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Effect of algae growth on aerobic granulation and nutrients removal from synthetic wastewater by using sequencing batch reactors

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Abstract

The effect of algae growth on aerobic granulation and nutrients removal was studied in two identical sequencing batch reactors (SBRs). Sunlight exposure promoted the growth of algae in the SBR (Rs), forming an algal-bacterial symbiosis in aerobic granules. Compared to the control SBR (Rc), Rs had a slower granulation process with granules of loose structure and smaller particle size. Moreover, the specific oxygen uptake rate was significantly decreased for the granules from Rs with secretion of 25.7% and 22.5% less proteins and polysaccharides respectively in the extracellular polymeric substances. Although little impact was observed on chemical oxygen demand (COD) removal, algal-bacterial symbiosis deteriorated N and P removals, about 40.7-45.4% of total N and 44% of total P in Rs in contrast to 52.9-58.3% of TN and 90% of TP in Rc, respectively. In addition, the growth of algae altered the microbial community in Rs, especially unfavorable for Nitrospiraceae and Nitrosomonadaceae.

Keywords: Aerobic granules; Sunlight; Algae; Nitrification; Phosphorus removal

1. Introduction

Twenty years ago aerobic granular sludge was first reported by Mishima and Nakamura (1991) in a continuous upflow aerobic sludge blanket bioreactor. Up to now much effort has been put on this promising biotechnology which possesses many incomparable advantages like excellent settleability, high biomass and ability to withstand toxicity and organic loading (Adav et al., 2008a; Huang et al., 2014) in comparison to conventional activated sludge processes. So far, research works on aerobic granular sludge are mainly focused on pollutants removal efficiency and granulation mechanism in lab-scale sequencing batch reactors (SBRs), from which the results can be utilized as guidance for its practical engineering applications (Lee et al., 2010; Maszenan et al., 2011). Moreover, aerobic granular sludge with good performance for pollutants removal has been successfully cultivated in pilot-scale SBRs (Ni et al., 2009; Long et al., 2014). Therefore, in the near future, it is expected that the aerobic granular sludge technology can be applied as one of major processing units in wastewater treatment plants.

On the other hand, natural water bodies worldwide have suffered for a long time from frequent algae blooms. Algae are also attracting researchers' attention in the field of wastewater treatment, partially because of their high capacity of nutrients uptake and oxygen production thus facilitating aeration in wastewater (Abdel-Raouf et al., 2012). Recently, the microalgal-bacterial symbiosis has been tested in ponds and photo-bioreactors, achieving cost-effective wastewater treatment (Boelee et al., 2014;

Marcilhac et al., 2014). De Godos et al. (2009) used a tubular biofilm-based photobioreactor to treat pretreated swine slurry, and found that the microalgal–bacterial symbiosis could achieve nitrogen (N), phosphorus (P) and chemical oxygen demand (COD) removal efficiencies up to 100%, 90% and 75%, respectively with no external O_2 supply. Still, some researchers pointed out that the microalgal-bacterial symbiosis had some impact on the metabolism and biodiversity of microorganisms. The activity of ammonia oxidizing bacteria (AOB) was decreased by 20% with the co-existence of algae due to algae were superior competitors for N uptake compared with AOB (Risgaard-Petersen et al., 2004). In addition, Su et al. (2012) found that the bacterial communities varied with different ratios of algae to sludge inoculation in the algal-bacterial system for wastewater treatment. Up to now, however, little information can be found in the literature about the effect of algae growth on aerobic granules.

This work aimed to investigate the effect of algae growth on aerobic granulation and nutrients (COD, N and P) removal. The influences of algae on COD, N and P removal performance, and bioactivity of aerobic granules were determined. The components of extracellular polymeric substances (EPS) in addition to the changes in microbial diversity of the granules were also analyzed in order to shed light on the mechanism involved in the influence of algae growth on the aerobic granules. It is expected that this work will be useful for the cultivation and application of aerobic granules in practice.

2. Materials and methods

2.1. Reactor set-up and operation strategy

Aerobic granules were cultivated in two identical sequencing batch reactors (SBRs) made of acrylic transparent plastic, 6 cm in diameter with a height of 60 cm. The working volume of each SBR was 1.4 L. One of the two SBRs, Rs, was placed near the window in the laboratory and irradiated around 4 hours per day (from 9:00 to13:00 due to the location of Rs) by natural sunlight from March to June, 2014. During the 100 days' operation, 61 days were sunny and the average visible light and UV-light intensity were 42 and 3 mW/cm^2, respectively during the irradiation period. Another SBR (Rc), without sunlight irradiation, was used as control.

