Performance and microbial community analysis of an algal-activated sludge symbiotic system: Effect of activated sludge concentration

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Abstract
It was focused on the effect of different sludge concentrations on the performances of an algal-activated sludge symbiotic system in terms of wastewater treatment, algal-activated sludge characteristics and community structure. The results showed that the highest nutrient removal efficiencies were obtained in the reactor R2 with soluble chemical oxygen demand (sCOD), ammonia nitrogen (NH4+-N) and phosphate (PO43−-P) removal efficiencies of (90.6 ± 2.3)%, (97.69 ± 2.6)% and (83.81 ± 2.3)% respectively. Further investigation exhibited that sludge concentration has a great effect on the dissolved oxygen (DO) concentration, the pH, the growth of algae and the extracellular polymeric substance (EPS) production, which resulted in influencing the settleability and the performance of symbiotic system. The denaturing gradient gel electrophoresis (DGGE) analysis demonstrated that the sludge concentration had a selective power for particular members of algae. Meantime, the stimulated algal population would selectively excite the members of bacteria benefited for the formation of algal-bacterial consortia. The variation of microbial compositions, which was influenced by the different sludge concentrations, might be ultimately responsible for the different treatment performances.

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Keywords:
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Sludge concentration
Wastewater treatment
Algal and bacterial communities

Introduction
The algal-based water treatment biotechnology has attracted many researches for decades of years because of high nutrient removal efficiencies, high oil content and synergy with CO2 biofixation. However, several drawbacks exist in the algae biotechnology, such as a relatively longer hydraulic retention time (HRT), inartificial biomass wash-out (Park and Craggs, 2011; Mara and Pearson, 1986) and big footprint (Jorquera et al., 2010), which resulted in the system failure and the increase of operating costs. Therefore, the development of an efficient algal-based water treatment biotechnology to solve these problems remains a formidable challenge.

Several solutions have been reported to solve the problems, including immobilization technologies (Robinson, 1998), microalgal flocculation (Molina Grima et al., 2003) and algal–bacterial system (Boelee et al., 2014; Bordel et al., 2009). Among them, algal–bacterial system is considered to be one of the most efficient solutions because of the shorter HRT (Valigore et al., 2012), low-energy (Tang et al., 2016) and good settleability compared with algae-
only system. Under the illuminated conditions, algae can produce O₂ by photosynthesis, which can be used by the bacterial mineralization. Meantime, bacteria release CO₂ by respiration, which is consumed by algal photosynthesis, thereby realizing simultaneously cost-efficient aeration (Muñoz and Guieysse, 2006). However, most studies for the algal–bacterial system pollutant treatment mainly focused on the single algae with bacteria to establish an algal–bacterial system. Eixler et al. (2006) investigated the relation Chlorella vulgaris strain with the amount of available inorganic phosphorus in the medium and it was considered to be a capacity of phosphorus “luxury” uptake. Mujtaba et al. (2017) explored the effect of Pseudomonas putida strain on the N, P and C removal efficiencies of C. vulgaris strain, which indicated that the nutrients uptake capability of C. vulgaris was enhanced in the presence of P. putida. Nevertheless, the single algae-bacteria system is not effective especially in the case of industrial wastewater without pretreatment and it is hard to keep algae pureness. In addition, some researches usually cultivated algae and low concentration of sludge in the photo-bioreactors. Roudsari et al. (2014) cultivated activated sludge with algae to investigate the nutrients removal efficiency of algae-sludge system and the sludge concentration was around 100–200 mg/L. Su et al. (2012) found that high sludge concentration contributed to the improvement of the settleability, while excellent nitrogen and phosphorus removal was obtained in high algal concentration. Besides, the sludge concentration used in their studies were still low with 60–500 mg/L. As is well known, high sludge concentration would probably decrease the supply of CO₂ and prevent the biomass wash out which reduce the operation costs and keep the symbiotic system stable. While, arbitrarily high sludge concentration would limit the availability of light resulted to inhibit the growth of algae. Bai et al. (2012) used the different concentrations of pig manure sludge for algal production and they found that undiluted liquid manure resulted to the low algal production because of the strong influence on the algal photosynthetic activity. Additionally, the initial sludge concentration may influence the cooperation relationship between algae and sludge bacteria and then result in different nutrient removal efficiencies. Generally, the interactions between algae and bacteria do not limit to a simple CO₂/O₂ exchange. Algae could supply nutrients to bacterial activity for synthesizing required products (Huménik and Hanna Jr., 1971) and algae could also do harm to bacteria activity by influence the surrounding conditions such as pH, dissolved oxygen (DO) concentration and releasing inhibitory metabolites (Oswald, 2003; Schumacher et al., 2003). Therefore, the initial sludge concentration is one of the most important factors affecting the biological system performance. However, how different sludge concentrations influence the relationship between the algae and sludge bacteria and the performance of wastewater treatment and which sludge concentration is better for the performance are still unknown.

