shows the changes of effluent TP concentration from the two reactors and their TP removal rate during the operation. An obvious difference in TP removal was noticed: R_{A-B} kept at a much higher and relatively stable TP removal (63.96±6.00%) in comparison to R_B (48.87±7.79%) ($p < 0.05$). More specifically, both

reactors first experienced some decrease in P removal during the initial 5 days, probably due to the adaptation of granules to the new environment, and then recovered their P removal efficiency. Inoculation of mature algal-bacterial AGS may ameliorate this adaptation effect, and the newly established algal-bacterial AGS system (R_{A-B}) could soon recover and always exhibited higher P removal capability than R_B, signaling the co-existing algae in R_{A-B} may also contribute to P removal from wastewater to a great extent.

3.2. Characteristics of granules in the two reactors

3.2.1. Morphological change

At the beginning, the yellowish seed bacterial AGS exhibited a compact and regular structure (Fig. S2A). After 5 days' operation, the granules in R_{A-B} started to turn green in color due to algae growth (Fig. S2B). On day 10, more algal-bacterial AGS were obviously observed besides the appearance of filamentous bacteria (Fig. S2C). Then it was noticed that algae started to grow from the outer layer to the inner part of AGS (Fig. S2D and 2b). From day 18 on, the granules became dark green in color with a dense and compact structure, and regular shape, around which some filamentous bacteria and algae were growing (Fig. S2E). Namely, the mature algal-bacterial granules were achieved. In this study, microalgae were found to easily grow in R_{A-B}, most probably attributable to the inoculated algal-bacterial AGS that may have higher potential for nutrients uptake and compete against the bacterial AGS, and then grew faster under light illumination. As a result, the rapid establishment of a new

algal-bacterial AGS system was realized. This finding to some extent agrees with He et al. [22] who claimed that algae and filamentous bacteria are the two key factors for the rapid granulation of algal-bacterial granules.

3.2.2. Granule size and distribution

Figure 2 demonstrates the changes of granular size and its distribution in the two reactors on day 0 and day 18. The mean diameter of the initial bacterial AGS was determined as 2.00 mm in both reactors. On day 18, significant difference in granular size and distribution was observed in the two reactors. According to the models (Table S2) obtained in this study, the granules in R_{A-B} reached a mean diameter of 2.24 mm, while the mean size of granules in R_B decreased to 1.36 mm. This difference in granular diameter partially agrees with the growth of algae in the granules of R_{A-B}, which may function as the core of granules or bind with filamentous bacteria [22]. Compared to the algal-bacterial AGS in R_{A-B}, the granules in R_B seemed to be relatively unstable regarding granular size and distribution.

3.2.3. Biomass growth and settleability

Biomass concentration in the two reactors was also monitored (Fig. 3a and Table S2). The initial MLSS in R_B and R_{A-B} were 2.46±0.03 g/L and 2.50±0.04 g/L with a same average MLVSS/MLSS ratio of 82%, respectively. During the whole operation, the MLSS in R_{A-B} kept increasing and reached the maximum of 5.77±0.08 g/L (MLVSS/MLSS =0.80), mainly contributed by the stable growth of algae and

formation of algal-bacterial AGS under the operation strategy. While in R_B, MLSS increased first to 4.30±0.16 g/L during the initial 5 days, and then an obvious decrease was detected on days 10 (3.01±0.12 g/L) and 18 (2.49±0.12g/L), most probably due to its relatively weak structure. This can be evidenced by the appearance of some small flocs from the broken granules, which may be lost through the effluent discharge. The granules in R_{A-B}, in contrast, kept at a definitely better and stable structure. In addition, a slightly higher MLVSS/MLSS ratio was detected in the granules of R_{A-B} (0.80±0.05) than that of R_B (0.78±0.07) during the 25 days' operation.

On the other hand, the SVI_{30} increased to some extent for the granules in R_{A-B} (63.96±3.65 ml/g) during the first 5 days (Fig. 3b), probably brought about by the competition for nutrients between algae and bacteria with more EPS excretion to some extent. The excreted substances from the inoculated algal-bacterial granules (to protect their structure) and the fast growth of algae may have negative effect on sludge settleability during this period. However, under the operation conditions, the settleability of the granules in R_{A-B} recovered soon and kept stable after day 10, about 45.48±0.92 ml/g on day 25 (Fig. 3b). While in R_B, the SVI_{30} was found to decrease to some extent (better settleability) during the initial 5 days, which increased sharply from day 5 to day 18 (78.64±3.92 ml/g with AGS disintegration being always observed), finally to about 71.30±3.03 ml/g on day 25 (Fig. 3b). The newly established algae-bacterial AGS system exhibited an excellent and stable sludge settleability from day 18 onwards.