The two reactors, namely Rs and Rc, were operated sequentially in a 4-h cycle at room temperature (25±2℃): 2 min of influent filling, 28 min of non-aeration period, 185-200 min of aeration, 5-20 min of settling, and 5 min of effluent discharge. The settling time was gradually decreased from 20 min to 5 min due to the increase in settleability of the sludge. The volumetric exchange ratio was kept at 50%, leading to a hydraulic retention time of 8 h. The airflow rate was 2.0 cm/s and controlled via a gas-flow controller to keep the dissolved oxygen (DO) level between 7-9 mg/L in each aeration cycle.

2.2. Seed sludge and synthetic wastewater

Each reactor was inoculated with 0.5 L of seed sludge sampled from a sedimentation tank of the Shimodate Sewage Treatment Plant, Ibaraki Prefecture,

Japan. On the sampling day the concentrations of chemical oxygen demand (COD), ammonia nitrogen (NH_4-N), and orthophosphate phosphorus (PO_4-P) were about 200, 30, and 3 mg/L in the plant influent, respectively. The treatment plant was under normal operation with high efficient COD, NH_4-N, and PO_4-P removals of 89%-95%. The seed sludge was dark brown in color before the start-up of granulation. The initial mixed liquor suspended solids (MLSS) concentration was 3.8 g/L with sludge volume index (SVI) of 87 ml/g and MLVSS/MLSS of 0.8 in the two reactors. After aerobic granules appeared, the mixed liquor was withdrawn daily from the reactors in order to keep their solids retention time (SRT) around 20 days.

Synthetic wastewater was used in this study, and its composition was as follows: COD 600 mg/L (50% of which was contributed by glucose and sodium acetate, respectively); 10 mg PO_4-P/L (KH_2PO_4); 100 mg NH_4-N/L (NH_4Cl); 10 mg Ca^{2+}/L ($CaCl_2$); 5 mg Mg^{2+}/L ($MgSO_4 \cdot 7H_2O$); 5 mg Fe^{2+}/L ($FeSO_4 \cdot 7H_2O$); and 1ml/L of trace element solution. The trace element 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., 2008b). The pH in the reactors was adjusted with sodium bicarbonate to be within 7.0-8.3.

2.3. Analytical methods

Mixed liquor (volatile) suspended solids (ML(V)SS), sludge volume index (SVI), COD, NH_4-N, nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), and phosphorus (PO_4-P) were measured in accordance with the standard methods (APHA,1998). Total

concentration of phosphorus in the liquid was determined with molybdenum blue method after digestion by potassium persulfate at 120℃. Dissolved oxygen (DO) concentration in the bulk liquor was measured with a DO meter (HQ40d, HACH, USA). pH was determined by a pH meter (Mettler Toledo FE20, Switzerland).

The microbial activity of activated sludge was indicated by specific oxygen uptake rate (SOUR), in terms of milligrams of oxygen consumed by per gram of sludge per hour. In this study, SOUR was determined at 25℃ in a 100-ml volumetric flask, which was filled with 20 ml of the mixed liquor taken from the SBR at the end of the operational cycle and 80 ml synthetic wastewater, and then sealed after insertion of a DO electrode (HQ40d, HACH, USA). The mixed liquor was agitated using a magnetic stirrer. DO level in the bulk liquor of the volumetric flask was continuously recorded by the DO meter. SOUR value was obtained by linear regression of the DO concentrations over time divided by the constant concentration of MLSS.

Batch tests were performed under DO $\geq$ 8.0 mg/L at 25℃ in order to obtain the maximum specific ammonium uptake rate (SAUR) and specific ammonium nitrite uptake rate (SNUR). Before testing, granules were taken from the two reactors respectively at the end of the operational cycle and aerated for 1 h to ensure that all ammonium ions were completely consumed and converted. Subsequently, the granules were washed with tap water, and then divided into three aliquots based on wet weight. Each aliquot was dosed into one 250 ml flask filled with the same synthetic wastewater used in this study (except for ammonium or nitrite

concentration). A pulse of concentrated stock solution of ammonium or nitrite was added at the beginning of test in order to achieve an initial concentration of 50 or 20 mg-N/L, respectively. Samples were collected at an interval of 20 min and then measured. Granular SAUR or SNUR was obtained by linear regression of the NH_4-N or NO_2-N concentrations over time divided by the constant concentration of MLSS.