The motivation of this research was, therefore, to investigate the effect of different sludge concentration on: (1) the nutrient removal, (2) the characteristics of algae and activated sludge in the consortium, and (3) the changes of community structures and diversities of algae and bacteria.

1. Materials and methods

1.1. Cultivation of algae and sludge

The sources of algae were collected from the secondary clarifier wall of the municipal wastewater treatment plant of Harbin, China. The algae was cultivated and enriched using artificial wastewater and the composition of artificial wastewater was shown in Table S1. The incoming light intensity was 4000 lx with 18 hr light–6 hr dark cycle for 6 days. After enrichment, the total suspended solids (TSS) of algae was 1000 mg/L which was used as algal inoculum.

Activated sludge was taken from the same wastewater treatment plant of Harbin, China. Before tests, activated sludge was fed twice each day with synthetic wastewater regularly for 15 days. After adaptation, the TSS of activated sludge was 3000 mg/L and used as activated sludge inoculum.

1.2. Experimental setup and operation

The laboratory-scale batch experiments were conducted in five reactors. The five reactors were made up of transparent glass for light transmitting. The maximal working volume of each reactor was 2 L. All bioreactors (R0–R4) were operated at room temperature (22 ± 3)°C, 4600 lx of light intensity, 12 hr of light and dark cycle along with constant stirring for 24 hr in order to avoid algae and activated sludge sedimentation. The five reactors of R0, R1, R2, R3 and R4 were inoculated with 300 mg/L (TSS) algae and 0, 300, 700, 1100 and 1500 mg/L activated sludge inoculum, respectively. The volatile suspended solids (VSS) of R0–R4 was 189, 402, 658, 945 and 1242 mg/L, respectively. The liquor mixture color of the five reactors was changed from light to dark with the increase of sludge concentration, which was light green in R0 and turned from brownness to dark brown in R1 to R4 (Fig. S2). Properties of artificial wastewater were shown in Table S1: Soluble chemical oxygen demand (sCOD) (440 ± 9.8) mg/L, ammonia nitrogen (NH₄-N) (50 ± 2.6) mg/L and phosphate (PO₄³⁻-P) (12.8 ± 1.1) mg/L.

