



## Comparing three methods for photosynthetic bacteria separation and recycling during wastewater treatment

Haifeng Lu<sup>a</sup>, Guangming Zhang<sup>b,\*</sup>, Xiao Dai<sup>c</sup>, Guoyang Yuan<sup>a</sup>, Wei Cao<sup>a</sup>, Yuanhui Zhang<sup>d</sup>, Baoming Li<sup>a</sup>

<sup>a</sup>College of Water Resource and Civil Engineering, China Agriculture University, Beijing 100083, China, Tel. +86 10 62737329; Fax: +86 10 62736904; email: [hfaucedu@163.com](mailto:hfaucedu@163.com) (H. Lu), Tel. +86 18810011680; Fax: +86 10 62736904; email: [18810011680@163.com](mailto:18810011680@163.com) (G. Yuan), Tel. +86 10 62736698; Fax: +86 10 62736904; email: [caowei@cau.edu.cn](mailto:caowei@cau.edu.cn) (W. Cao), Tel./Fax: +86 10 62736904; email: [libm@cau.edu.cn](mailto:libm@cau.edu.cn) (B. Li)

<sup>b</sup>School of Environment and Natural Resources, Renmin University of China, Beijing 100872, China, Tel. +86 13520956445; Fax: +86 10 62511645; emails: [zgm200@126.com](mailto:zgm200@126.com), [zgm@ruc.edu.cn](mailto:zgm@ruc.edu.cn)

<sup>c</sup>Civil and Environmental Engineering, University of California, Irvine 92617, CA, USA, Tel. +1 408 896 8778; Fax: +1 888 203 4539; email: [woshidaixiao@gmail.com](mailto:woshidaixiao@gmail.com)

<sup>d</sup>Department of Agricultural and Biological Engineering, University of Illinois at Urbana-Champaign, 1304 W Pennsylvania Avenue, Urbana, IL 61801, USA, Tel. +1 217 333 2693; Fax: +1 217 244 0323; email: [y Zhang1@illinois.edu](mailto:y Zhang1@illinois.edu)

Received 21 December 2014; Accepted 15 May 2015

---

### ABSTRACT

Photosynthetic bacteria (PSB) wastewater treatment technology can simultaneously treat wastewater and produce valuable materials. However, PSB are typically difficult to collect from wastewater, which limits their utilization in wastewater treatment and resource recovery. In this study, three different methods (e.g. immobilization, coagulation and membrane separation) were investigated and compared for PSB collection to recover bioresources. A strain of PSB, Z08 (*Rhodobacter sphaeroides*) was used. Results showed that PSB hardly attached to the seven support materials tested during immobilization. Aluminium polychloride was shown to be effective at PSB separation via coagulation at a dosage of 5,000 mg/L; however, coagulants might cause the second pollution. Also, the membrane was effective at Z08 collection. Ninety-nine per cent of PSB was collected from water; this PSB liquid was then concentrated by a factor of 12.4, and the water production ratio reached 93.0%. The optimal Z08 dosage was 1,682.1 mg/L, which generated 165,396.0 mg/L of biomass within a 50 h water cycle. Compared with activated sludge, the water cycle that combines the PSB and the membrane can function up to 60 min with an initial PSB concentration of 10,000 mg/L, which will mitigate membrane fouling and achieve bioresource recovery.

*Keywords:* Photosynthetic bacteria; Bioresource recovery; Membrane separation; Coagulation; Immobilization

---

\*Corresponding author.

## 1. Introduction

Photosynthetic bacteria (PSB) have been applied in wastewater treatment due to their many advantages, which include purifying wastewater effectively and recovering valuable bioresource [1]. In the 1960s, Kobayashi found that during wastewater self-purification processing [2], PSB were important component and could degrade most organic matter in wastewater. Therefore, using PSB for wastewater treatment has since been developed significantly. Researchers have used PSB to treat many types of wastewater, such as aquarium water [3], sewage wastewater [4], olive mill wastewater [5,6], shrimp farms wastewater [7], poultry slaughterhouse wastewater [8], dairy wastewater [9], palm oil mill effluent [10], soybean wastewater [11–13], beer wastewater [14], latex rubber sheet wastewater [15] and pharmaceutical wastewater [16]. Results showed that PSB can effectively decrease 50–90% of COD, 60–80% of TP and 40–80% of TN [11–14]; using PSB to treat wastewater can thus diminish secondary pollution and achieve resource recovery. Compared with traditional wastewater treatment processes, the biomass generated using PSB is only PSB, which contain lots of single-cell proteins, biopolymers and carotene. These valuable extractions can be recovered as resources and used in fertilizers or fishery industries [17,18]; this can help mitigate sludge disposal costs and secondary pollution.

However, PSB are typically difficult to collect from wastewater due to their small size (e.g. 0.5–3.0  $\mu\text{m}$ ) and resistance to forming flocs [19], which limit their utilization in wastewater treatment and resource recovery. To solve these problems, immobilization has been used in PSB wastewater treatment and hydrogen production to avoid PSB lose [20,21]. There are two types of this method: embedding and biofilm. The embedding materials used are usually polyvinyl alcohol beads and PVA–boric acid gel granules [3,22], sodium alginate or agar [23] and porous ceramic [4]. The materials used in the biofilm method are packed glass beads [20] and other materials that have porous

structures [21]. Some fixation and separation methods used in water supply and wastewater treatment for water purification and solid–liquid separation can also be used for PSB collection. Except for water and wastewater treatment, coagulation is also usually used for microalgae collection [24–26]. During flocculation, single cells tend to form larger aggregates that can be separated from the medium by simple gravity sedimentation [26]. Different coagulants, dosages and dosing methods are usually investigated to obtain the optimal coagulation effect [24–26]. PSB are also microbes and have some common characteristics with microalgae; thus, coagulation might be used for PSB collection. Ultrafiltration membrane separation is well known for its excellent retention of microsize particles [27]; therefore, ultrafiltration membranes also can be used in a PSB wastewater treatment system to separate PSB and to further purify the effluent. Although there are many types of methods for immobilizing PSB, most of these methods attempt to mitigate PSB loss, promoting wastewater treatment efficiency and hydrogen production efficiency. The recover or collection of PSB has not been studied systematically.

In this study, three typical methods for PSB bioresources recovery (e.g. immobilization, which uses materials with porous structure; coagulation; and membrane separation) were investigated and compared; the optimal separation conditions for PSB condensation were also studied.

## 2. Materials and methods

### 2.1. PSB and the culturing method

A strain of PSB (*Rhodobacter sphaeroides*) called Z08 that was isolated from local soil was used in this study. This strain is a Gram-negative bacterium that is spherical with a diameter of 0.3–1.0  $\mu\text{m}$ . Z08 can survive under light-anaerobic, natural light-micro aerobic and dark-aerobic conditions [12]. Z08 was cultured via the shaking culture method using an HCH medium (Table 1).