Extracellular polymeric substances (EPS) were extracted from the sludge by using ultrasound-formaldehyde-sodium hydroxide method (Adav and Lee, 2008). Extracellular proteins (PN) in the extracted EPS were determined by Bradford method with bovine serum albumin (BSA) as standard (Bradford, 1976). Extracellular polysaccharides (PS) were measured by using phenol-sulfuric acid method with glucose as standard (Dubios et al., 1956).

The mean granular size was measured by a stereo microscope (STZ-40TBa, SHIMADZU, Japan) with a program Motic Images Plus 2.3S (Version 2.3.0). Morphology characteristics of the granules were observed using a scanning electron microscope (JSM6330F, Japan).

2.4. High-throughput sequencing

The total DNA (100 μl) of granular sludge samples harvested on day 80 from Rs and Rc were extracted by using Mo Bio PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., USA) according to the manufacturer's protocol. The electrophoresis of genomic DNA was then performed in 0.8% agarose gels and quantified by spectrometry (NanoDropetry-1000).

The hypervariable region (~200 bp) of the bacterial 16S rRNA gene was amplified. The primer set composed of a forward primer V4F, 5′-AYTGGGYDTAAAGNG-3′ and an equimolar mixture of four reverse primers, i.e. V4R1 5′-TACCRGGGTHTCTAATCC-3′, V4R2 5′-TACCAGAGTATCTAATTC-3′, V4R3 5′-CTACDSRGGTMTCTAATC-3′, and V4R4 5′-TACNVGGGTATCTAATCC-3′ based on the RDP pyrosequencing pipeline (http://pyro.cme.msu.edu/pyro/help.jsp). The PCR conditions were as follows: 95℃ for 7 min, followed by 32 cycles at of 95℃ for 1 min, 55℃ for 1 min, 72℃ for 1 min and a final extension step at 72℃ for 10 min. After quantification using Qubit 2.0 kit, the PCR products of all samples were taken for high-throughput sequencing on Ion Torrent PGM System (Life Technology, USA). Mothur (version: 1.31.2) was used for analyzing microbial biodiversity in the granules.

2.5. Calculations

Free nitrous acid nitrogen (FNA-N) concentration was calculated according to Eq. (1) after total nitrite nitrogen (TNO_2-N) and solution pH being measured (Anthonisen et al., 1976).

$$[\text{FNA-N}](\text{mg/L})/[TNO_2\text{-N}](\text{mg/L})=1/(1+e^{-2300(273+T)}\cdot 10^{pH}) \quad (1)$$

where T is the room temperature in 25℃.

3. Results and discussion

3.1. Formation and characterization of granules in the reactors

The initial seed sludge had irregular and loose structure with a mean floc size of 0.14 mm. After 15 days' cultivation, yellowish granules with clear boundary were observed in the two reactors and grew gradually. Interestingly, the surface of granules in Rs gradually turned into green from day 19 on, due to the growth of algae resulted from sunlight exposure (Figs. S1a and S1b). SEM observation on day 90 clearly shows that bacteria prevailed all over the granules with compact and dense structure in Rc (Fig. S1c). The granules in Rs, however, with symbiotic growth of bacteria and algae had rough surface and loose structure (Fig. S1d). This phenomenon implies that the community structure of microorganisms in Rs should be different due to the growth of algae, which was further investigated in this study.

The physical characteristics such as MLSS, SVI and particle size of the sludge in the two reactors were monitored (Table 1). After 40 days' operation, the MLSS in Rs kept around 7.5 g/L, higher than that in Rc (about 6.4 g/L), probably brought about by the rapid growth of algae due to sunlight irradiation. It is worth noting that the SVI_{30} value of granular sludge decreased from initial 87 ml/g to 38 ml/g in Rs while to 25 ml/g in Rc at the end of experiment, suggesting the growth of algae in Rs has some negative effect on the settleability of granules. This observation is to some extent in agreement with previous findings that the settleability of bacterial sludge is much better than algae (Choi et al., 2010; Su et al., 2012). In addition, different increase in granular diameter was noticed between the granules in Rs and Rc along with the operation. After 100 days' cultivation, the average diameter of granules in Rs was about 0.78 mm, much smaller than that of granules in Rc (1.21 mm). Therefore, it

could be inferred that the growth of algae in Rs affected the settleability of granular sludge, slowing down the growth of granules to a certain extent.

3.2. Performance of Rs and Rc

3.2.1. COD and TP removals

As shown in Fig. 1a, COD removal was lower than 90% for both reactors during the initial 10 days, most probably due to low MLSS in the reactors and some adaptation period was necessary for the seed sludge (used to treat domestic wastewater) to effectively treat the synthetic wastewater prepared in this study. From day 12 on, however, the COD removal efficiencies in Rc and Rs were averagely 96.1% and 95.2%, respectively with effluent COD < 30 mg/L till the end of experiment, suggesting the granular sludges in both reactors have good potential for organics removal.