3.2.4. Changes in EPS and chlorophyll contents

EPS including proteins (PN) and polysaccharides (PS) are the major extracellular substances secreted from the cells, which are considered to contribute to the granulation process [24]. The major difference in EPS content in the granules from the two reactors was noticed during the first 5-10 days' operation (Fig. 4a). Specifically, obviously higher PN and PS contents were detected in the granules from R_{A-B} (61.7±18.4 and 19.23±5.05 mg/g-MLVSS versus 38.71±8.57 and 11.12±2.41 mg/g-MLVSS in R_B) on day 5, to some extent in agreement with the changes in biomass growth and its settleability. Restated, algae growth may stimulate granules to secrete more EPS during the first 5 days' operation, which could help to re-aggregate bacteria and algae to form new algal-bacterial granules. It was noticed that the PN was the dominant component in the EPS, which might be crucial for a stable granule structure. The PS/PN ratio of the granules reflected a similar trend in the two reactors during the 25 days' operation (Fig. 4a), most probably attributable to that mature bacterial AGS and algal-bacterial AGS were used as seed in this study.

All green plants contain chlorophyll a (Chl-a), and Chl-a can be used as an algal biomass indicator [23]. In this study, the change of Chl-a content in the algal-bacterial granules is shown in Fig. 4b. As seen, the Chl-a content was detected to remarkably increase from 0 to 5.31±0.89 mg/g-MLVSS on day 5. After some slight decrease on day 10 (4.92±0.17 mg/g-MLVSS), the Chl-a content continued to increase and stabilized at 6.3-6.5 mg/g (days 18 and 25). This observation indicated that algae content in the granules might be a good indicator for the maturation of algae-bacterial

granules.

Seen from the obtained results, compared to the seed algal-bacterial AGS, the newly established algal-bacterial AGS system almost exhibited the similar efficiencies in nutrients removal, and other characteristics including morphology, granular size and distribution, etc.

3.3. Biological community analysis

3.3.1. Microbial richness and diversity

The richness and diversity of microbial community in algal-bacterial AGS (R_{A-B}) and bacterial AGS (R_B) are illustrated by the estimators (Table 1). The sequences increased during the rapid establishment of algal-bacterial AGS from conventional bacterial AGS. Besides, both ACE and Chao estimators indicated that this rapid formation process for algal-bacterial AGS also had obvious enhancement effect on the community richness. However, very little discrepancy in Shannon and Simpson estimators was observed during this rapid establishment period, in which algal-bacterial AGS seemed to have some slight increase in community diversity on day 18. Therefore, the newly formed algal-bacterial AGS could maintain similar microbial community richness and diversity with the bacterial AGS during the 18 days' establishment period. This observation is different from He et al. [22] who claimed that both richness and diversity of microbial were decreased during the granulation period, which might be mainly attributable to the differences in light source and other operation conditions. As stated, a strong summer sunlight might inhibit the growth of

microorganism such as nitrite oxidizing bacteria (NOB) [27].

Table 1

Similarity-based Operational Taxonomic Units (OTUs), richness and diversity estimators of microbial communities in the granules from R_{A-B} and R_B on day 18, respectively.

Samples	Sequences	OTUs	Community richness		Community diversity		Coverage [d]
			ACE [a]	Chao [a]	Shannon [b]	Simpson [c]	
R_B	39,313	504	554.13	557.63	4.38	0.03	0.997
R_{A-B}	47,470	513	584.88	598.87	4.40	0.03	0.997

[a]A larger number → greater richness. [b] A larger number → more diversity. [c] A larger number → less diversity. [d] Sampling depth.

3.3.2. Biological community

The taxonomic affiliations of the dominant bacteria at both phylum and class levels are shown in Fig. 5. Clearly, during the rapid establishment of algal-bacterial AGS from conventional bacterial AGS, much change was observed in two dominant phyla in the two reactors. (1) *Proteobacteria* (including classes *Alpha-, Beta-, Gamma-,* and *Delta-proteobacteria)* were dominant in R_B, while they declined obviously during the formation of new algal-bacterial AGS in R_{A-B}. Among these classes, the abundant *Gamma-* and *Delta-proteobacteria* may contribute to the low effluent NO_2-N concentration in the two reactors (Fig. 1a), as NOB capable of converting nitrite to nitrate belong to these two classes [25]. (2) *Cyanobacteria* increased dramatically in R_{A-B}, which are closely associated with the rapid growth of

algae (Fig. 4b). Results show that the relative abundance of *Cyanobacteria* in the granules from R_{A-B} and R_B were 20.90% and 0.20%, respectively. In addition, the relative abundance of phylum *Planctomycetes* decreased in the granules of R_{A-B}. This phylum may compose anaerobic ammonium oxidation (anammox) *Planctomycetes* and non-anammox *Planctomycetes*. The former can oxidize ammonia to dinitrogen without oxygen, and play a major role in the global nitrogen cycle and the newly developed biological processes for high strength ammonia wastewater treatment [26]. Currently, it is unknown which *Planctomycetes* were decreased during the re-aggregation process. However, seen from the relatively higher and stable TN removal by R_{A-B} (Fig. 1a), the enhanced nutrients removal by R_{A-B} may be attributable to its increasing growth of algae (Fig. 4b). In conclusion, the rapidly established algal-bacterial AGS system from conventional bacterial AGS can maintain diverse microbial communities, indicating its outstanding potentials for nutrients removal.