Table 1  
Composition of HCH medium

Components	Concentration (g/L)	Components	Concentration
DL-malic acid	4.0	Yeast extract	100 mg/L
MgSO <sub>4</sub>	0.12	Fe <sup>3+</sup>	0.0025 mol/L
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>2</sub>	1.0	Mn <sup>2+</sup>	0.0090 mol/L
CaCl <sub>2</sub>	0.075	Zn <sup>2+</sup>	0.0033 mol/L
KH <sub>2</sub> PO <sub>3</sub>	0.5	Co <sup>2+</sup>	0.0024 mol/L
K <sub>2</sub> HPO <sub>3</sub>	0.3	Cu <sup>2+</sup>	0.0024 mol/L
Na <sub>2</sub> EDTA	0.020	pH	6.8

HCH medium measuring 50 mL was placed in a 250 mL flask. The flask mouth was sealed by an oxygen-enrichment membrane to keep out dust and infectious microbes. The medium was sterilized in an autoclave at 120°C for 20 min. Z08 with a volume of 15 mL was inoculated into the flask; the biomass concentration was approximately 840.0 mg/L dry weight after the medium had cooled. Then, Z08 was cultured in a thermostat shaker at 140 rpm and 28°C with a light intensity of 3,000 lux. The Z08 used in the following experiments were growing during the logarithmic growth phase.

The Z08 biomass dry weight was plotted vs. OD<sub>660</sub> in Fig. 1 and can be described by the following fitted equation:

$$\text{Biomass (dry weight)} = 6.0046 + 835.0469 \times \text{OD}_{660}, \\ R^2 = 0.9904$$

## 2.2. Ultrafiltration membrane and the membrane filtration set-up

A hollow fibre crossflow ultrafiltration membrane module was used (LU8-4A, Litree Company, China).

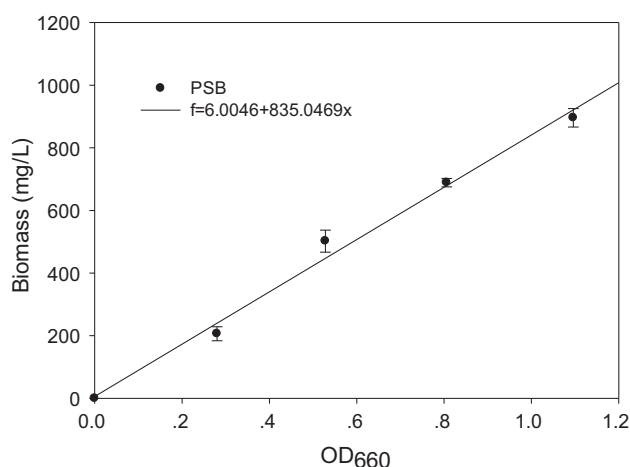


Fig. 1. Biomass dry weight vs. OD<sub>660</sub>.

Table 2  
Components and weights of the support materials used

Supporting materials	Components	Weight (g)	Source
QS	SiO <sub>2</sub>	10	Tianjin Biboquan Water Filtration Materials Science and Technology Ltd.
CS	SiO <sub>2</sub>	10	
AC	Active carbon	10	
VR	SiO <sub>2</sub>	10	
PVC	Polyvinyl chloride	1.5	
PP	Polypropylene	4.0	
LS	Plant fibre	3.0	

The membrane was made of hydrophilized polyvinyl chloride and had an effective membrane surface area of 0.16 m<sup>2</sup>. The internal and external diameters of the hollow filament fibre were 1.00 and 1.66 mm, respectively. The nominal pore size was 0.01 μm and the molecular weight cut-off was 100,000 Daltons. The manufacturer's specified operating pressure range was 0.1–0.2 MPa, the maximum flow rate was 200 L/h and the minimum flux was 10 L/(m<sup>2</sup> h).

## 2.3. PSB immobilization

For immobilization, seven support materials including quartz sand (QS), cobblestone (CS), active carbon (AC), volcanic rock (VR), polyvinyl chloride (PVC), polypropylene (PP) and loofah sponge (LS) were used. All support materials were pretreated in an ultrasonic cleaner (KQ2200DV, 100 W, Kunshan Ultrasonic Cleaner Co. Ltd) three times to remove impurities; the support materials were then weighed after drying in an oven for 48 h at 70°C. The weights of the seven support materials were recorded in Table 2.

The support materials and the 150 mL of HCH medium were first placed in 250 mL flasks. The flask mouth was sealed by an oxygen-enrichment membrane to keep out dust and infectious microbes. The medium was sterilized in an autoclave at 120°C for 20 min. After the medium and support materials were cooled, Z08 was inoculated into the 250 mL flasks. The initial inoculation dosage was 12.8 mg dry cell weight.

After inoculation, the flasks were placed in a thermostat shaker set to 140 rpm and 30°C for 10 h. After 10 h, Z08 was cultured statically and lit by a 60 W incandescent lamp to maintain a temperate near 30°C; the light intensity was also controlled to be near 2000 lux. The total culturing time was 30 d. During this period, the medium was renewed with 50 mL of new medium every three days. For the medium replacement, a 50 mL mixture of Z08 cells and medium in the

experimental group was first measured and centrifuged at 12,000 rpm for 10 min to collect the Z08 cells. Then, the collected Z08 cells were added back into the sample with 50 ml of new sterile medium. Replacement of the medium was performed in a sterile bench with sterile tubes and flasks. The support materials were flushed on the 30th day to remove the biomass that was not attached stably and were then dried in an oven to constant weight before being weighted.

#### 2.4. Coagulation of PSB

The three coagulants of aluminium sulphate, ferric sulphate and aluminium polychloride (Tianjin Biboquan Water Filtration Materials Science and Technology Ltd) were used in this study. These coagulants were first dried to constant weights at 120°C and were then used to prepare 10% liquid reserves. In the experiments, Z08 was placed in 100 mL flasks with a concentration of approximately 420.0 mg/L dry weight. Coagulant concentrations were controlled by grades to be 100, 500, 1,000, 5,000, 10,000 and 20,000 mg/L. The flasks were then placed in a shaker at a mixing speed of 200 rpm for 1 min and then at 100 rpm for 15 min. After the Z08 cells were allowed to settle for another 30 min,  $OD_{660}$  and pH in the supernatant were tested.

In this experiment, the PSB separation ratio was calculated by subtracting the PSB concentration in liquid after coagulation from the initial PSB concentration and then dividing this result by the initial PSB concentration; water production was also calculated by considering the volume ratio of the clean water produced via membrane filtration vs. the total influent. The clean water was collected in a barrel during the membrane separation process and its volume was measured. The volume of the total influent was also measured during the membrane separation process.