1.3. Extraction of extracellular polymeric substance (EPS) and chlorophyll-a (Chl-a)

A one-step heat extraction method was adopted to extract EPS from the sludge sample. Sludge samples were centrifuged at 4000 × g for 5 min, and then the extracted supernatant was filtered through a 0.45-μm membrane filter. The sediment was then diluted with the distilled water and sheared by a vortex mixer for 1 min immediately. After that the sludge suspension was heated to 80°C in a water bath for 30 min and then centrifuged at 5000 × g for 5 min and filtered by 0.45 μm membrane. The filtrate was labeled as EPS. EPS was known as the extracellular polymeric substances collective and their protein and carbohydrates contents were analyzed. The phenol-sulfuric acid method (Dubois et al., 1956) was used to monitor carbohydrates using glucose as standard. The modified Lowry method (Lowry et al., 1951) was used to monitor proteins using bovine serum albumin (BSA) as standard.
As for the analysis of chlorophyll-α content. The samples were centrifuged at 4000 × g for 10 min. The sediment was mixed with 5 mL of 90% acetone plus little CaCO₃. Solution was mixed well by constant shaking with the volume up to 15 mL. The mixture was vortexed and then kept in the refrigerator at 4°C for 24 hr. After, the solution was mixed for 5 min and centrifuged at 4000 × g for 10 min. Optical density (OD) value of the supernatant was measured at 750, 663, 645 and 630 nm by UV/V (UV-2550, Shimadzu, Japan). The chlorophyll-α content was calculated from the standard formula. The analyses were conducted in triplicate, and their average values were reported.

1.4. Analytical methods

DO and pH were measured continuously in the middle of each reactor using DO meter (Oxi3210, WTW, Germany) and a pH meter (FE20K, Mettler Toledo, Switzerland). Before analysis of water quality and EPS, the samples were filtered by membrane of 0.45 μm. NH₄-N, sCOD, PO₄³⁻-P, and TSS were measured according to standard methods (APHA, 2005).

1.5. Data analysis

The chlorophyll-α content was calculated by Eq. (1):

\[ C_{\text{Chl-α}} = \frac{(11.64 \times (\text{OD}_{750} - \text{OD}_{645}) - 2.16 \times (\text{OD}_{663} - \text{OD}_{645}) + 0.10 \times (\text{OD}_{630} - \text{OD}_{645})) \times V_1}{V \times \delta} \]  

(1)

where, \( C_{\text{Chl-α}} (\text{mg/m}^3) \) is the chlorophyll-α content; \( V \) (L) is the filtered volume; \( \text{OD} \) (nm) is the optical density; \( V_1 \) (mL) is the extraction volume; \( \delta \) (cm) is the cuvette light-path.

The above batch tests were conducted in triplicate. The Shapiro-Wilk and Levene’s test was used to ensure the normal distribution and the equality of variances of the data, respectively. The Student t-test was used to examine the statistically significant difference (\( p < 0.05 \)) between groups. All values which was satisfied with t-test were the averages of three independent repetition ± standard deviations (SD). The statistical correlation was analyzed to evaluate possible associations among environmental factor, microbial parameter, and nutrient removal using Excel 2013.

1.6. Community analysis

The genomic DNA of activated sludge was extracted by a bacterial genome extraction kit (Shanghai Hua Shun, China) and the method was according to the manufacturer’s instructions. The sample of algae was damaged the cell wall with liquid nitrogen before extracted the DNA of algae. Then the genomic DNA of algae was extracted using the EMNE method (Kim et al., 2012). The polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) procedures were based on the methods (Liu et al., 2017). The analysis of the similarity between different lanes and the quantity of each detected band was through the Quantity One software (version 4.1.0, Bio-Rad Laboratories). Nucleotide sequences of these dominant DGGE bands were compared in Genbank using the BLAST program (http://www.ncbi.nlm.nih.gov).