Figure 6 shows the relative abundance of algae species at genus level in the seed algal-bacterial AGS and R_{A-B} on day 18. Obviously, *Leptolyngbya* is the dominant algae species in the seed algal-bacterial AGS. However, after 18 days' inoculation and operation in R_{A-B}, *Jaaginema* dominated the algal-bacterial AGS. The observation may be brought about by the differences in reactor dimension/configuration and photoperiod between the reactors used for the cultivation of seed algal-bacterial granules and for this study. The above-mentioned differences may have effect on light intensity, transmittance and distribution, thus the changes in algae species [20]. *Jaaginema* is likely to grow faster than other algae species in the small and high

illumination intensity reactor. Whether the increase of *Jaaginema*, probably a polyphyletic genus [28], contributed to the rapid establishment of the new algal-bacterial AGS system or not remains unknown. As shown in Fig. 6, in this study more than half of the algal species were still unclassified (60-70%) in both systems. In order to add insights into the mechanisms involved, further works are necessary on the identification of algae species and their functions during the rapid establishment of algal-bacterial AGS system.

3.4. Preliminary analysis on the mechanisms involved in the rapid establishment of new algal-bacterial AGS system

Based on the above observations, the establishment of a new algal-bacterial AGS system from the conventional bacterial AGS by inoculating algal-bacterial AGS can be explained as the following three stages (Fig. 7). (1) Adaptation stage (≤ 5 days): during the initial 5 days algae can grow quickly due to light illumination and compete against the bacteria in AGS because of its higher nutrients uptake potential and faster growth rate. In response to the co-existence of algae, although more EPS are excreted to keep its structure, the bacterial AGS is easily broken with small pellets or flocs generated. During this period, C and N removals are not affected, while P removal and sludge settleability are affected to some extent due to the AGS disintegration. (2) Re-aggregation and re-organization stage (days 5-10): under the designed operation conditions, algae can stably grow from the out layer of bacterial AGS to the inner part, and some algae can function as the core of the new algal-bacterial AGS through

re-aggregation. The newly formed algal-granules gradually recover their P removal and settleabililty. (3) Maturation stage (up to 18 days): with the stable growth of algae and further increase of EPS secretion, the algal-bacterial AGS becomes mature with a dense and compact structure, demonstrating stably efficient nutrients removal and excellent settleability. This maturation process may be promoted by the co-existence of filamentous bacteria and algae. More detailed information of dynamic changes in bacterial and algal species during this rapid establishment of algal-bacterial AGS will be included in another work, which is expected to shed more light on the mechanisms involved.

4. Conclusions

Newly mature algal-bacterial granules were achieved within 18 days by inoculating 4.3% (w/w, dry mass basis) of mature algal-bacterial granules into the conventional bacterial AGS reactor. The new algal-bacterial granules exhibited better and stable performance in TN (66.21±5.18%) and TP (63.96±6.00%) removals and settleability (45.48±0.92ml/g) than the bacterial AGS. Algae content in granules can be used as a good indicator for maturation of algal-bacterial AGS during this process. The established algal-bacterial AGS system possessed the similar nutrients removal and other characteristics as the seed algal-bacterial AGS. This work provides a feasible and simple means to realize the rapid transformation of conventional bacterial AGS to algal-bacterial granular system.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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Figures