#### 2.5. Membrane collection of PSB

The membrane collection process of PSB can be divided into two steps: filtration (also can be called as separation), which separates PSB from wastewater via membrane filtration; and collection (also can be called as recovery), which collects or recycles PSB via backwash from the membrane. In this experiment, Z08 was kept in a 4.0-L round bottom flask with an initial biomass of approximately 841.1 mg/L dry weight. The schematic diagram of the ultrafiltration and backwash equipment is shown in Fig. 2.

During the ultrafiltration process (separation), the inflow rate and pressure were controlled by valves 4

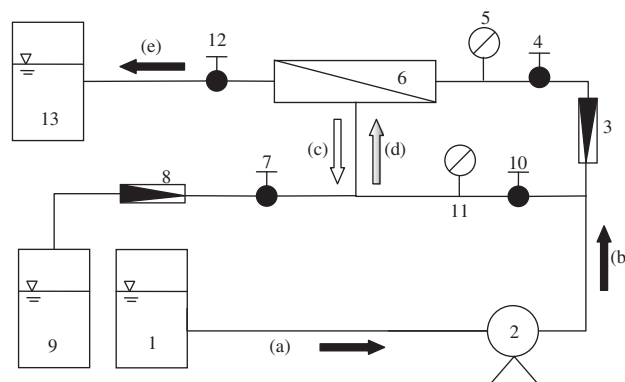


Fig. 2. Schematic diagram of the ultrafiltration and backwash equipment.

Note: 1. PSB storage tank; 2. electric diaphragm pump; 3. inflow meter; 4. inflow control valve; 5. inflow pressure gauge; 6. ultrafiltration membrane; 7. drainage valve; 8. drainage meter; 9. reclaimed water storage tank; 10. backwash valve; 11. backwash pressure gauge; 12. backwash valve; 13. condensed PSB storage tank; (a) inlet pipe; (b) shunt pipe; (c) filtrated water drainage pipe; (d) backwash inlet pipe; (e) concentrated bacterial liquid drainage pipe.

and 7. The bacterial liquid was stored in the PSB storage tank (1). Valves 4 and 7 were then opened and valves 10 and 12 were closed. Pump (2) was then activated, pumping the bacterial liquid into the system via pipe (a). The bacterial liquid flowed through pipe (b) and then entered the ultrafiltration membrane (6). The clear water flowed out through pipe (c) to the reclaimed water storage tank (9).

The operational pressure and inflow rate during the membrane filtration process were 0.1 MPa and 15 L/h, respectively. When the PSB concentration in the effluent was constant, the separation cycle was complete. During one separation cycle, the biomass in the inflow and effluent were tested, and the membrane separation ratio was calculated. The COD of the effluent was also tested. The membrane separation ratio was calculated by subtracting the PSB concentration of the effluent after membrane filtration from the PSB concentration of the influent and then dividing the results by the PSB concentration of the influent.

During the backwash process (PSB collection), the PSB storage tank (1) was filled with backwash water. The pressure and flow rate were controlled by valves 10 and 12, which were opened; then, valves 4 and 7 were closed and pump (2) was activated. Backwash water was transferred by pump (2) into pipe (a) and then directed through pipes (b) and (d). Then, the backwash water flowed through the ultrafiltration membrane (6). The concentrated bacterial liquid was then discharged through pipe (e) into the condensed PSB storage tank (13).

The operational pressure and inflow rate for the backwash were 0.2 MPa and 20 L/h, respectively. The biomass in the effluent was tested at 0, 10, 20, 30, 40, 50, 60, 120, 180, 240 and 300 s, and the biomass collection ratio was calculated by subtracting the PSB concentration in the effluent from the PSB concentration retained in the membrane and then dividing the result by the PSB concentration retained in the membrane.

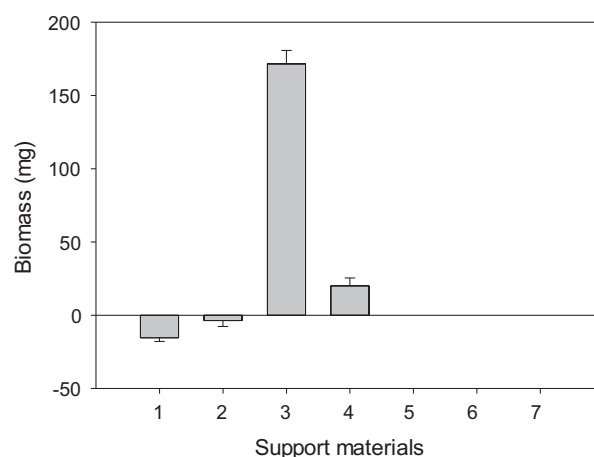
## 2.6. Analysis methods

OD<sub>660</sub> was tested using the spectrophotometric method (APHA, 2005). The biomass and COD were tested using the APHA standard methods [28]. The pH and light intensity were monitored regularly using a PSH-3 pH meter (Shanghai Precision and Scientific Instrument Company, China) and a Li-250A light meter (Li-COR Inc., Canada), respectively. The zeta potential was tested using a Zeta sizer (Nano-z, Engima Business Park, UK). The amount of extracellular polymeric substance (EPS) was tested using the high-speed centrifugation method [29–31]. The amount of carbohydrates present was measured using the phenol–sulphuric acid method [30]. The amount of proteins present was measured using the Lowry method [32]. The amount of nucleic acids present was measured using UV–visible spectrometry method [31]. Each experiment was repeated three times, and all of the results shown are the averages of the results of the three corresponding tests.

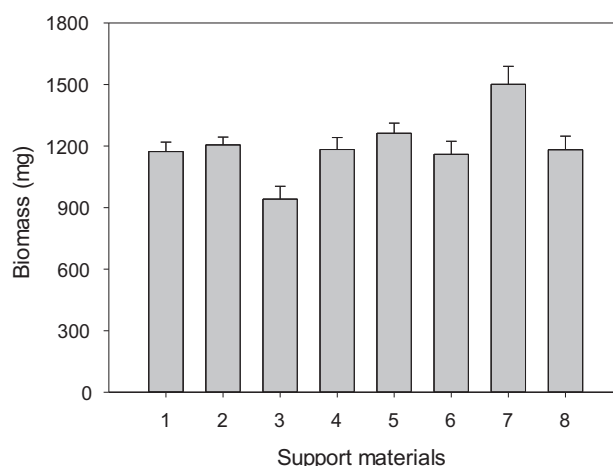
## 3. Results and discussion

### 3.1. PSB immobilization

The previous studies in the literature showed that PSB could adhere to the surface of support materials [33]. However, in this study, Fig. 3(a) showed that PSB did not adhere well to the seven common support materials tested during the 30 d cultivation period. Except for AC and VR, no Z08 adhered to the other supports. The final biomasses of Z08 on the QS, CS, AC, VR, PVC, PP and LS were –15.4, –3.6, 171.6, 20.1, 0, 0 and 0 mg, respectively. These findings indicated that immobilization could not concentrate Z08 effectively. Fig. 3(a) showed that the PSB weight on QS was –15.4 mg after 30 d of accumulation; this result might be caused by a measurement error or by the dissolution of QS into the water used in this experiments. The biomass in the AC experimental group was found to be the highest; however, the initial AC weight was 10.0 g, and thus, the small change in weight measured could be considered negligible.