On the other hand, from day 5 on, a different trend in TP removal was detected between the two reactors. The effluent TP from Rc was always below 1.0 mg/L after 20 days' operation with P removal rate > 90%. The granular P release rate in Rc during non-aeration stage was around 4.6 mg-P/(g-MLSS·h), signaling the presence and high activity of polyphosphate accumulating organisms (PAOs) in Rc. However, only a small amount of P release was monitored during non-aeration period and the effluent TP concentration was averagely 5.6 mg/L in Rs, possibly attributable to the inhibited activity of PAOs by free nitrous acid (FNA) (Fig. 1b). Pijuan et al. (2010) detected 50% inhibition on PAOs growth, phosphate uptake and glycogen production

at FNA concentration of approximately 0.5×10^{-3} mg HNO_2-N/L and pH 7.0, while complete inhibition occurred at FNA concentration of about 6×10^{-3} mg HNO_2-N/L. In this study, the FNA concentration in Rs was about 6.3-9.8 $\times10^{-3}$ mg HNO_2-N/L from day 10 on, most probably brought about by the inhibited nitratation process (the conversion of produced NO_2-N into NO_3-N) due to algae growth thus resulted in NO_2-N accumulation (Fig.1b). This observation implies that algae growth might affect the activity of NOB (nitratation process), resulting in FNA accumulation and subsequent inhibition on PAOs.

3.2.2. Nitrogen profiles

Fig. 1b shows the changes of N species and N removal performance in Rs and Rc during the 100 days' operation. The granules in Rc exhibited excellent performance in removing NH_4-N, with removal ratio > 99% from day 10 on to the end of experiment. However, it took about 40 days for the NH_4-N removal rate in Rs to increase from 43% to 98%. Moreover, an obvious difference in the effluent NO_2-N concentration was noticed between Rc and Rs. NO_2-N was observed to rapidly accumulate in Rs and reached the maximum 43.6 mg/L on day 20. Although decreased to some extent from day 20 to the end of experiment, the NO_2-N concentration in Rs was still at high level (> 28 mg/L). In contrast, only transient NO_2-N accumulation was detected in Rc during the first 10 days, and the effluent NO_2-N concentration maintained below 0.3 mg/L from day 20 on.

In addition, much higher granular SAUR and SNUR were detected in the

granules taken from Rc in the batch experiments, especially SNUR (NO_2-N uptake rate, Table S1). These results confirm that algae growth inhibited the activity of nitrifying bacteria in Rs, especially nitrite oxidizing bacteria (NOB). Stated, the TN removal efficiency in Rs varied between 40.7 % - 45.4% after mature granules formed, lower than that in Rc (52.9 % - 58.3%), indicating that the denitrification process in the granules from Rs was also impacted to some extent by algae growth.

3.3. EPS and SOUR of granules

Extracellular polymeric substances (EPS), sticky metabolic products secreted by bacteria, are mainly composed of proteins (PN), polysaccharides (PS), humic acids and lipids. Adav et al. (2008b) pointed out that EPS were beneficial for aerobic granulation and granular stability, especially PN and PS, and the latter formed the backbone of the granules. The contents of PN and PS extracted from granules at different stages are presented in Fig. 2a. Both compounds from the granular sludge in the two reactors increased a lot when compared with the seed sludge (day 0). In addition, PN/PS ratio was about 3.3 for the granules in Rc and 3.1 for those in Rs, increasing more than 2 times compared to the seed sludge. This observation is in agreement with Zhang et al. (2007) who found that the protein content was significantly high in aerobic granules with PN/PS ratios ranging between 2.3 and 4.9. A meaningful finding is that the PN and PS contents in the granules from Rs were determined to be 49.6 and 15.9 mg/g-VSS on day 100, i.e. decreased by 25.7% and 22.5% respectively in comparison to Rc, leading to the significant decrease in EPS

production and further slowing down the growth of aerobic granules.