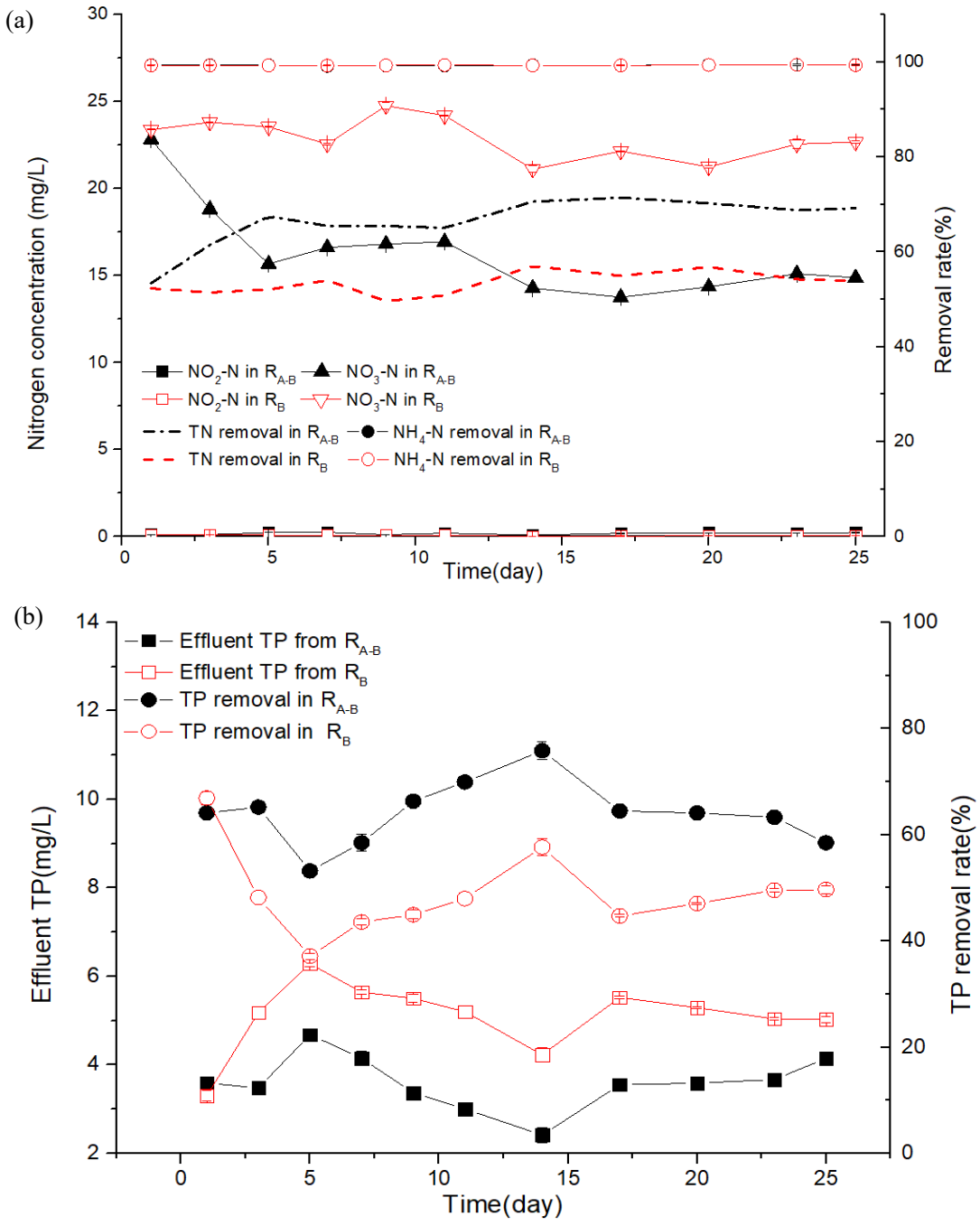
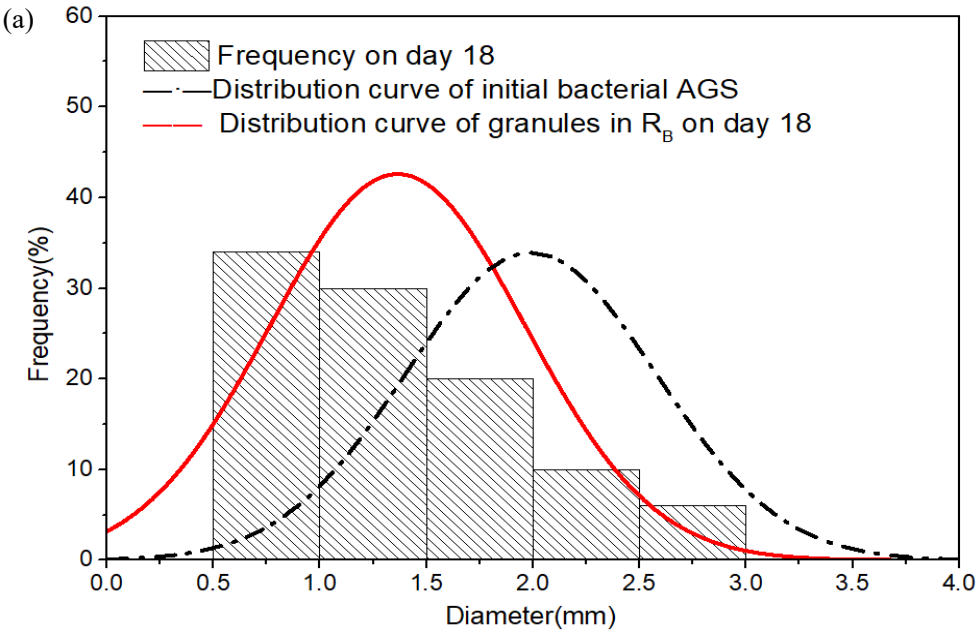


Fig. 1. Nitrogen (a) and phosphorous (b) profiles in R_{A-B} and R_B during 25 days' operation, respectively.



