

# **An efficient microalgal biomass harvesting method with a high concentration ratio using the polymer-surfactant aggregates process**

Y. H. Wu <sup>a, b, #</sup>, L. C. Shen <sup>c, #</sup>, H. Y. Hu <sup>b</sup>, N. P. Hankins <sup>c, \*</sup>, W. E. Huang <sup>a, \*\*</sup>

<sup>a</sup> Department of Engineering Science, University of Oxford, Parks Road, Oxford, OX1 3PJ, United Kingdom

<sup>b</sup> Environmental Simulation and Pollution Control State Key Joint Laboratory, State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (SMARC), School of Environment, Tsinghua University, Beijing 100084, P.R. China

<sup>c</sup> Laboratory of Sustainable Water Engineering, Department of Engineering Science, The University of Oxford, Parks Road, OX1 3PJ, United Kingdom

<sup>#</sup> These authors contributed equally to this work.

<sup>\*</sup> Corresponding author. Email address: [nick.hankins@eng.ox.ac.uk](mailto:nick.hankins@eng.ox.ac.uk) (N. P. Hankins).

<sup>\*\*</sup> Corresponding author. Email address: [wei.huang@eng.ox.ac.uk](mailto:wei.huang@eng.ox.ac.uk) (W. E. Huang)

## **Abstract**

The high cost and energy consumption related to the downstream harvesting and dewatering process is one of the most important bottlenecks limiting the commercial production of microalgal bioenergy. In this study, a novel microalgal biomass harvesting technique has been developed using polymer surfactant aggregates (PSAs). This approach has been applied to three different microalgal strains and two cyanobacterial strains with a recovery efficiency of over 80%. In particular, the recovery efficiency of *Chlorella* sp. ZTY4 with a biomass

Abbreviations: PSA, polymer surfactant aggregates; PAA, poly (acrylic acid); CPC, cetylpyridinium chloride; poly DADMAC, poly (diallyl dimethylammonium) chloride; PEI, poly (ethyleneimine); OD, optical density; TOC, Total organic carbon

concentration of  $1.43 \text{ g}\cdot\text{L}^{-1}$  can be as high as 99.9% using  $360 \text{ mg}\cdot\text{L}^{-1}$  poly (acrylic acid) (PAA) and 4 mM ( $1432 \text{ mg}\cdot\text{L}^{-1}$ ) cetylpyridinium chloride (CPC). In addition, with this PAA and CPC dosage, the recovery efficiency of *Chlorella* sp. ZTY4 remains above 90% for biomass concentrations up to  $2.5 \text{ g}\cdot\text{L}^{-1}$ . Furthermore, the water content in the harvested biomass is below 70% with a corresponding concentration ratio of 231. The total flocculation time needed for this technique is 20 minutes. The optimum dosage ratio for PAA to CPC ranges from 90 to 100 mg/mmol. Based on these results, an efficient harvesting method with a high concentration ratio is proposed to simplify the whole downstream harvesting and dewatering processes of microalgal biomass.

**Keywords:** microalgal bioenergy; harvesting; flocculation; polymer-surfactant aggregates

## 1. Introduction

Microalgal biomass-based bioenergy is considered to be one of the most promising substitutes for fossil fuels due to a series of advantages, including an attractive energy density, compatibility with the existing sophisticated distribution and storage infrastructure, and higher energy production efficiency and lower land demand compared with terrestrial crops [1-3]. Several processes have been developed to convert microalgal biomass into bioenergy products, such as biodiesel, bio-crude oil, biogas and so on. However, regardless of the conversion process, the water content of microalgal biomass must be reduced below at least 90% before being converted into bioenergy [4, 5], which means the biomass in an autotrophic cultivation system must be concentrated by a factor over one hundred. With the decrease of the water content in the harvested biomass, the conversion process would then be much easier and more efficient. Actually, one of the key factors limiting the commercial production of microalgal bioenergy is the high cost related to the downstream harvesting and dewatering processes [6], which typically contributes some 20%-30% to the total cost of the microalgal

biomass production [7]. Microalgal biomass is difficult to recover from the culture, mainly because of: (1) low biomass concentration in the medium (typically around 1 g·L<sup>-1</sup> in an autotrophic cultivation system), (2) the small size of microalgal cells (5-50 µm), (3) their negative surface charge and, (4) the similarity of the density of microalgal cells to the growth medium [8, 9].