The stimulated EPS excretion in aerobic granules can be associated with the bioactivity of bacteria. The granular SOUR of Rc was 76.4 mg-O_2/g-VSS·h on day 20 and slightly decreased to 67.8 mg-O_2/g-VSS·h on day 100, much higher than the granular SOUR of Rs during the whole process (Fig. 2b). Results from Yang et al. (2004) showed that the PN/PS ratio had a negatively linear relationship with the SOUR of bacteria in the granules under varied influent FA conditions. In this study, the PN/PS ratio was also found to be negatively correlated with the granular SOUR during operation (R^2=0.99 and 0.92 for the granules in Rs and Rc, respectively), implying that high metabolic activity of bacterial cells favors the production of PS rather than PN. Moreover, the following two aspects may have some contribution to the difference in SOUR values: (1) The growth of algae may decrease the quantity of co-existing bacteria in granules. Algae are photoautotrophic microorganisms and have lower bioactivity than bacteria under no sunlight condition (during the testing of SOUR), while most bacteria in granules belong to heterotrophic and have high bioactivity on organics degradation. (2) Algae may influence the activity of bacteria and thus the distribution of bacterial community in the granules, which needs further investigation.

3.4. Changes in microbial biodiversity in granules

The difference in nutrients removal and physical and biochemical properties between the granules from Rs and Rc is probably attributable to the difference in

microbial diversity in the aerobic granules from the two reactors. As shown in Fig. 3, the predominant bacteria cover Actinobacteria, Bacteroidetes (Flavobacteria and Sphingobacteria), Nitrospira, Proteobacteria (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria) and Firmicutes, accounting for 92.1% and 90.4% respectively in the granules of Rs and Rc. Significant difference in the proportion of dominant bacteria has also been found in the granules from the two reactors. The family of Chitinophagaceae from class Sphingobacteria is about 27.9%, occupying the largest portion of the bacteria in Rs, while Rhodocyclaceae from class Betaproteobacteria is about 30.8% dominating the granules in Rc. Moreover, the bacteria from families Rhodobacteraceae and Comamonadaceae, are about 15.6% and 10.5%, respectively in the granules in Rc, much higher than those in Rs (10.1% and 4.4%, respectively), indicating that algae growth inhibits the growth of these bacteria. Three genera from family Rhodocyclaceae, i.e. *Thauera*, *Zoogloea*, and *Rhodocyclus* are reported to closely associate with EPS excretion, the formation of activated sludge flocs and P removal, respectively (Allen et al., 2004; Shao et al., 2009; Wong et al., 2005). The genus *Lampropedia* from family Comamonadaceae has been identified as the major PAOs responsible for P removal in wastewater treatment (Stante et al., 1997). In addition, genera *Paracoccus denitrificans* and *Comamonas*, from families of Rhodobacteraceae and Comamonadaceae respectively, can oxidize NH_4-N to nitrite or nitrate under aerobic condition and reduce nitrate and nitrite to N_2 under anoxic condition (Baumann et al., 1996; Crossman et al., 1997; Gumaelius et al., 2001). Therefore, it was deduced that because of the algal-bacterial symbiosis Rs became

unfavorable for the growth of these bacteria related with N and P removal, leading to the deterioration in nutrients (N and P) removal and the physicochemical properties of the granules. Also, it is noticeable that the percentage of Nitrospiraceae and Nitrosomonadaceae in Rc are about 2.2% and 1.5% respectively, much higher than that in Rs (<0.1%), signaling that nitrifying bacteria(AOB and NOB) were significantly inhibited under the symbiotic growth of bacteria and algae in Rs, resulting in decreased nitrification performance (Fig. 1b). On the other hand, the bacteria from families Chitinophagaceae, Flavobacteriaceae and Bdellovibrionaceae, are about 27.9%, 9.0% and 16.5% respectively in Rs, much higher than those in Rc (9.7%, 5.1% and 5.6%, respectively), suggesting that the growth of these bacteria was favored due to the co-existence of algae. Except some genera from family Flavobacteriaceae could remove N or P (Bernardet and Nakagawa, 2006), little information could be found about the correlation of Chitinophagaceae and Bdellovibrionaceae with nutrients removal. In addition, the Bdellovibrionaceae, a family of Proteobacteria, have many genera which are bacterial parasites: they can enter into the periplasmic space of other bacteria and feed on the biopolymers like proteins and nucleic acids of the host bacteria (Strauch et al., 2006). From this study, the Bdellovibrionaceae are considered to have negative effect on the growth of microorganisms in the granules. The above preliminary results clearly demonstrate that the symbiotic growth of algae and bacteria greatly influence the biodiversity of microbial community in the aerobic granules. Some specific species might also be responsible for the different performance of the granules in two reactors, which is still

under followed-up investigation.