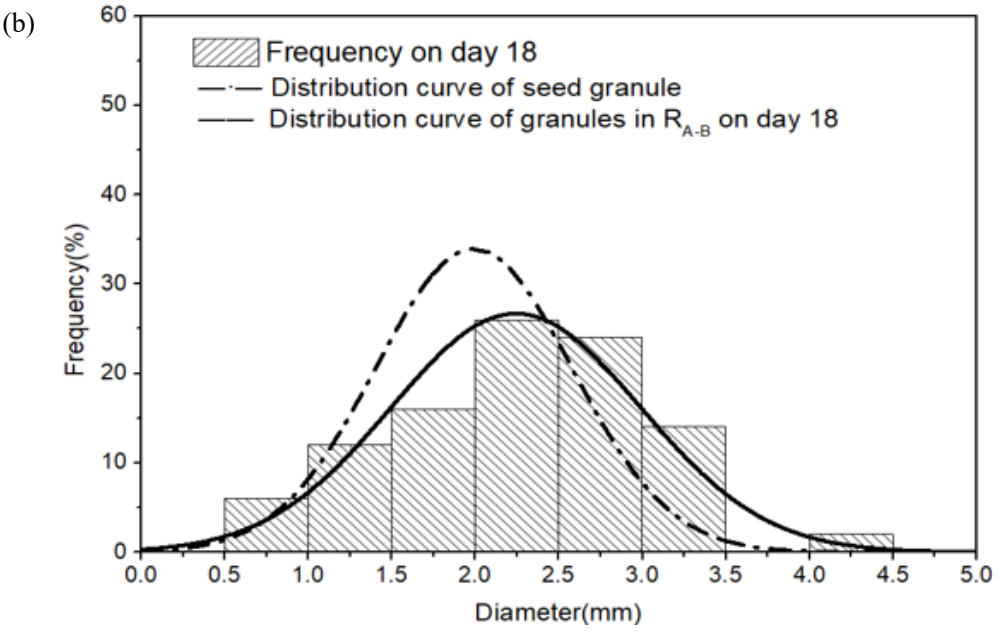
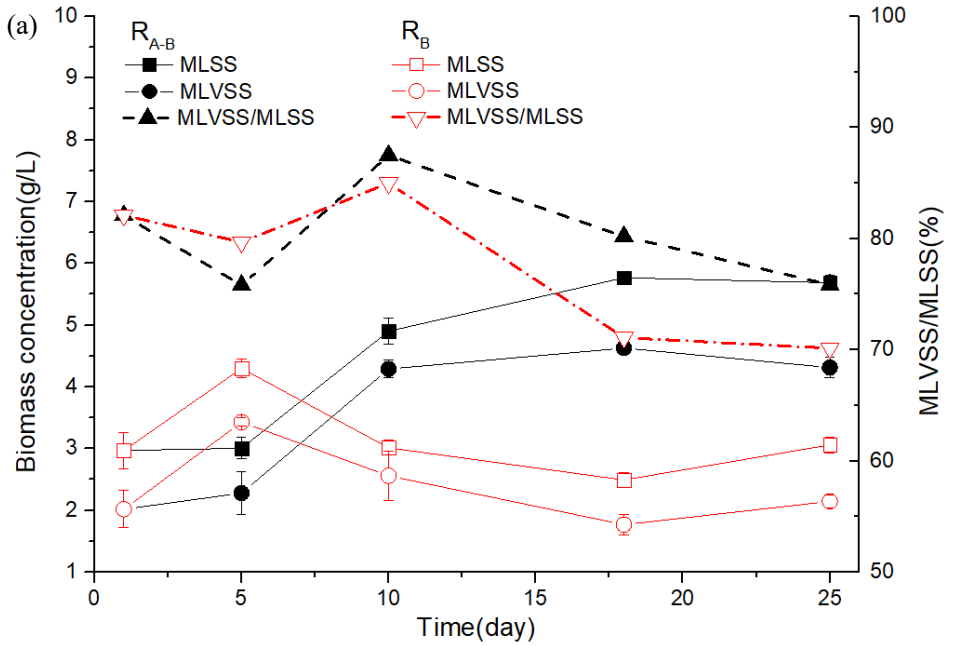


Fig. 2 Changes in granular size and distribution in R_B (a) and R_{A-B} (b) on day 0 and day 18, respectively.