Several harvesting and dewatering approaches have been developed for microalgal biomass, including but not limited to micro-filtration, centrifugation, and flocculation followed by sedimentation or air flotation [8, 9]. Micro-filtration and centrifugation are effective in recovering and concentrating microalgal biomass, but are energy-intensive and expensive to operate. These methods are acceptable only when harvested microalgal biomass is used to produce high-value products, such as long-chain polyunsaturated fatty acids (PUFAs), astaxanthin, carotenoid and so on [7, 10]. For low-value bulk products, such as biodiesel, flocculation and flotation are the preferable options. However, there are lots of hydrophilic species on the microalgal cell membrane, such as membrane proteins and polysaccharides, which form a hydration layer. Furthermore, large amounts of water are trapped in the harvested microalgal biomass due to the intercellular capillary effect. During the conventional flocculation or flotation process, the removal of intercellular water in a hydrophilic system is difficult to achieve. As a result, flocculation alone followed by sedimentation or flotation could only achieve a concentration ratio ranging from 5 to 50. Further dewatering processes, such as filter pressing, drum drying and sun drying [9], are required before converting the harvested microalgal biomass to bioenergy. Considering the operational cost, energy consumption and complexity of the current harvesting approaches, it is necessary to develop novel cost-effective and consolidated techniques which can permit a high recovery efficiency of microalgal biomass with a sufficient concentration ratio for further conversion processes.

To achieve this purpose, a novel microalgal harvesting process has been developed using a colloidal structure called polymer-surfactant aggregate (PSA). The cationic surfactant, which contains a hydrophilic and cationic ‘head’ and a hydrophobic ‘tail’, can bind to the microalgal cell membrane via electrostatic and hydrophobic attractions. Due to the electrostatic attraction, the surfactant head is bound to the hydrophilic and negatively charged species of the cell membrane, such as polysaccharides and some proteins. Due to the hydrophobic attraction, the surfactant tail can bind to the hydrophobic parts of the cell membrane as well as to other surfactant tails, thus forming a surfactant bilayer. Both forces of attraction lead to the surfactant headgroups coating the microalgal cell membrane and displacing the associated water. The anionic polymer acts as a flocculant and a backbone for the formation of PSAs. The positively charged surfactant initially binds electrostatically to the negatively charged polymer, which leads to an increase in the local concentration of surfactant in the vicinity of the polymer chain. When the local concentration of surfactant is relatively high, small aggregates start to form onto the polymer chain as the backbone. This process has been successfully applied to recover charged species, such as heavy metal ions and metallic anions, from aqueous solutions [11-14].

There are three main characteristics of the PSA process that can be advantageous for harvesting microalgae: in-situ formation of nano-scale PSAs, self-flocculation and hydrophobicity. Firstly, PSAs contain both positive and negative charges in one structure when they form in-situ in an aqueous solution. Individual nano-scale PSAs contain a high surface to volume ratio, which can effectively bind to microalgal cells. In addition, when the microalgal cells are bound to PSAs, they also associate inter-cellularly with each other to form large flocs. This subsequently leads to self-flocculation of the microalgae loaded PSAs, forming visible-size flocs. The flocs can be easily recovered by coarse filtration, which

obviates the need for an expensive membrane process or the relative long residence time for a sedimentation process. Lastly, when the microalgae-loaded PSAs flocculate, the polymer shrinks to form compacted flocs. The formation of a hydrophobic surfactant coating layer on the microalgal membrane cell also squeezes the intercellular water out of the flocs. Both effects reduce the amount of final intercellular water in the harvested algal biomass.

In this paper, 6 different microalgal strains, including *Synechococcus* sp. PCC 7002, *Microcystis aeruginosa*, *Scenedesmus* sp. LX1, *Chlorella* sp. ZTY4, *Selenastrum capricornutum* and *Chlamydomonas reinhardtii*, were used to test the applicability of the PSA process. *Chlorella* sp. ZTY4 was selected for detailed investigation because this strain could grow mixotrophically using different kinds of wastewater as a resource and synchronously accumulate lipids within the cells [15, 16]. Comparative studies in recovery efficiency and concentration ratio were conducted between the PSA process and several conventional flocculation methods using cationic polymers, the aluminum ion and ferric ion. The dosage between polymer and surfactant was optimised, based on previous application in the treatment of dilute solutions of metallic ions [13]. The effects of mixing time and cell density were examined to evaluate the potential industrial applicability. Based on the results, an efficient microalgal biomass harvesting method with a high concentration ratio was proposed.