2. Results and discussion

2.1. Effect of sludge concentration on the wastewater treatment

2.1.1. sCOD removal

The sCOD concentrations in the five reactors with different sludge concentrations were investigated and the results were shown in Fig. 1. It was seen that the sCOD removal efficiencies in R1-R4 (84.1 ± 2.1)%, (90.6 ± 2.3)%, (86.5 ± 1.9)% and (84.1 ± 2.4)%, respectively in carbon with both algae and sludge were significantly higher than those of R0 (67.8 ± 1.8)% without sludge. The lower removal efficiency in R0 might be due to the lack of activated sludge to enhance organic carbon mineralization. Similar result was also observed by Su et al. (2012) report, where the sCOD removal with both algae and activated sludge was higher than that of only algae. As for reactors with both activated sludge and algae, the furnishing O₂ of algal photosynthesis contributed to the heterotrophic bacteria mineralization leading to higher removal efficiencies of sCOD. A maximal COD removal efficiency was achieved within 7 days, during which the COD concentration obtained minimum level. Among the minimum levels, the sCOD concentration in R2 was the lowest one compared with other reactors. It is well known that the DO concentration was one of the limiting factors removing the sCOD for heterotrophic aerobic bacteria respiration and nutrient degradation (Valigore et al., 2012). However, it was found that R2 (700 mg/L) had the highest sCOD removal efficiency among the five reactors, while the DO concentration (Fig. S1a) in R2 reactor was not the highest. Thus, there may be a mutual beneficial interaction between algae and activated sludge not limit to a simple CO₂/O₂ exchange, which ameliorated the activated sludge mineralization resulting in the highest sCOD removal efficiency in R2. Interestingly, the profile of sCOD concentration started to decrease rapidly in the early stage reaching the minimum level, and increased thereafter with similar patterns. The increase of sCOD concentration in the later stage could be probably explained by the disintegration of microorganism caused by endogenous respiration. Generally, microbial intracellular substances would be consumed by themselves under starvation conditions leading to the deterioration of bioreactor performance. Meantime, the biomass balance of COD exhibited that the activity of mineralization and assimilation were significantly decreased in the later stage (Appendix A). Thus, it is logical to suppose that the activity of activated sludge is decreased after extreme sCOD concentration decline, which resulted in the accumulation of sCOD. The similar phenomenon was also found by Roudsari et al. (2014), who demonstrated the sCOD accumulation occurred after decline was because of the recession of activated sludge activity.

2.1.2. Phosphorus removal

The phosphate concentrations and removal efficiencies were shown in Fig. 2. The highest phosphate removal efficiency was found in R2 with (83.81 ± 2.3)% followed by the reactor R3 (74.28 ± 1.6%). While, only (30.4 ± 1.6)% was removed in the reactor R4 with inoculated the highest sludge concentration. This might be attributed to the lowest DO concentration in R4 (Fig. S1a), which resulted in the limitation of the phosphate assimilation into biomass and bacteria respiration. The severe
light inhibition among algae cells led to the low DO concentration in R4 due to weak photosynthesis. In addition, the changing trend of phosphate concentration of R2–R4 was similar to that of COD removal. The concentration of phosphate was first decreased in the early stage and then increased to (3.07 ± 0.6) mg/L, (5.18 ± 0.7) mg/L and (9.49 ± 1.2) mg/L, respectively. However, R0 and R1 showed different changing trends of phosphate with R2–R4, where they presented a continuously decreasing trend during the whole operation. The different profiles of phosphate concentrations can be probably explained by the phosphate release from some bacteria endogenous respiration and it was reused by algae and bacteria in R0 and R1. Besides, the phosphorus precipitation (occurred above pH 8.5) can also account for the decrease in P removal in R0 which the pH was approximately 9 (Fig. S1b). Therefore, the phosphate removal was attributed to the interaction between algae and sludge bacteria influencing the condition like DO concentration and pH and there may be an optimal ratio of algae and activated sludge existing in the symbiotic system.

2.1.3. Nitrogen removal
The nitrogen removal abilities were investigated (Fig. 3). The maximal NH$_4^+$-N removal efficiency of R2 and R3 was (97.69 ± 2.6)% and (96.37 ± 3.0)%, respectively. However, it was noted that the NH$_4^+$-N removal efficiency was only (60.96 ± 2.9)% in R4. Generally, the DO concentration is significantly influence the activity of nitrification microorganism. The DO concentration in R4 was the lowest among the five reactors, which would result the lower nitrification efficiencies further lowering NH$_4^+$-N removal efficiency. However, although the DO concentration in R1 was highest among the five reactors, the NH$_4^+$-N removal efficiency was still lower than that of R2 and R3. Moreover, concerning the nitrogen removal in R0 without inoculation activated sludge, (36.88 ± 3.5)% NH$_4^+$-N was removed even though the nitrate bacteria was little to negligible. Li et al. (2011) demonstrated that NH$_4^+$-N could be directly assimilated into the algae cell for the growth of algae. Thus, the higher NH$_4^+$-N removal