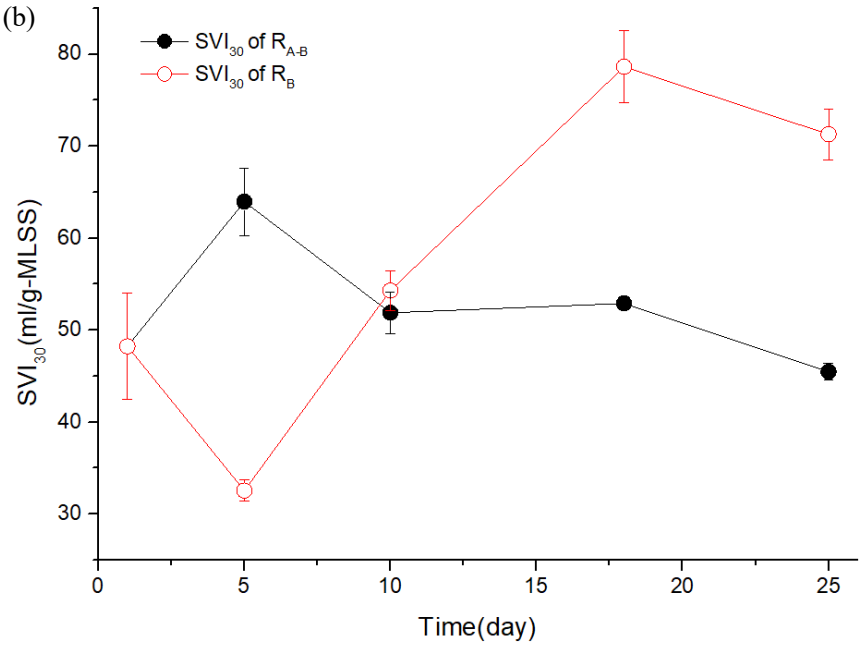


Fig. 3 Variations of biomass concentration (MLSS and MLVSS, a) and sludge settleability (SVI_{30}, b) in the two reactors during the 25 days' operation.