(a) Biomass immobilized on different support materials, mg



(b) Biomass accumulated in the liquid of different support materials, mg

Fig. 3. Biomass dry weight immobilized on supporting materials and accumulated in liquid, 30 d of cultivation.

Notes: 1. Quartz Sand (QS); 2. Cobblestone (CS); 3. Active Carbon (AC); 4. Volcanic Rock (VR); 5. Polyvinyl Chloride (PVC); 6. Polypropylene (PP); 7. Loofah Sponge (LS); 8. Blank.

The support materials were shown to only slightly affect Z08 growth. After 30 d of accumulation, the final biomasses in the liquid were 1,173.2, 1,205.6, 941.3, 1,183.5, 1,262.2, 1,159.4, 1,500.8 and 1,182.1 mg for the QS, CS, AC, VR, PVC, PP, LS and the blank experimental groups, respectively, as shown in Fig. 3(b). The biomass in the liquid of the LS experimental group was 26% higher than that of the blank experimental group, which indicated that Z08 primarily grew in the liquid phase. The loofah sponge was composed by fibre and contained some nutrients such as polysaccharides and other nutrients. After a long soaking time, the nutrients in the loofah sponge might have dissolved into the water, which could have promoted the growth of Z08.

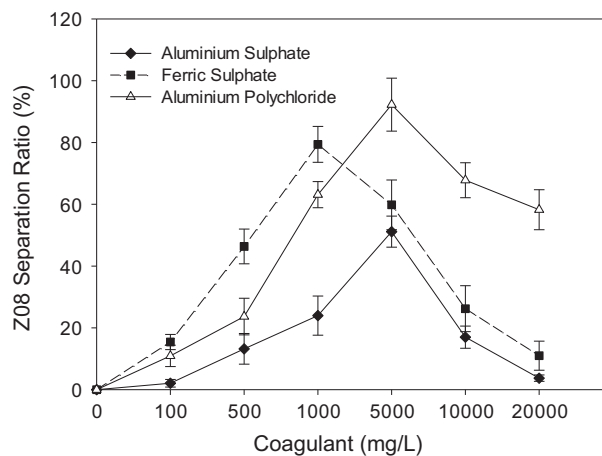


PSB did not form a biofilm easily and did not adhere to the surface of the support materials well; these findings might be caused by the following reasons. Firstly, Z08 cell do not have a capsule [34], which is sticky and typically allows cells to adhere to each other or other materials. Because the surface of Z08 cells is smooth, the cells tended to suspend in the water without adhering to each other or the surface of the support materials. Secondly, the zeta potential of the Z08 cells was low:  $-20$  mV at pH 9.0 with an  $OD_{660}$  of 0.50. A low zeta potential typically generates a significant repulsion effect to other Z08 cells dispersed in water, which can prevent clustering of Z08 cells. Thirdly, many studies have shown that low EPS concentrations can cause poor PSB flocculation [35,36]. Watanabe et al. [35] and Sheng et al. [36] found that the EPS concentration of PSB was 16–90 mg/L when PSB grew under normal conditions, which was insufficient for flocculation. Conversely, EPS was much higher when PSB was in the presence of a high concentration of metal ions [35,36]; the flocculating ratio of PSB was shown to be above 50%. In this study, Z08 grew in a normal environment, and the EPS of Z08 was much lower (9.0 mg/L) than that in the previous studies [35,36] and of activated sludge, which cannot absorb  $Ca^{2+}$  and binds the water and polysaccharide effectively [37,38]; as a result, the Z08 cells in this study did not form a biofilm easily. Finally, the biotic community is unitary in this study, which prevents the formation of a symbiotic ecosystem. Therefore, Z08 cannot rely on each other to form ecological communities, leading to poor accumulation and clustering [39].

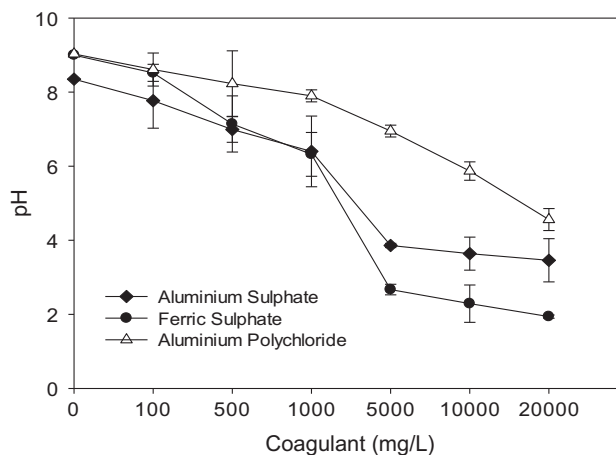
### 3.2. Coagulation of PSB

The above results showed that the PSB immobilization by the support materials tested was not effective; thus, other methods might be used for PSB collection. Coagulation is usually used in microalgae collection [24–26]. Some characteristics of PSB are very similar to those of microalgae, including a low zeta potential and a small diameter; thus, coagulation might be used for PSB collection. Three coagulants, aluminium polychloride, aluminium sulphate and ferric sulphate were investigated with regard to increasing the zeta potential of Z08 and improving Z08 clustering.

Fig. 4(a) showed that all three of the coagulants tested could effectively separate PSB from water. The optimal coagulation dosage were determined to be 5,000, 5,000, 1,000 mg/L for aluminium polychloride, aluminium sulphate and ferric sulphate, respectively. Table 3 showed that the final Z08 separation ratios were 92.2, 51.1 and 79.4% for aluminium polychloride,



(a) Z08 separation ratios at different coagulants dosages



(b) pH at different coagulation dosages

Fig. 4. Coagulation effect and changes in pH at different coagulation dosages.

aluminium sulphate and ferric sulphate, respectively. These findings meant that to achieve ideal coagulation, the dosage of the coagulant must be above 1,000 mg/L. For aluminium polychloride, the total cost per kg will near 1.55 \$/kg Z08, which is not economical. During coagulation, the pH dropped significantly as the dosage increased, as shown in Fig. 4(b).