## **2. Materials and methods**

### **2.1 Microalgal strain**

The strains used in this study are listed in Table 1.

All the microalgal strains except *Synechococcus* sp. PCC 7002 were maintained in a liquid BG11 medium as well as on agar plates containing BG11 medium in an artificial climate chamber (Incubators SI60, Stuart Equipment). *Synechococcus* sp. PCC 7002 was cultivated in A plus medium.

Colonies picked from the agar plate were first cultured in 200 mL of liquid BG11 medium (A

plus medium for *Synechococcus* sp. PCC 7002) in an artificial climate chamber (Incubators SI60, Stuart Equipment) until the end of the initial growth phase (cultivated for 7 days). After this, a 10 mL sample of pre-cultivated algal culture was inoculated into 400 mL of BG11 medium (A plus medium for *Synechococcus* sp. PCC 7002) in a 500 mL Duran bottle with filtered air-bubbling. The cultivation conditions were as follows: light intensity  $70 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  to  $80 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ; light/dark ratio 14 h:10 h, and temperature  $25^\circ\text{C}$  ( $30^\circ\text{C}$  for *Synechococcus* sp. PCC 7002). After being cultivated for 15 days, the microalgal culture was used to test the harvesting methods.

## **2.2 Harvesting microalgal biomass by the PSA process**

Poly (acrylic acid) (PAA) was prepared by diluting stock PAA solution (Sigma Aldrich, average MW  $\sim 250,000$ , 35 wt.% in  $\text{H}_2\text{O}$ ). A stock solution of 1 M cetylpyridinium chloride (CPC) was prepared using the powder (purity  $\geq 99\%$ , purchased from Sigma Aldrich UK). A calculated amount of PAA and CPC solutions were added to an algal culture with a known cell density (described in detail in the results). The solution was stirred by a magnetic stir bar at 200 rpm for a period of time (investigated in detail in this study) to achieve equilibrium, which was indicated by a transparent solution with precipitates at the bottom of the flask. After the equilibrium was achieved, these solutions were then coarse-filtered by a  $100 \mu\text{m}$  filter. The dosage was optimised for a fixed amount of CPC with different amounts of PAA. Microalgal recovery and polymer/surfactant usage efficiencies were the primary criteria for the optimal dosage. The effects of stirring time and initial microalgal biomass concentration on the recovery efficiency were also investigated.

All solutions were filtered by a Millipore model 8050 dead-end filtration cell. The feed passed through the  $100 \mu\text{m}$  filter under gravity, such that entities larger than the filter pore size, i.e. polymer–surfactant precipitates together with the bound microalgal cells, were retained in the

filtration cell, while free cells, individual surfactant monomers and polymers which were smaller than the pore size passed into the collection vessel.

The experiments were carried out in triplicate (3 independent replications).

### **2.3 Harvesting microalgal biomass by conventional flocculation methods**

In order to compare the PSA process with a conventional flocculation method, different types of flocculants were used to harvest *Chlorella* sp. ZTY4, including ferric chloride, aluminium sulfate, poly (diallyl dimethylammonium) chloride (polyDADMAC) and poly (ethyleneimine) (PEI). PolyDADMAC and PEI solutions were prepared by diluting stock polyDADMAC solution (Sigma Aldrich, average MW <100,000, 35 wt.% in H<sub>2</sub>O) and PEI solution (Sigma Aldrich, average MW ~ 750,000, 50 wt.% in H<sub>2</sub>O) , respectively. In the comparative study, the dosage of Fe<sup>3+</sup> and Al<sup>3+</sup> was 1 mM and 2 mM, respectively, and the dosage of both polyDADMAC and PEI were 360 mg·L<sup>-1</sup>. These dosages were selected by preliminary experiments (data not shown) to achieve higher microalgal biomass recovery efficiency. After the addition of a certain amount of flocculant (as mentioned above) into the microalgal culture, the solution was stirred by a magnetic stir bar at 150 rpm for 3 min, then at 50 rpm for 17 min, and then allowed to settle for 20 min. After that, a sample was taken from the supernatant to measure the microalgal biomass concentration remaining in the culture after harvesting. The settled microalgal biomass was collected via a liquid separating funnel and used to determine the water content. Besides gravitational settling, coarse filtration was also attempted in order to harvest the microalgal biomass after flocculation.