![Fig. 1 – The variation of soluble chemical oxygen demand (sCOD) concentration in the reactors.](image1)

![Fig. 2 – The phosphate (PO$_4^{3-}$-P) concentration and removal efficiency in the reactors.](image2)
efficiency in R2 and R3 was probably not only attributed to the increase of DO concentration resulting in the improvement of nitrification, but also the assimilation of NH$_4^+$-N by algal cell. The above results indicated that different sludge concentrations have a strong influence on nutrient removal efficiency. Either too high or too low sludge concentration was not good for the performance of wastewater treatment. The better performance was obtained in R2 with initial sludge concentration of 700 mg/L, in which the proper composition of algae-sludge consortia influenced the conditions and promoted the activities of both items.

2.2. Effect of sludge concentration on the algal–bacterial characteristics

2.2.1. Variation of Chl-a in the symbiotic system

The Chl-a concentration was monitored to characterize algal density and it was shown in Fig. 4a. The profile showed an increasing trend during the whole operation period which increased from (3.0 ± 0.1) mg/L to (5.2 ± 0.09) mg/L, (4.6 ± 0.11) mg/L, (4.8 ± 0.12) mg/L, (4.0 ± 0.09) mg/L and (5.0 ± 0.1) mg/L in R1, R2, R3, R4 and R0, respectively. The content of Chl-a in R4 was the lowest among the five reactors because of the inhibition of light availability for algal growth. High sludge concentration in R4 would lead to the shading of the light within the algal population, the decrease of photosynthetic efficiency and the increase of O$_2$ consumption due to the algal dark respiration, which resulted to the reduction of algal growth. Moreover, the statistical correlation between Chl-a concentration and various parameters, including sCOD removal rate, NH$_4^+$-N removal efficiency and PO$_4^{3-}$-P removal efficiency, was studied as shown in Table 1. It can be seen that the Chl-a concentration exhibited stronger relationships with NH$_4^+$-N removal and PO$_4^{3-}$-P removal, while no significant relationship with sCOD removal. It was indicated that algae preferred using nitrogen and phosphate rather than organic carbon when all the three nutrients were presented in the medium. In addition, the relative coefficient between NH$_4^+$-N removal efficiency and Chl-a concentration (except R4 and R0) was larger than PO$_4^{3-}$-P removal rate, indicating that nitrogen was more preferred for assimilation into algae cells compared to phosphate. The results were consistent with previous study in the literature (Lee et al., 2015), showing that the relative coefficient of nutrient removal with Chl-a concentration was in the declining order of N > P > sCOD. In addition, the relative coefficient between the removal of phosporus and the removal of nitrogen was higher than that of carton (Table S2). It indicated that P removal is more influenced by N concentration than that by the removal of C. Higher P concentration could allow for bacterial and algal growth and higher uptake of N, which is consist with the statistical correlation between Chl-a concentration and various parameters.