### 3.3. Membrane separation

Although coagulation showed a high efficiency during PSB collection, it consumed significant amounts of chemical reagents, and was thus not economical. Also, the biomass collected via coagulation cannot be used directly as a feed supplement because it contains large amount of chemical coagulants. Compared to immobilization and coagulation, the

Table 3

Dosages, Z08 separation ratios and prices of the three chemical coagulants tested for Z08 separation

	Aluminium sulphate	Ferric sulphate	Aluminium polychloride
Dosage (mg/L)	5,000	1,000	5,000
Z08 separation ratio (%)	51.1	79.4	92.2
Price (\$/kg)	0.29	0.71	0.13
Price (\$/kg Z08)	3.45	1.69	1.55

membrane separation method was more effective for PSB collection, and the biomass could be directly used after separation.

The membrane separation method could be used to remove macromolecules, chemical compounds, polymeric compounds, colloids and viruses. Then, ultrafiltration membranes could be used to remove all 0.0012–0.05  $\mu\text{m}$  solute molecules from a liquid mixture because this technology is currently used to separate bacteria and mycoproteins. The diameter of Z08 was  $1 \mu\text{m} \times 1.5 \mu\text{m}$  [16], and the aperture of the membrane was 0.01  $\mu\text{m}$ ; thus, the ultrafiltration membrane could separate the Z08 cells effectively.

### 3.3.1. Feasibility of the ultrafiltration method for PSB filtration

Fig. 5 showed that the membrane separation method is particularly effective. With an operational pressure of 0.1 MPa and an initial inflow rate of 15 L/h, the Z08 dry cell weight decreased from 841.1 to 7.7 mg/L. The separation ratio of the membrane separation method thus reached 99.1%.

During this period of the experiment, the flux, which refers to the volume of water passing through the membrane during a unit time across a unit mem-

brane area, was monitored and recorded in  $\text{L}/(\text{m}^2 \text{ h})$ . In this study, the effective membrane surface area was 0.16  $\text{m}^2$ , and the maximum flow rate was 200 L/h; thus, the minimum flux was 10  $\text{L}/(\text{m}^2 \text{ h})$ . If the flux decreased below 10  $\text{L}/(\text{m}^2 \text{ h})$ , the membrane would be considered to be blocked and is in need of cleaning. Fig. 5 shows that the flux remained above 10  $\text{L}/(\text{h m}^2)$  (i.e. the minimum design flux) for approximately 30 h. After that time, the flux dropped below 10  $\text{L}/(\text{h m}^2)$ . Under this condition, the membrane must be cleaned to recover the separation efficiency. The biomass was retained in the membrane reactor; to clean the membrane and recover biomass, the backwash method was applied. The separation cycle was thus set to 30 h.

### 3.3.2. Clean water production and biomass recovery efficiency

During the membrane separation process, significant amounts of residual water were generated. Although the water generated from the PSB membrane separation process contained some metabolites, the production was simple, and the ingredients did not seriously block the membrane. Fig. 6(a) showed that the COD of the effluent was 20.6 mg/L at 30 h, which meets the national standards [40]. The water production reached 93.0%; therefore, the residual wastewater separated by the membrane can be collected and used as reclaimed water in industries ( $\text{COD}_{\text{Cr}} \leq 60 \text{ mg/L}$ ), agriculture, forestry, animal husbandry ( $\text{COD}_{\text{Cr}} \leq 40 \text{ mg/L}$ ) and landscaping ( $\text{COD}_{\text{Cr}} \leq 30 \text{ mg/L}$ ) [40], producing significant water resource.

PSB was collected by the backwash method (i.e. condensing). The cleanliness of the membrane was measured by testing the PSB concentration of the effluent. Fig. 6(b) showed that the PSB concentration was 55,890.5 mg/L at the beginning of the process and quickly dropped to 1,639.7 mg/L after 10 s. After 240 s, the PSB concentration was 6.6 mg/L (i.e. 0.01% of the initial PSB concentration). This showed that 99.0% of the Z08 cells were collected via the backwash method; thus, the recommended backwash time was determined to be 300 s. After backwashing, the

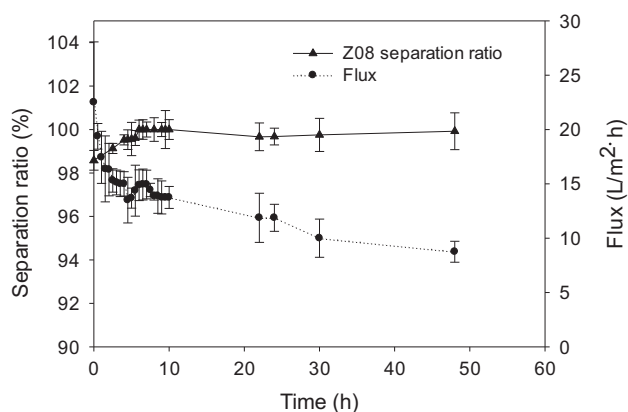


Fig. 5. Z08 separation ratio and flux changes during separation at 0.1 MPa and 15 L/h.

collected Z08 concentration was 10,421.5 mg/L, which meant that the PSB liquid has been concentrated 12.4 times compared to the initial PSB concentration.

### 3.3.3. PSB concentration optimization during the membrane separation process

PSB separation using the membrane separation process can collect biomass. However, PSB cells also block the membrane pores, which generate membrane fouling; a larger initial PSB concentration tends to produce more serious membrane blockages more quickly, possibly shortening the separation cycle. Thus, the effect of the initial PSB concentration was investigated.

Three PSB concentration experimental groups (e.g. 841.1, 1,682.1 and 2,523.2 mg/L) were investigated.