All the experiments were carried out in triplicate (3 independent replications).

### **2.4 Analytical methods**

For all the microalgal strains used, the optical density (OD) method was used to determine the biomass concentration. The absorbance at a wavelength of 650 nm (OD<sub>650</sub>) for all the culture except *Synechococcus* sp. PCC 7002 was measured by a microplate reader (Synergy

HT, BioTek). For *Synechococcus* sp. PCC 7002, OD<sub>730</sub> was used. For *Chlorella* sp. ZTY4, the cell counting method using a counting chamber and the filter membrane method was used as well. Forty mL of culture was filtered by a pre-weighed 0.22 µm membrane filter, and then the filter was dried at 60 °C until reaching a constant weight. The relationship between the biomass concentration of *Chlorella* sp. ZTY4 and the OD<sub>650</sub> value was established, and is shown as follow:

$$N=3.18 \times OD_{650}, R^2=0.93 \quad (1)$$

where  $N$  (g·L<sup>-1</sup>) is the microalgal biomass concentration.

The calibration curve is shown in the supplementary material.

The total carbon content of solutions was measured by a total organic carbon analyser (TOC–VCPH, Shimadzu) to indirectly measure the degree of flocculation and de-flocculation. A reduction in the total carbon in the filtrate indicated that the PAA and CPC had formed precipitates or colloids which had subsequently been filtered out. Before the filtrate was used to determine the TOC content, it was filtered by a 0.45 µm membrane to remove free microalgal cells. Assuming that the volume of solution before and after treatment is consistent, the polymer-surfactant usage efficiency and microalgal recovery efficiency are defined by Equations 2 and 3, respectively.

$$u=(1-\frac{C_F}{C_A}) \times 100\% \quad (2)$$

where  $u$  (%) is the polymer-surfactant usage efficiency,  $C_F$  (mg·L<sup>-1</sup>) is the total organic carbon content in the filtrate, and  $C_A$  (mg·L<sup>-1</sup>) is the total organic carbon content added into the solution.



$$r = (1 - \frac{B_R}{B_O}) \times 100\% \quad (3)$$

where  $r$  (%) is the microalgal biomass recovery efficiency,  $B_R$  is the biomass concentration remaining in the culture after harvest,  $B_O$  is the biomass concentration in the original culture.

The water content of the original culture and the harvested microalgal biomass were measured by conventional methods. Microalgal culture or the harvested microalgal biomass were put into a pre-weighed weighing boat, and then the boat was dried at 60 °C to a constant weight. The water content could be calculated based on the change in the weight. The concentration ratio of the harvesting process could be calculated by Equation 4:

$$\eta = \frac{1 - w_H}{1 - w_O} \quad (4)$$

where  $\eta$  is the concentration ratio,  $w_H$  (%) is the water content of the harvested microalgal biomass, and  $w_O$  (%) is the water content of the original culture.

The determinations were carried out in triplicate (3 independent replications).

## 2.5 Statistical analysis

All statistical analysis was carried out using SPSS statistics 13.0. Independent-Samples T test were used for significant difference analysis.

## 3. Results

### 3.1 Recovery of different microalgal strains using polymer-surfactant aggregates

The recovery efficiency of different microalgal strains using the PSA process is shown in Fig.

1. Four microalgal strains and two cyanobacteria, each of different phylum and genus, were used to test the universal applicability of the PSA process. After 15-day cultivation, the OD<sub>650</sub> of *Chlamydomonas reinhardtii*, *Scenedesmus* sp. LX1, *Chlorella* sp. ZTY4, *Microcystis aeruginosa* and *Selenastrum capricornutum* was 0.30, 0.59, 0.41, 0.44, and 0.31 respectively.

The OD<sub>730</sub> of *Synechococcus* sp. PCC 7002 was 1.18. Although the recovery efficiency of

*Scenedesmus* sp. LX1 was 70%, the recovery efficiencies of the other five strains were all greater than 80%, which was higher than the results achieved by some other methods reported so far, such as flocculation by some metal salts or chitosan [17, 18]. With regard to *Chlorella* sp. ZTY4 and *Synechococcus* sp. PCC 7002, the recovery efficiencies were even as high as 96%. The variation in the performance of the PSA process in treating different microalgal strains may be due to the difference in the initial microalgal biomass concentration, the surface charge of the cells, and the sub-optimised chemical dosage (400 mg·L<sup>-1</sup> PAA and 4 mM/1432 mg·L<sup>-1</sup> CPC). With further optimization of the chemical dosage, the recovery efficiency of *Scenedesmus* sp. LX1 and of *Chlamydomonas reinhardtii* was expected to be higher. These results demonstrate that the PSA process has great potential to be applied as a universal method in microalgal biomass harvesting.