Notably, the relative coefficient between Chl-a concentration and various parameters (Table 1) were decrease with the increase of sludge concentration. The increase of sludge concentration inhibited algae growth because of the limitation of photosynthesis caused by light inhibition. Additionally, oxygen concentration was a crucial factor for respiration and mineralization of bacteria. The increase of sludge concentration deteriorated algal photosynthesis further decreasing dissolved oxygen. Thus, the decrease of sludge concentration not only promoted algae growth, but also drove bacteria to rely on the O$_2$ produced by algae for subsistence. However, decreasing sludge concentration unboundedly would have a negative effect on nutrient removal efficiencies. As mentioned above, the removal efficiencies of sCOD, NH$_4^+$-N and PO$_4^{3-}$-P were decreased under decreasing sludge concentration due to variation of pH, DO, and the cooperation performance of algae and bacteria. Thus, there was a trade-off between algae growth and nutrient removal under different sludge concentration. The impact of sludge concentration on the characteristics of both sludge and algae should be considered together. In this study, R2 might be the more suitable sludge concentration, which achieved high nutrients removal efficiencies and proper Chl-a concentration ((3.0 ± 0.1) mg/L to (4.6 ± 0.11) mg/L) in the symbiotic system.

Fig. 3 – Ammonia nitrogen (NH$_4^+$-N) removal in the five reactors.
2.2.2. Variation of TSS in the symbiotic system
The TSS first increased by approximately 65% (R1), 39% (R2), 24.3% (R3) and 16.7% (R4), and then decreased by 16.6% (R1), 34.8% (R2), 37.2% (R3) and 35% (R4) at the end, respectively (Fig. 4b). Contrarily, the profile of R0 showed an increasing trend during the whole operation period from (320 ± 80) mg/L to (710 ± 83) mg/L. The decline trend (R1–R4) of TSS in the late period was probably due to the death and degradation of bacteria and algae as mentioned previously. Nevertheless, Chl-a representing the algae content exhibited an obvious increasing trend among the five reactors as shown in Fig. 4a, indicating the disintegration of sludge bacteria should be the main reason contributing to the decline of TSS in the symbiotic system. As discussed above, the insufficient of nutrients and oxygen reduced the activity of bacteria for subsistence and production, which led to the disintegration of bacteria.

Moreover, under the situation of nutrient deficiency, the algae maybe displayed an important role for nutrient utilization and this assumption was in accordance with the whole increasing profile of Chl-a. The decrease of sludge concentration could balance the growth of algae and bacteria. However, decreasing sludge concentration unrestrainedly also had a detrimental effect on algal-activated sludge symbiosis system. From the variation of SV% (representing the settleability of sludge flocs) in five reactors, SV% was increased with the decreasing sludge concentration, suggesting sludge settleability was deteriorated with decreased sludge concentration (Su et al., 2011). The deterioration of sludge settleability would cause a secondary pollution to the effluent and instability of algae-sludge system. The key to

<p>| Table 1 - Relationship between Chlorophyll-a (Chl-a) and nutrient removal parameters. |
|----------------------------------|----------------------------------|----------------------------------|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>sCOD removal rate</th>
<th>NH₄⁺-N removal rate</th>
<th>PO₄³⁻-P removal rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>R0 Chl-a</td>
<td>r = 0.876</td>
<td>r = 0.931</td>
<td>r = 0.969</td>
</tr>
<tr>
<td>R1 Chl-a</td>
<td>r = 0.601</td>
<td>r = 0.928</td>
<td>r = 0.918</td>
</tr>
<tr>
<td>R2 Chl-a</td>
<td>r = 0.536</td>
<td>r = 0.891</td>
<td>r = 0.836</td>
</tr>
<tr>
<td>R3 Chl-a</td>
<td>r = 0.426</td>
<td>r = 0.887</td>
<td>r = 0.829</td>
</tr>
<tr>
<td>R4 Chl-a</td>
<td>r = 0.364</td>
<td>r = 0.614</td>
<td>r = 0.821</td>
</tr>
</tbody>
</table>

sCOD: soluble chemical oxygen demand.
improve the stability is that algae could be driven to adhere or captured by activated sludge and settled down together (Ekama and Wentzel, 1999; Tang et al., 2016). Consequently, too high or too low sludge concentration in the inoculum was not good for biomass production and sedimentation of algal-activated sludge flocs.