3.5. Preliminary analysis of mechanisms involved in this study

The current study investigated the effect of algae growth on aerobic granulation, nutrients removal and biodiversity of the microbial community in aerobic granules. Fig.4 illustrates the possible influences of algae growth on aerobic granules. High effluent NO_2-N concentration with lower TN removal was always detected in Rs during the 100 days' operation (Fig. 1b), clearly indicating that the co-existence of algae suppressed the nitratation and denitrification processes in the granules under the tested conditions. Guerrero and Jones (1996) and Kaplan et al. (2000) noticed that the activity of nitrifying bacteria exposed to sunlight was significantly inhibited, and NOB was more sensitive to sunlight than AOB. Previous studies attributed this greater sensitivity of NOB to the relatively low cytochrome c content of Nitrobacter compared to Nitrosomonas (Philips et al., 2002). As a possible mechanism, Barak et al. (1998) suggested that light may destroy the electron transfer from cytochrome c to nitrite reductase. However, in this study it is still not clear whether and to what extent the sunlight impacted NOB in the granules, due to the fact that a temporary inhibited nitratation was also detected in Rc during the first 10 days' operation (Fig. 1b).

After 19 days' operation, algae were clearly observed, forming algal-bacterial symbiosis in Rs. The impact of algae growth on the granular performance is complicated. Previous work shows that a proper algae/sludge inoculation ratio (1:5) could yield the maximum N and P removal efficiencies along with better settleability of the sludge in an algal-bacterial culture (Su et al., 2012). Most recently, Marcilhac et

al. (2014) found that the activity and abundance of AOB decreased with algae growth leading to a decreased nitrification in a microalgal-bacterial ecosystem, which is attributable to that algae is a better competitor for N compared with AOB. In this study the growth of algae in aerobic granules deteriorated nutrients removal, especially P, which might be resulted from accumulated NO_2-N due to an inhibited nitratation process. As can be seen from Fig. 1b, averagely high concentration of NO_2-N (>28 mg NO_2-N/L since day 10) was detected in Rs and FNA was estimated to be 6.3-9.8 $\times 10^{-3}$ mg HNO_2-N/L), greatly inhibiting the activity of PAOs (Fig. 1a). Besides, O_2 possibly generated by algae may affect P release by PAOs during non-aeration phase. On the other hand, the microbial biodiversity and distribution in the aerobic granules was altered due to the symbiotic growth of bacteria and algae, leading to the suppression of functional bacteria in the granules to some extent with resultant decreased bioactivity, such as *Thauera* and *Zoogloea*, further slowing down the granulation process.

4. Conclusion

The symbiotic growth of algae greatly decreased the bioactivity of granules with less EPS production in the granules. The results demonstrated that the activity and growth of nitrifying bacteria (especially Nitrospiraceae and Nitrosomonadaceae), denitrifying bacteria and PAOs were inhibited in algal-bacterial symbiosis to a great extent, resulting in lower TN and TP removal efficiencies of the granules. Further research will be related with how to ameliorate these inhibition effects brought about

by algae growth. And quantification and classification of the algae in the granules will also be done, which will help to further disclose the mechanisms involved.

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Tables

Table 1- Physical characteristics of granules in the reactors during 100 days' operation

Operation duration (day)	MLSS (g/L)		SVI_{30} (ml/g)		Average diameter (mm)	
	Rc	Rs	Rc	Rs	Rs	Rc
0	3.4	3.3	87	87	0.14	0.14
20	4.7	4.8	41	56	0.35	0.31
40	6.1	7.2	28	40	0.61	0.53
60	6.4	7.5	26	37	0.97	0.69
80	6.5	7.3	27	39	1.13	0.74
100	6.3	7.7	25	38	1.21	0.78

Figure captions

Fig. 1. Variations of COD removal and effluent TP concentration (a), and effluent NO_3-N and NO_2-Nconcentrations, and NH_4-N and TN removals (b) during the operation of the two reactors.

Fig. 2. Changes in extracellular polymeric substances (EPS) extracted from the sludge in Rs and Rc (a) and specific oxygen utilization rate (SOUR) of the granules (b) during the operation, respectively.

Fig. 3. Abundance of families in the granules of Rc and Rs on day 90

Fig. 4. Schematic diagram of algae growth and its effect on aerobic granules. FNA, free nitrous acid; PAOs, polyphosphate accumulating organisms.