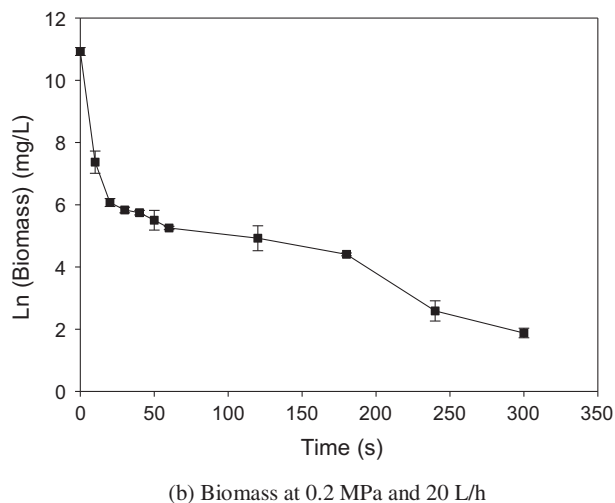
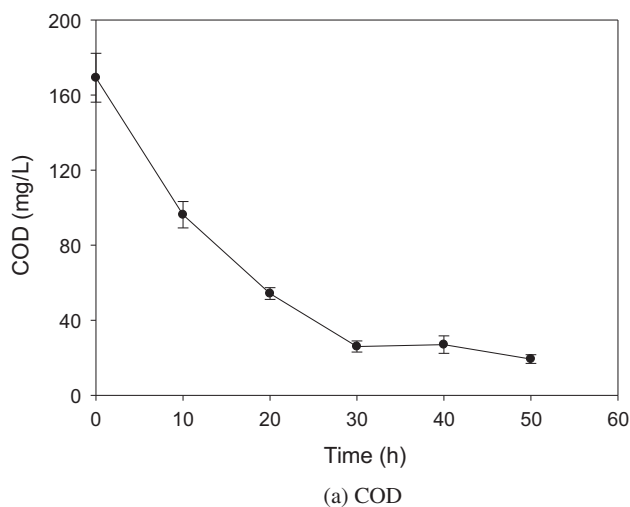


Fig. 6. COD changes during PSB filtration processing and biomass concentration changes during the backwash process.

Fig. 7(a) showed that the separation ratio had no effect among the different initial PSB concentration experimental groups; the separation ratios of all three experimental groups were above 96.0%. Although the 2,523.2 mg/L experimental group showed a higher separation ratio than the 841.1 and 1,682.1 mg/L experimental groups, the flux of the 2,523.2 mg/L experimental group decreased more quickly than those of the other experimental groups. Fig. 7(b) also shows that the 841.1 mg/L experimental group maintained the flux above 10 L/(h m<sup>2</sup>) (i.e., the minimum design flux) compared to the other experimental groups; this could prolong the separation cycle time. In the 841.1 mg/L experimental group, the flux was kept above 10 L/(h m<sup>2</sup>) for more than 70 h; however, in the 1,682.1 and 2,523.2 mg/L experimental groups,

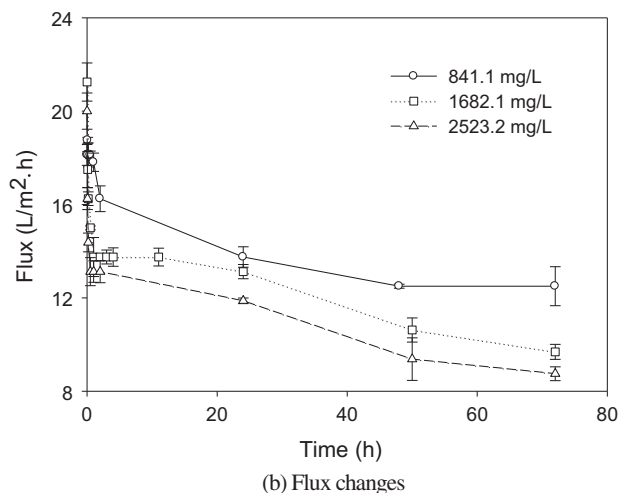
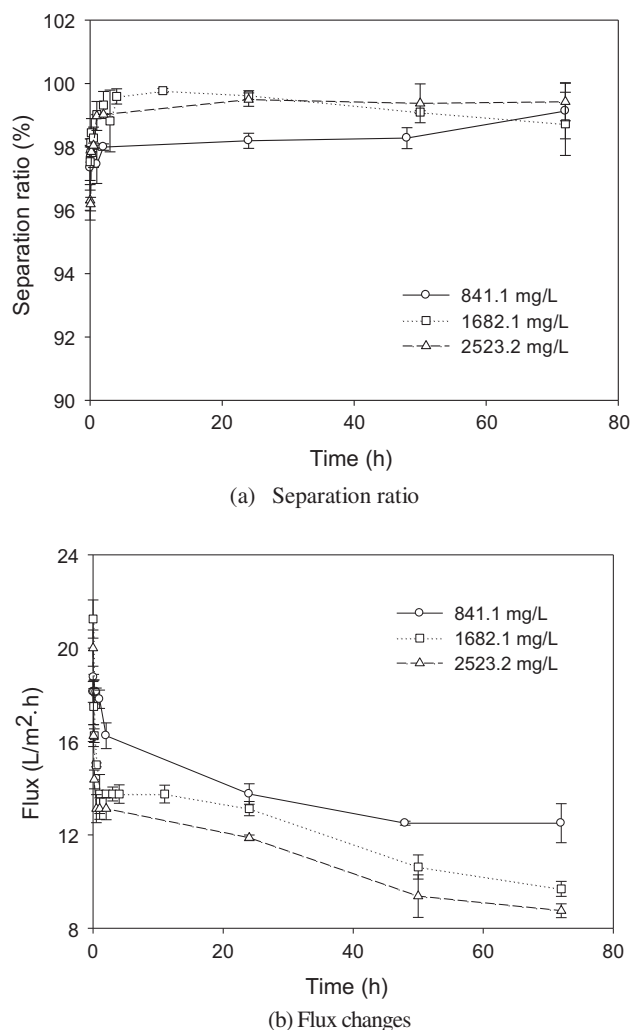


Fig. 7. Separation ratio and flux changes in the three PSB concentration experimental groups at 0.1 MPa and 20 L/h.



the fluxes fell below  $10 \text{ L}/(\text{h m}^2)$  after 50 and 24 h, respectively. The final biomass production was 114,634.8, 165,396.0 and  $127,260.0 \text{ mg}/\text{L}$  for the 841.1, 1,682.1 and 2,523.2 experimental groups, respectively. Comparing the results of the separation cycle times and the biomass productions, the  $1,682.1 \text{ mg}/\text{L}$  group was shown to be optimum.

### 3.4. PSB combined membrane system compared to a traditional membrane bioreactor

A membrane bioreactor (MBR) is a wastewater treatment technology that combines activated sludge and a membrane. In an MBR, pollutant removal ratios are typically high and the effluent can be directly used as recycled water, which is superior to traditional activated sludge technology. However, the sludge produced by this process cannot mitigate the problems generated by the traditional activated sludge (i.e. secondary pollution), even though the activated sludge production of this process was less than that of traditional wastewater treatment processes. In this study, PSB and membrane technologies were combined to form a new system. In this system, PSB was substituted for activated sludge; no activated sludge was used, and PSB was the generated biomass from this system. The PSB biomass can be used as raw materials for livestock, aquaculture and medicine, which avoids the problems caused by activated sludge.