2.2.3. Variation of EPS in the symbiotic system

EPS production was regarded as a survival mechanism of microorganism, which was composed of polysaccharides (Ps), proteins (Pr), nucleic acids, lipids, and humic acid (Di Pippo et al., 2009; Romaní et al., 2008). As shown in Fig. 5a, the highest EPS concentration was found in R2, which increased

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**Fig. 5** – The variation of extracellular polymeric substance (EPS) in the reactors, volatile suspended solid (VSS).
from 29.24 to 99.79 mg/g VSS during the operation time. Xiao and Zheng (2016) reported that the uptake of C, N and P, which were important components of polysaccharides and proteins in EPS, would promote the production of EPS. Meantime, the EPS has a positive influence on the nutrients uptake (Chen et al., 2015; Wang et al., 2014). Thus, the highest nutrient removal efficiencies in R2 was probably associated with the highest EPS concentration and vice versa. Besides, the profile of EPS concentration displayed a nearly stable state at the end of operation in all the reactors, which suggested that there was a stronger mineralization in the early stage and then limited metabolism in the late stage. This phenomenon was consist with the increase profile of sCOD, NH4+-N and PO4−-P concentrations in the post period of the operation days. The EPS concentration of R0 was lower compared to R1-R3. Interestingly, the SV% in R0 was higher than others indicating the poor settleability. Therefore, the lower EPS was partly responsible for the decrease settleability of algae and activated sludge flocs. Similar results were reported by Tolhurst et al. (2002), who found that the stability of sediment with EPS was higher than that without EPS, and the increase of EPS could not only increased physical and chemical stability, but also bound water molecules. Moreover, it was found that the ratio of polysaccharides to proteins (Ps/Pr) in R2 was higher than others expect R0 during. It appeared that polysaccharides were more crucially to the sediment stability (Huang et al., 2015; Di Pippo et al., 2012). Therefore, higher nutrient removal and preferable settleability in R2 might be related positively to its higher EPS production in the symbiotic system.

2.3. Insight into the community structures of symbiotic system

In this study, DGGE was used to monitor the variation of bacterial and algal communities (Figs. 6 and 7). The gene library results were summarized in Tables 2 and 3. It was seen that both the structure and diversity of algae and bacteria were changed after operation.

The DGGE profile of algae communities in Fig. 6 showed that the diversity of algae was decreased with the increase of sludge concentration except R0. This result was probably attributed to the light inhibition induced by the superabundant bacteria concentration which had an inhibitory effect on the growth of algae. However, the diversity in R0 without activity sludge was still lower even though no light inhibition of sludge for algal growth. Thus, those results suggested that the sludge bacteria had a crucial effect on diversity of algae and either too high or too low sludge concentration was not good for diversity of algae. In order to provide deeper insight into

![Fig. 6 – Denaturing gradient gel electrophoresis (DGGE) profile of algae communities in the algae samples (R0–R4). Band 1 to band 20: the principal bands that were excised and sequenced.](image)
algal communities, the six predominant species extracted from DGGE bands were sequenced (Table 2). They were *Prorocentrum* (Band 1 and Band 4), *Karlodinium* (Band 3 and Band 6), *Akashiwo* (Band 7), *Heterosigma* (Band 8), *Takayama* (Band 9), and *Symbiodinium* (Band 10), respectively. Some researches reported that *Prorocentrum* sp. and *Karlodinium* sp. were positively related to the removal of dissolved organic nitrogen and phosphorus (Rothenberger et al., 2009), and *Heterosigma* sp. and *Symbiodinium* sp. were perfer to form algae and bacteria symbionts in wastewater (Caron et al., 2015; Viswanath and Bux, 2012). Based on the above analysis, the activity sludge has a great influence on the algal community structure and the algal functional community, which may be play an important role for the performance of wastewater treatment.