Figures

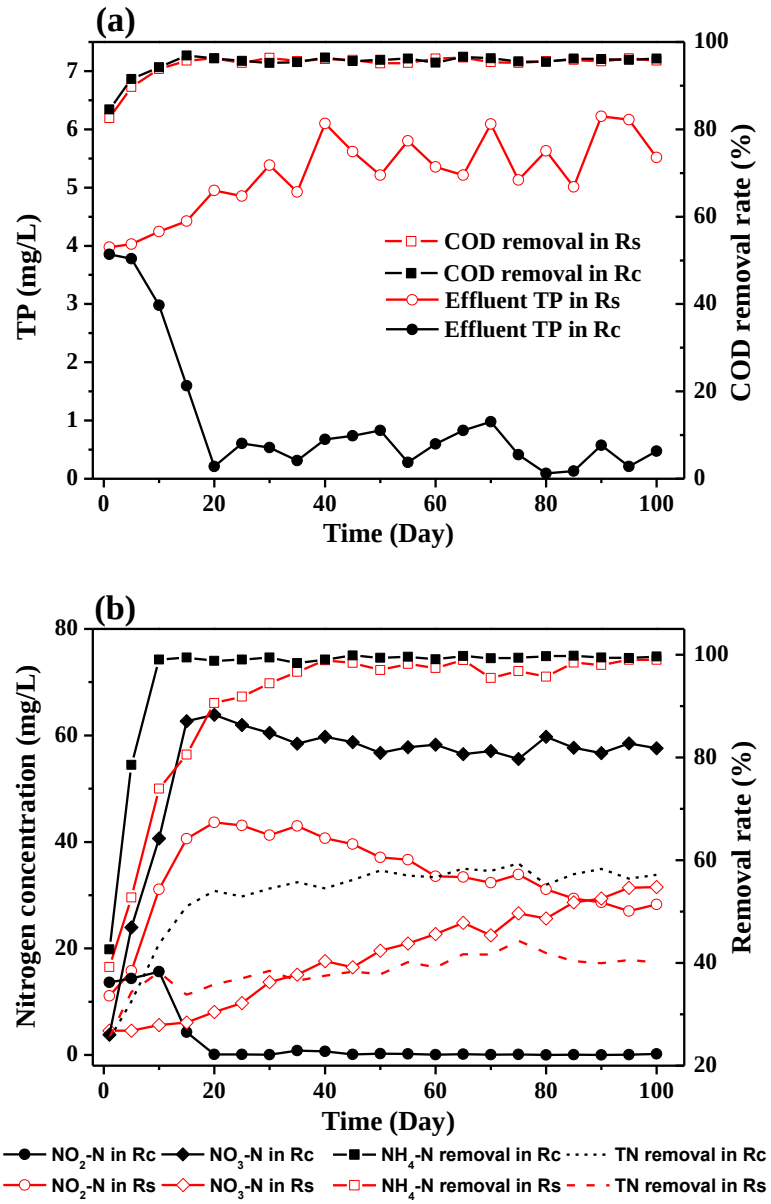
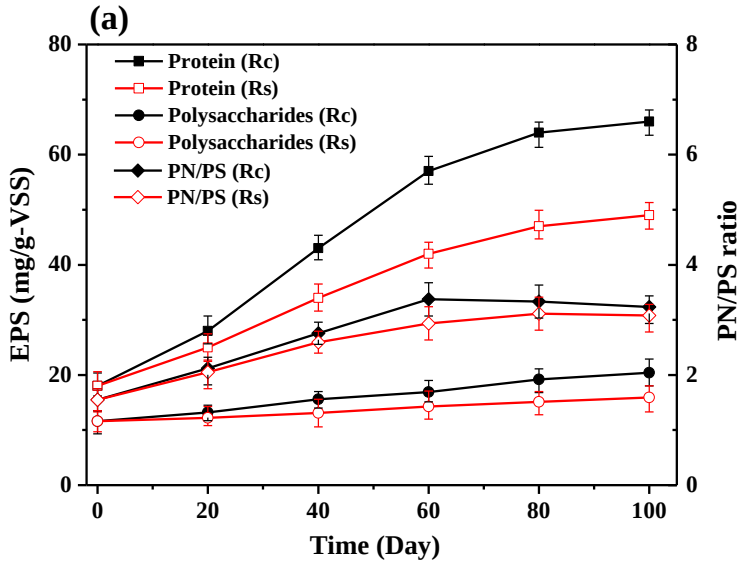