However, membrane fouling is a serious problem in traditional MBR wastewater treatment technology. In this study, membrane fouling was also present. To evaluate the membrane fouling, the PSB combined membrane system and the traditional MBR system were compared. The initial concentrations of PSB and activated sludge were  $10,000$  and  $6,000 \text{ mg}/\text{L}$ , respectively. Fig. 8 shows that the flux of PSB combined membrane system changed from  $93.8 \text{ L}/(\text{m}^2 \text{ h})$  at 0 min to  $13.8 \text{ L}/(\text{m}^2 \text{ h})$  at 15 min, and the flux remained above  $10 \text{ L}/(\text{m}^2 \text{ h})$  for 60 min. For the activated sludge–MBR system, the flux reached  $13.8 \text{ L}/(\text{m}^2 \text{ h})$  at 6 min and at 20 min, the flux finally reached  $9.4 \text{ L}/(\text{m}^2 \text{ h})$ . The concentration of PSB was higher than that of the activated sludge, but the flux of the PSB combined membrane system performed for longer than the traditional MBR system. Thus, the operational period of the PSB combined membrane system was longer compared to that of the system using activated sludge.

Membrane fouling is primarily caused by the EPS of the activated sludge [37]. In traditional wastewater treatment processes, the EPS of the activated sludge was approximately  $100 \text{ mg}/\text{L}$ . Watanabe et al. [35]

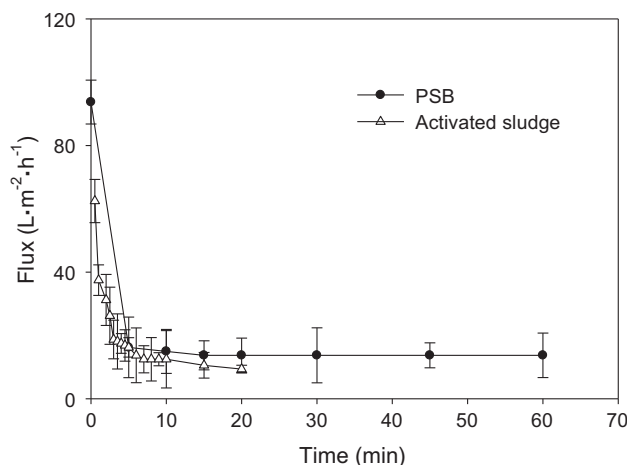


Fig. 8. Flux changes in the PSB combined membrane and traditional MBR systems at  $0.1 \text{ MPa}$  and  $25 \text{ L}/\text{h}$ .

and Sheng et al. [36] found that the EPS of PSB was  $16\text{--}90 \text{ mg}/\text{L}$  when PSB grew normally. In this study, under normal conditions, the EPS of Z08 was approximately  $9.0 \text{ mg}/\text{L}$ , which was much lower than the activated sludge. Therefore, the membrane fouling in this study might be lower than that in traditional MBR wastewater treatment processing. Besides, PSB had no capsule, had a low zeta potential, and could not form clusters (Section 3.1). All of these characters might also mitigate membrane fouling.

## 4. Conclusions

Three types of PSB separation methods (immobilization, coagulation and membrane filtration) were investigated and compared. The membrane system used for PSB separation was shown to be more effective compared to the immobilization and coagulation methods. The membrane system can treat wastewater, recover bioresources and produce usable water resources. The collection process was divided into two steps: filtration and condensing. Throughout the process, the PSB recovery ratio reached  $99.0\%$  with a water production ratio of  $93.0\%$ ; the biomass was also condensed 12.4 times, and the optimal initial PSB concentration was found to be  $1,682.1 \text{ mg}/\text{L}$ . With an operational pressure of  $0.1 \text{ MPa}$  and an initial inflow rate of  $20 \text{ L}/\text{h}$ , the water cycle duration and biomass concentration reached 50 h and  $165,396.0 \text{ mg}/\text{L}$ , respectively. Besides, compared to the traditional MBR wastewater treatment processing, the PSB combined membrane system also showed a longer operational duration and could manage higher initial biomass concentrations, indicating its significant potential and

efficiency in wastewater treatment, water resource reclamation, membrane fouling mitigation and bioresource recovery.

### Acknowledgements

The authors would like to thank the National Natural Science Foundation of China (51308535, 51278489) and MARC (MARC2012D011) for their financial support.