An analysis of the relative quantity (Fig. 7b) and seven predominant species (Table 3) of bacteria communities was conducted in this section. The results showed that the bacterial communities between the five reactors and original sludge
inoculation was changed, which indicated that a stimulated algal would selectively excite the members of bacteria that cooperatively interacted with algal growth. Additionally, the diversity of bacteria in R2 was the highest among the five reactors. As is well known, microbial community structure and population composition are closely related to the wastewater treatment, algal-bacterial characteristics and microbial community compositions, which resulted to the built community succession. Previous researches reported that Microbacterium sp. was found to increase the growth of C. vulgaris (Kim et al., 2015). Sphingobium sp. was isolated from aerobic granular biomass which was used for biodegradation of phosphate (Reddy et al., 2014). Rhodanobacter sp. and Thermomonas sp. were reported to be capable of nitrate reduction (Green et al., 2015). As shown in Fig. 7b, the relative abundance of Microbacterium sp. was increased after introducing algae. Sphingobium sp. was more enriched in R2 and R3 than other reactors, which is consistent with their high phosphate removal efficiencies. Moreover, the relative abundance of Rhodanobacter sp. in R2 was the highest among the five reactors, which might be associated the highest nitrogen removal efficiency of R2. Hence, it seems that the higher abundant of predominant species in R2 was attributed to the promotion of nutrient removal efficiency. Generally, microorganisms could lead to the variation of nutrient concentration under the uptake of biomass and mineralization, while on the other hand, the variation of C, N and P would also change the micro-environment of the sludge bacteria, which induced the community succession.

In summary, it was clearly seen that both algae and bacteria population were changed compared with the original inoculum after operation days. Some specific bacteria and algae were enriched during the operation, which were few in the original inoculum. Additionally, the algal and bacterial predominant species has been observed that they were closely associated with the removal of C, N and P in many previous studies. Thus, it was suspected that those species might be positively responsible for the nutrient removal efficiency of reactors observed in the results as mentioned above.

### 2.4. An overall mechanism analysis

In the present study, the effects of sludge concentration on the wastewater treatment performance (Figs. 1–3), the algal growth (Fig. 4), the flocs characteristics (Fig. 5) and the microbial community structure (Figs. 6 and 7) were investigated. Fig. 8 exhibits schematic diagram of the effect of sludge concentration on the algal-bacterial system. Based on the above results, it was found that either too high or too low sludge concentration in the inoculum was not good for the wastewater treatment performance. The system of R2 with the initial sludge concentration of 700 mg/L were more favored to obtain the algal-bacterial system synergism advantages. Proper sludge concentration would improve the formation of cooperative interaction and optimize the microbial community compositions, which resulted to the built of a mutually beneficial relationship between algae and bacteria. Meanwhile, the relationship and community structure have a strong influence on the external conditions (e.g., DO and pH) and the flocs characteristics (e.g., EPS and settleability). Because of the two main aspects, the reactor, which inoculated with proper sludge concentration (R2) and algae, showed an excellent nutrient removal. Too high or too low sludge concentration would break the system biological relationship leading to the poor bioreactor performance. Therefore, sludge concentration is an important factor to evaluate the synergistic effect between bacteria and algae.

### 3. Conclusions

An algae-activated sludge system was constructed in this study to investigate the effect of different sludge concentrations on the wastewater treatment, algal-bacterial characteristics and community structures. R2 with the initial sludge concentration
of 700 mg/L was favored for achieving higher nutrient removal of sCOD (90.6% ± 2.3%), NH$_4^+$-N (97.69% ± 2.6%) and PO$_4^{3-}$-P (83.81% ± 2.3%). The increase of sludge concentration, on one hand, enhance the nutrient removal efficiency and then improve the folcs settleability, while, on the other hand, inhibited algae growth and then destroyed the correlation between the algae and bacteria. Furthermore, different sludge concentration lead to different algal and bacterial community compositions and diversity, which were closely associated with the variation of EPS production, the changed of Ps/Pr and the different nutrient removal efficiency. Consequently, the variation of microbial community was ultimately responsible for the different performance of the reactors.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.04.010.

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