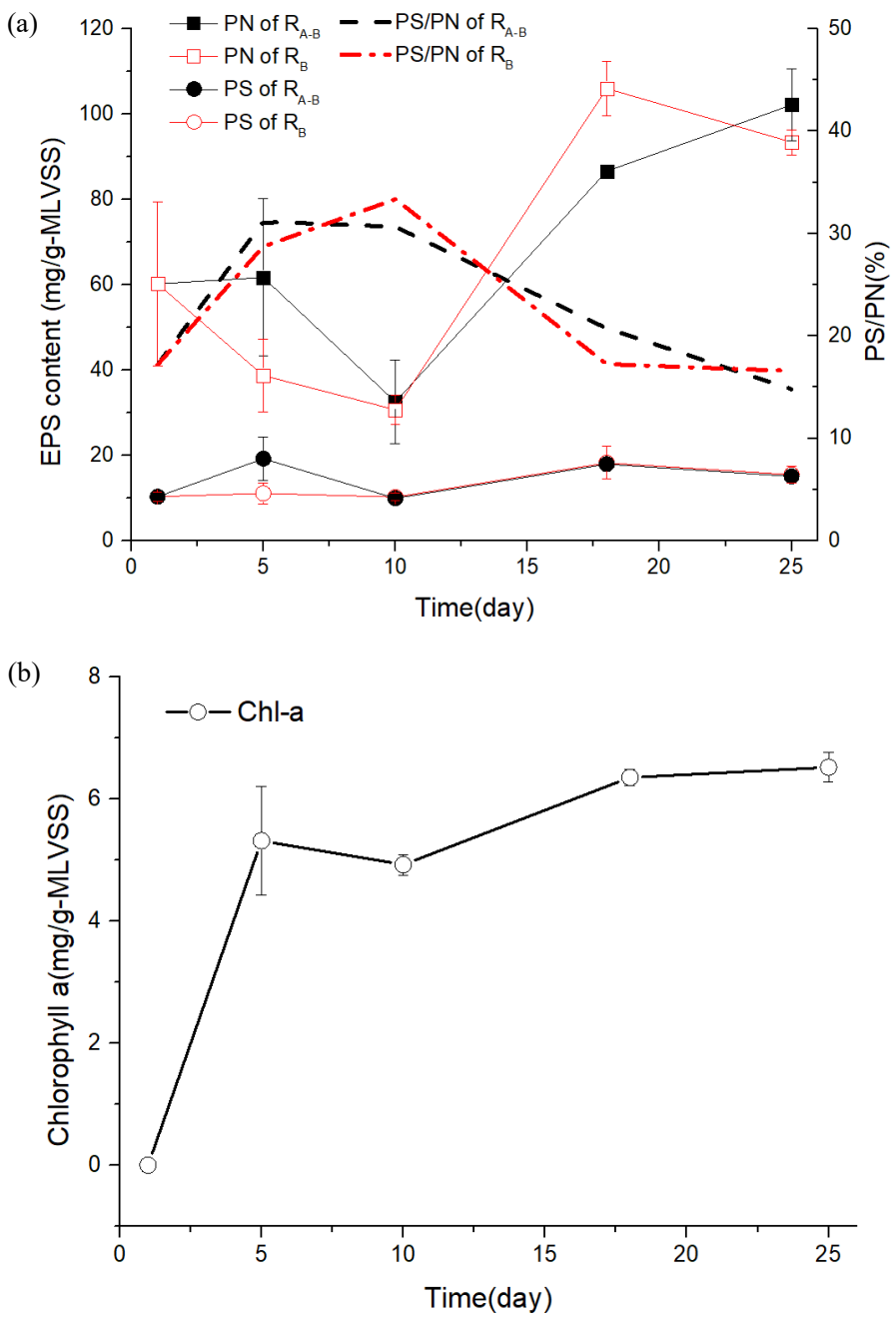


Fig. 4 (a)Changes in extracellular polymeric substances (EPS) components, proteins (PN) and polysaccharides (PS) in the granules from R_{A-B} and R_B; (b) Changes in chlorophyll a (Chl-a) content in the algal-bacterial granules (R_{A-B}) during the operation.

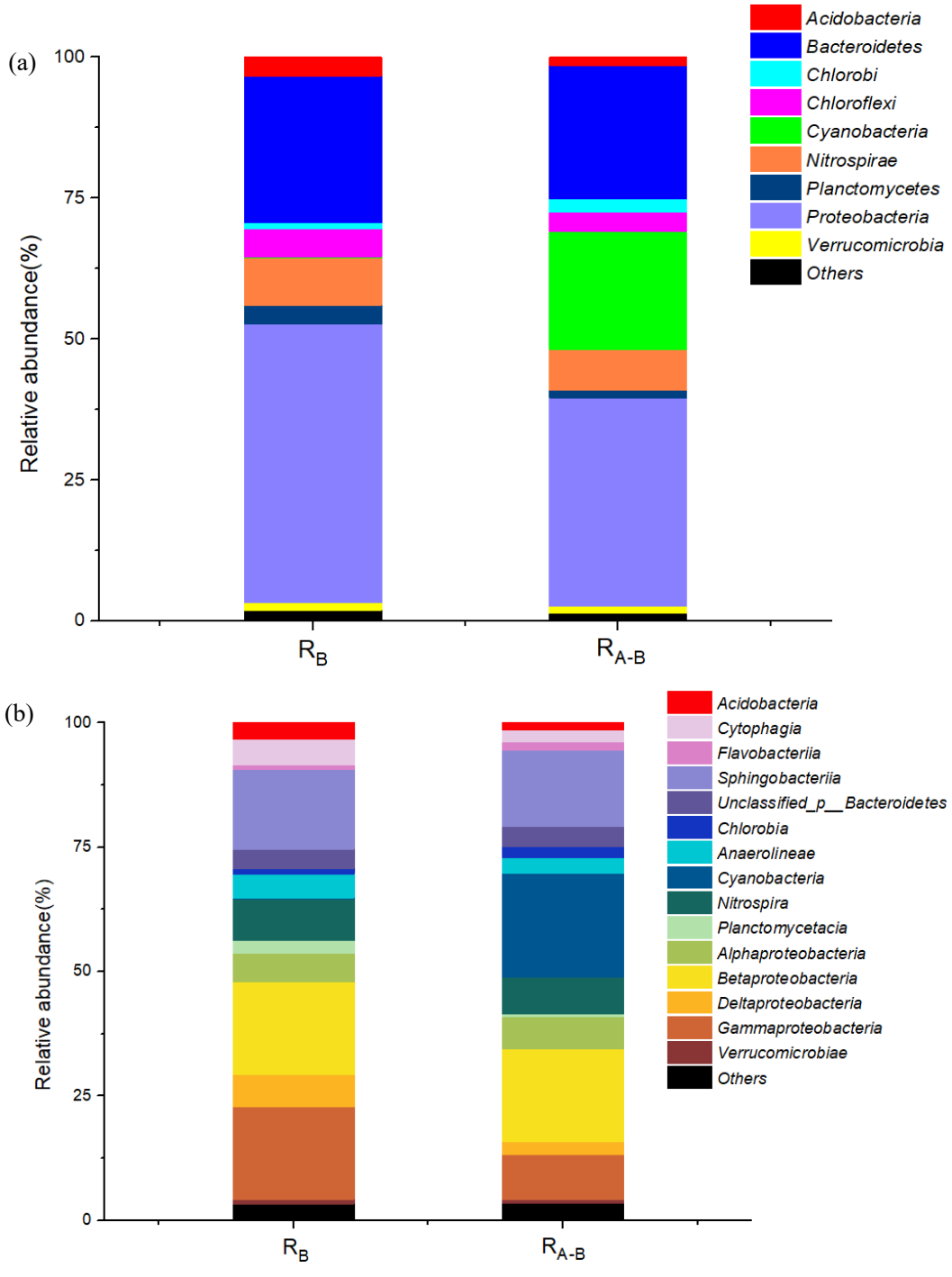


Fig. 5 Microbial communities in the granules from R_{A-B} and R_B at phylum level (a) and class level (b) on day 18.