### References

- [1] M. Kobayashi, S. Kurata, Mass culture and cell utilization of photosynthetic bacteria, *Proc. Biochem.* 13 (1978) 27–30.
- [2] M. Kobayashi, *Microbial Energy Conversion*, Pergamon Press, New York, NY, 1977.
- [3] H. Nagadomi, T. Hiromitsu, K. Takeno, M. Watanabe, K. Sasaki, Treatment of aquarium water by denitrifying photosynthetic bacteria using immobilized polyvinyl alcohol beads, *J. Biosci. Bioeng.* 87(2) (1999) 189–193.
- [4] H. Nagadomi, T. Kitamura, M. Watanabe, K. Sasaki, Simultaneous removal of chemical oxygen demand (COD), phosphate, nitrate and H<sub>2</sub>S in the synthetic sewage wastewater using porous ceramic immobilized photosynthetic bacteria, *Biotechnol. Lett.* 22 (2000) 1369–1374.
- [5] U. Gündüz, M. Yüce, I. Eroğlu, Photosynthetic bacterial growth and productivity under continuous illumination or diurnal cycles with olive mill wastewater as feedstock, *Int. J. Hydrogen Energy* 35(11) (2010) 5293–5300.
- [6] E. Eroğlu, I. Eroğlu, U. Gündüz, M. Yüce, Effect of clay pretreatment on photofermentative hydrogen production from olive mill wastewater, *Bioresour. Technol.* 99(15) (2008) 6799–6808.
- [7] W. Luo, X.Y. Deng, W.T. Zeng, D.H. Zheng, Treatment of wastewater from shrimp farms using a combination of fish, photosynthetic bacteria, and vegetation, *Desalin. Water Treat.* 47(1–3) (2012) 221–227.
- [8] E.H.G. Ponsano, C.Z. Paulino, M.F. Pinto, Phototrophic growth of *Rubrivivax gelatinosus* in poultry slaughterhouse wastewater, *Bioresour. Technol.* 99(9) (2008) 3836–3842.
- [9] J. Kaewsuk, W. Thorasampan, M. Thanuttamavong, G.T. Seo, Kinetic development and evaluation of membrane sequencing batch reactor (MSBR) with mixed cultures photosynthetic bacteria for dairy wastewater treatment, *J. Environ. Manage.* 91(5) (2010) 1161–1168.
- [10] M. Suwansaard, W. Choorit, J.H. Zeilstra-Ryalls, P. Prasertsan, Isolation of anoxygenic photosynthetic bacteria from Songkhla Lake for use in a two-staged biohydrogen production process from palm oil mill effluent, *Int. J. Hydrogen Energy* 34(17) (2009) 7523–7529.
- [11] J. He, G. Zhang, H. Lu, Treatment of soybean wastewater by a wild strain *Rhodobacter sphaeroides* and to produce protein under natural conditions, *Front. Environ. Sci. Eng. Chin.* 4(3) (2010) 334–339.
- [12] H. Lu, G. Zhang, T. Wan, Y. Lu, Influences of light and oxygen conditions on photosynthetic bacteria macromolecule degradation: Different metabolic pathways, *Bioresour. Technol.* 102(20) (2011) 9503–9508.
- [13] H. Lu, G. Zhang, X. Dai, C. He, Photosynthetic bacteria treatment of synthetic soybean wastewater: Direct degradation of macromolecules, *Bioresour. Technol.* 101(19) (2010) 7672–7674.
- [14] E.I. Madukasi, H.F. Lu, W. Zhao, Influence of metal ions on biodegradation of high organic load wastewater by photosynthetic bacteria, 2010 International Conference on Challenges in Environmental Science and Computer Engineering, Wuhan, 2010.
- [15] D. Kantachote, S. Torpee, K. Umsakul, The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater, *Electron. J. Biotechnol.* 8(3) (2005) 314–323.
- [16] E.I. Madukasi, X. Dai, C. He, J. Zhou, Potentials of phototrophic bacteria in treating pharmaceutical wastewater, *Int. J. Environ. Sci. Technol.* 7(1) (2010) 165–174.
- [17] R. Muñoz, B. Guieysse, Algal-bacterial processes for the treatment of hazardous contaminants: A review, *Water Res.* 40 (2006) 2799–2815.
- [18] C. Lorrunguang, J. Martthong, K. Sasaki, N. Noparatnaraporn, Selection of photosynthetic bacterium *Rhodobacter sphaeroides* 14F for polyhydroxyalkanoate production with two-stage aerobic dark cultivation, *J. Biosci. Bioeng.* 102 (2006) 128–131.
- [19] J.F. Imhoff, H.G. Trüper, Purple nonsulfur bacteria, in: J.T. Staley (Ed.), *Bergey's Manual of Systematic Bacteriology*, Williams & Wilkins, Baltimore, 1989, pp. 1658–1682.
- [20] X. Tian, Q. Liao, X. Zhu, Y. Wang, P. Zhang, J. Li, H. Wang, Characteristics of a biofilm photobioreactor as applied to photo-hydrogen production, *Bioresour. Technol.* 101(3) (2010) 977–983.
- [21] C. Zhang, X. Zhu, Q. Liao, Y. Wang, J. Li, Y. Ding, H. Wang, Performance of a groove-type photobioreactor for hydrogen production by immobilized photosynthetic bacteria, *Int. J. Hydrogen Energy* 35(11) (2010) 5284–5292.
- [22] X. Tian, Q. Liao, W. Liu, Y.Z. Wang, X. Zhu, J. Li, H. Wang, Photo-hydrogen production rate of a PVA-boric acid gel granule containing immobilized photosynthetic bacteria cells, *Int. J. Hydrogen Energy* 34(11) (2009) 4708–4717.
- [23] K. Takeno, Y. Yamaoka, K. Sasaki, Treatment of oil-containing sewage wastewater using immobilized photosynthetic bacteria, *World J. Microbiol. Biotechnol.* 21 (2005) 1385–1391.
- [24] L. Brennan, P. Owende, Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products, *Renewable Sustainable Energy Rev.* 14 (2010) 557–577.
- [25] A. Schlesinger, D. Eisenstadt, A. Bar-Gil, H. Carmely, S. Einbinder, J. Gressel, Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production, *Biotechnol. Adv.* 30 (2012) 1023–1030.
- [26] D. Vandamme, I. Foubert, K. Muylaert, Flocculation as a low-cost method for harvesting microalgae for bulk biomass production, *Trends Biotechnol.* 31(4) (2013) 233–239.

- [27] S. Judd, *The MBR Book: Principles and Applications of Membrane Bioreactors in Water and Wastewater Treatment*, second ed., Elsevier Science Publishers, Amsterdam, 2011.
- [28] APHA, AWWA, WEF, *Standard Methods for the Examination of Water and Wastewater*, 21st ed., American Public Health Association, American Water Works Association and Water Environment Federation, Washington, DC, 2005.
- [29] A. Eldyasti, G. Nakhla, J. Zhu, Development of a calibration protocol and identification of the most sensitive parameters for the particulate biofilm models used in biological wastewater treatment, *Bioresour. Technol.* 111 (2012) 111–121.
- [30] A.S. Gong, C.A. Lanzl, D.M. Cwiertny, S.L. Walker, Lack of influence of extracellular polymeric substances (EPS) level on hydroxyl radical mediated disinfection of *Escherichia coli*, *Environ. Sci. Technol.* 46(1) (2012) 241–249.
- [31] G.P. Sheng, H.Q. Yu, X.Y. Li, Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review, *Biotechnol. Adv.* 28(6) (2010) 882–894.
- [32] B. Frølund, R. Palmgren, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, *Water Res.* 30 (1996) 1749–1758.
- [33] T. Sun, L. Li, Removal of nitrogen and phosphorus in wastewater using photosynthetic bacteria immobilized on activated carbon, *J. Agro-Environ. Sci.* 25 (2006) 211–213.
- [34] N. Basak, D. Das, The prospect of purple non-sulphur (PNS) photosynthetic bacteria for hydrogen production: The present state of the art, *World J. Microbiol. Biotechnol.* 23(1) (2007) 31–42.
- [35] M. Watanabe, K. Sasaki, Y. Nakashimada, T. Kakizono, N. Noparatnaraporn, N. Nishio, Growth and flocculation of a marine photosynthetic bacterium *Rhodovulum* sp., *Appl. Microbiol. Biotechnol.* 50 (1998) 682–691.
- [36] G.P. Sheng, H.Q. Yu, Z.B. Yue, Production of extracellular polymeric substances from *Rhodospseudomonas acidophila* in the presence of toxic substances, *Appl. Microbiol. Biotechnol.* 69 (2005) 216–222.
- [37] H.F. Jenkinson, H.M. Lappin-Scott, Biofilms adhere to stay, *Trends Microbiol.* 9(1) (2001) 9–10.
- [38] J. Wingender, T.R. Neu, H.-C. Flemming, *Microbial Extracellular Polymeric Substances*, Springer, New York, NY, 1999.
- [39] G. Roeselers, M.C.M. Loosdrecht, G. Muyzer, Phototrophic biofilms and their potential applications, *J. Appl. Phycol.* 20 (2008) 227–235.
- [40] Standards of reclaimed water quality, SL 368-2006, The Ministry of Water Resources of the People's Republic of China, Beijing, 2006. Available from: <http://www.anykeen.cn/pic/4-8.pdf>.