The cells of different microalgal strains vary in shape and size, but the surface of the cells are usually negatively charged. The latter is due to the presence of proteins and polysaccharides on the cell membrane, and the alkaline pH of most microalgal cultures (pH>7) which is also higher than the isoelectric points of these compounds. As previously reported, PSAs were efficient in absorbing negatively charged particles [13, 14], and therefore this process could be used to harvest microalgal biomass.

### **3.2 Comparison between the polymer-surfactant aggregate process and conventional flocculation methods in recovering *Chlorella* sp. ZTY4**

In previous studies, *Chlorella* sp. ZTY4 grew well in both domestic wastewater and reverse osmosis concentrate, showing promising abilities for pollutant removal as well as lipid accumulation [15, 16]. This strain, therefore, was selected for further investigation. Ferric chloride and aluminium sulfate have commonly been used in wastewater treatment and microalgal biomass harvesting [8, 9]. Cationic polyelectrolytes were considered as the most effective flocculants for the recovery of microalgal biomass by some researchers [19]. Hence,

the performance of two cationic polyelectrolytes, PolyDADMAC and PEI, was also examined.

The recovery efficiency of *Chlorella* sp. ZTY4 using the PSA process and the four conventional flocculants is shown in Fig. 2. When the conventional flocculants were used to harvest *Chlorella* sp. ZTY4 from the culture, the recovery efficiency was below 70%, whereas the recovery efficiency using the PSA process was  $96 \pm 0.2\%$  (Fig. 2).

In addition to the high recovery efficiency, another advantage of the PSA process was that the biomass harvested by this technique was highly concentrated. The water content of the microalgal biomass harvested by the PSA process and by conventional flocculants and the corresponding concentration ratio are shown in Figs. 3 *a* and *b*, respectively. Before the harvesting process, the initial biomass concentration in the culture was  $1.43 \text{ g}\cdot\text{L}^{-1}$  with a water content as high as 99.8%. When recovered by conventional flocculation and sedimentation, the water content of the harvested microalgal biomass was still over 99%, and the corresponding concentration ratio was below 10. In the case of aluminium sulfate used as the flocculant, the water content of the harvested microalgal biomass drops to 90% with a concentration ratio of 69 when the filtration method was also used to recover the microalgal biomass flocs. However, the flocs formed by the conventional flocculation process were loose and fragile. When filtered by a coarse filter, it was observed that lots of the flocs were crushed and subsequently went through the filter. Hence, only 60-70% of the flocs were recovered by the coarse filter. This result suggests that coarse filtration might not be suitable for recovery of the biomass flocs after conventional flocculation. But by comparison, the flocs formed by the PSA process were so compact that they could be easily recovered by a coarse filter without any leakage. The water content of the harvested biomass was  $66.6 \pm 7.5\%$  with a concentration ratio as high as  $231.3 \pm 45.9$  (Fig. 3). These results indicated that no dewatering process was needed for the microalgal biomass harvested by the PSA process

because it was already suitable for further conversion processes, such as lipid extraction [4] or hydrothermal liquefaction [5].

Granados et al. [18] reported the performance of several flocculants for the recovery of microalgal biomass. Similar to this study, metal salts were found to be inefficient (the recovery efficiency was below 40%) whereas cationic polyelectrolytes, such as EM1, EM 16 and EM22, could achieve a recovery efficiency over 90%. However, in all cases investigated by Granados et al., the concentration ratio was reported to be below 40, which was nearly 5 times lower than that of the PSA process (Fig 3b). A concentration ratio as high as 200-800 was achieved using a non-ionic polymer Madnafloc LT-25 after the adjustment of pH to between 10 and 10.6 [20]. This method needed a long settling time (over 24 h) to obtain such a high concentration ratio. The adjustment of pH, nevertheless, may not be a cost-effective choice at a large scale. Furthermore, it was unclear whether these flocs could be strong enough to be recovered by coarse filtration.