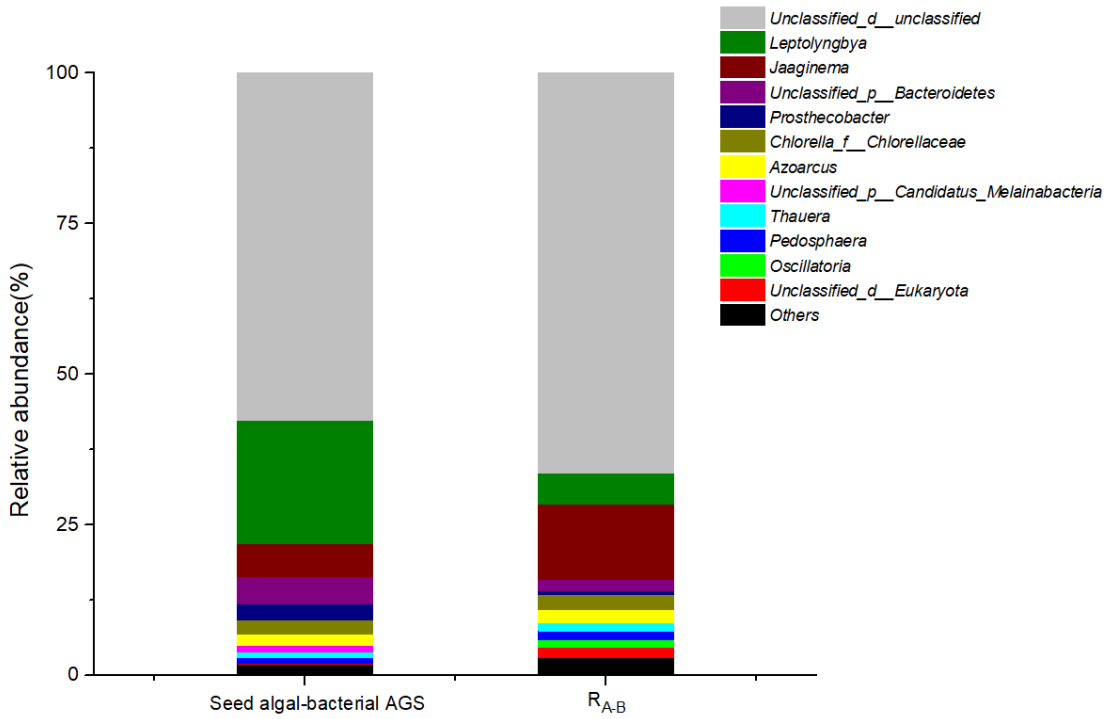


Fig. 6 Algae species at genus level in the seed algal-bacterial AGS and granules from $R_{A\text{-}B}$ on day 18.

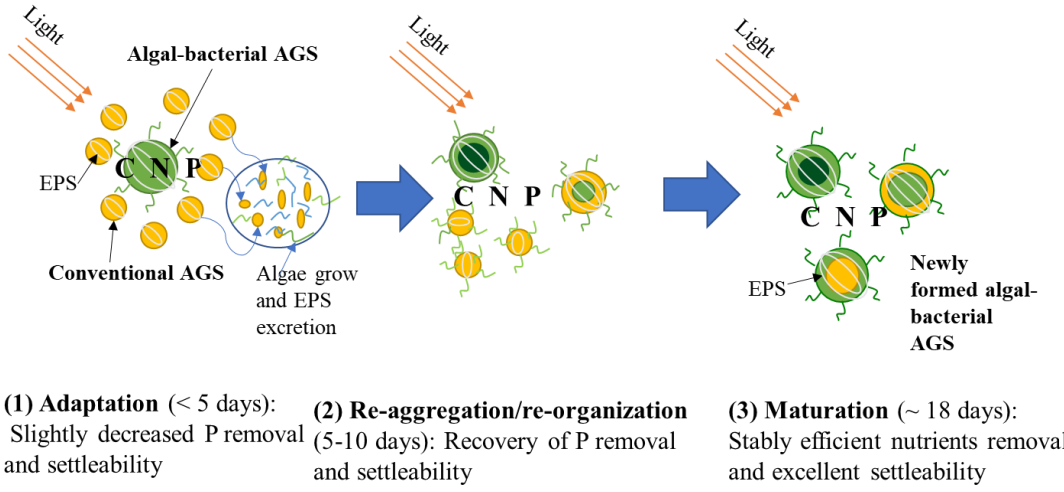


Fig. 7 Schematic diagram of possible mechanisms proposed for the rapid formation of algal-bacterial AGS in this study.