Fig. 1 Huang *et al.*



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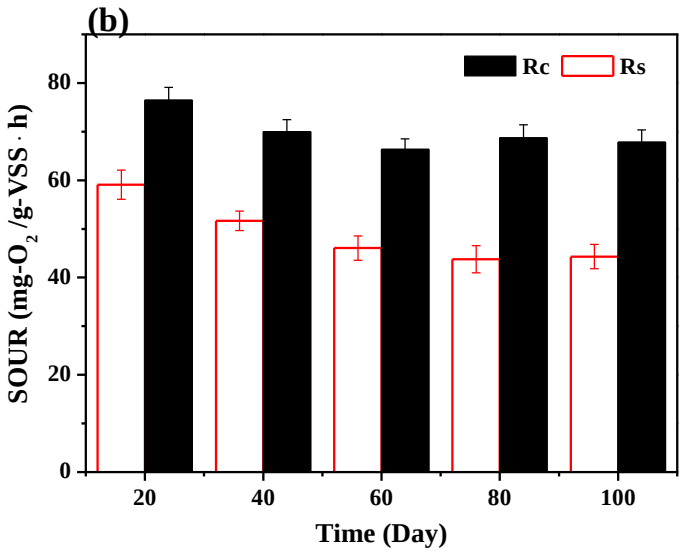

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5 Fig. 2 Huang *et al.*

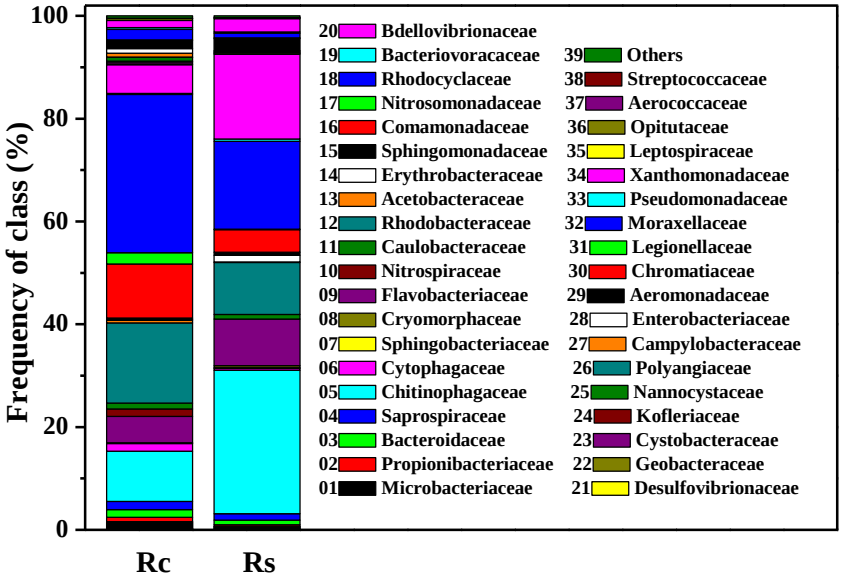

Fig. 3 Huang *et al.*

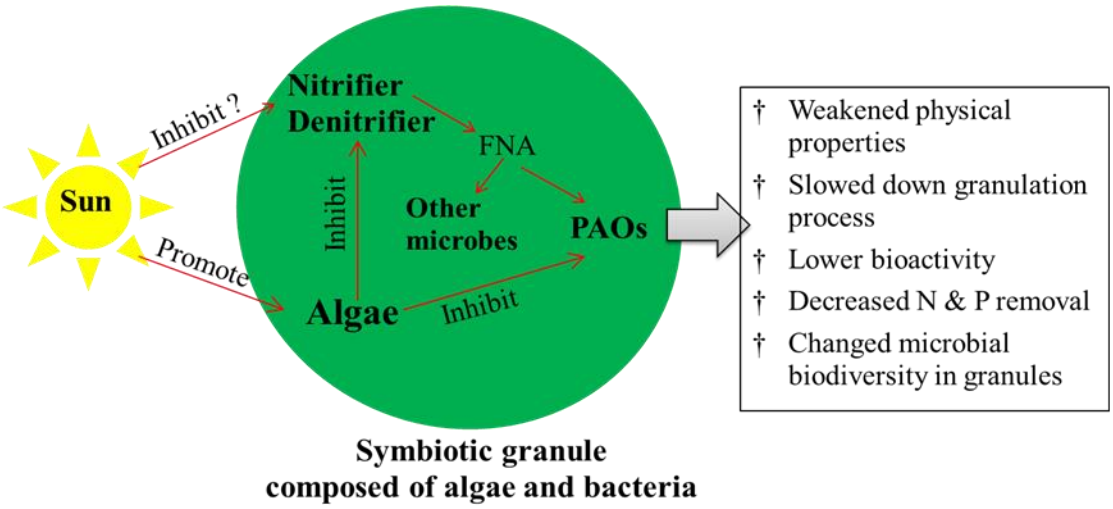

Fig. 4 Huang *et al.*