### **3.3 Optimization of the polymer-surfactant aggregate process for industrial application**

In order to further investigate the potential of industrial application, the PSA process was optimised in this study using *Chlorella* sp. ZTY4 as a testing strain. The dosage of PAA was optimised at 1 mM (358 mg·L<sup>-1</sup>) CPC and 4 mM (1432 mg·L<sup>-1</sup>) CPC, and the results are shown in Figs. 4 *a* and *b*, respectively.

In Fig 4 *a*, the optimum dosage for 1 mM (358 mg·L<sup>-1</sup>) CPC was 100 mg·L<sup>-1</sup> PAA, which was determined by the highest algal recovery efficiency (92%) and a good polymer/surfactant usage efficiency (78%). In fact, with a small variation in the polymer dosage, the performance of the PSA process was stable. This means that a margin for error in the dosage is allowed in industrial applications, which decreases the operational difficulty.

To enhance the performance of algal harvesting and understand the optimum dosage ratio between CPC and PAA, the chemical dosage was also optimised for 4 mM (1432 mg·L<sup>-1</sup>) CPC, and the results are shown in Fig 4 b. A PAA final concentration of 360 mg·L<sup>-1</sup> was found to yield the best performance: 97% of microalgal recovery efficiency and 98% of polymer/surfactant usage efficiency. A slightly higher recovery efficiency (98%) was found at 440 mg·L<sup>-1</sup> PAA, but it may be not cost-effective to spend 20% more on polymer dosage for a 1% increase in recovery efficiency. It was worth noting that all the optimum ratios for polymer to surfactant were around 90-100 mg/mmol. According to the results mentioned above, 1 mM (358 mg·L<sup>-1</sup>) CPC and 100 mg·L<sup>-1</sup> PAA were enough for the microalgal biomass concentration of a typical autotrophic cultivation system (about 1-1.5 g·L<sup>-1</sup> microalgal biomass). The higher overall dosage usually offered a better recovery efficiency, because a larger amount of PSAs were available to flocculate microalgal cells. However, it was also important to note that a higher chemical cost was associated with a higher dosage, even if the polymer and surfactant were potentially recyclable [12, 14]. Upon further expanding the range of optimum dosage ratios, there was less than a 10% decrease in the recovery performance. This suggested that there was a reasonable flexibility in the dosage ratio between polymer and surfactant, which was beneficial for industrial applications. Besides recovery efficiency and polymer/surfactant usage efficiency, the effect of chemical dosage on the water content in harvested biomass was also investigated. With the change of the PAA and CPC dosages, the water content in all the harvested microalgal biomass was between 60% and 70%. The effect of chemical dosage on the water content was thus found to be insignificant.

After understanding the range of optimum dosage, another important factor in field applications was the effect of mixing time. The effect of mixing time on the recovery efficiency of *Chlorella* sp. ZTY4 and polymer and surfactant usage efficiency are shown in

Fig. 5. The results suggest that most of the microalgal biomass was recovered by the PSA process in 20 min. The quick flocculation process implies low residence time and allows the design of small mixing tanks, which in turn lowers the capital cost and footprint of the plant. Alternatively, the treatment capacity can be increased for the same size of mixing tanks. Some other approaches which could achieve a similar concentration ratio as the PSA process were proposed for microalgal biomass harvesting, using a non-ionic polymer Madnafloc LT-25 [20] or extracellular polymeric substances produced by flocculating microbes [21]. However, these methods required either a long settling time (over 24 h) or a long mixing time (24 h).

In Fig 5, the results show that 90% of the PAA and CPC was flocculated within 1 min and the rest was flocculated within 15 min. When the PAA and CPC had not been fully flocculated, the solution was cloudy. The reason was that the repulsion of electrical double layers between PSAs was stronger than the van der Waals attraction forces, which resulted in the PSAs presenting in a colloidal status. As a consequence, the colloids were also considered as unrecovered microalgal biomass by the optical density method, which significantly reduced the actual recovery efficiency of microalgae. A direct counting method was applied to mitigate this problem. Most importantly, the results showed that within 20 min 99.9% of the microalgal biomass was harvested. The optical density method verified that microalgal biomass was fully flocculated by PAA and CPC.

In industrial applications, the microalgal biomass concentration may vary when harvesting. Thus it is essential to understand the range of microalgal biomass concentrations that one dosage can cope with. The effect of microalgal biomass concentration on the recovery efficiency is shown in Fig. 6. The recovery efficiency remained above 90% when the biomass concentration was below  $2.5 \text{ g}\cdot\text{L}^{-1}$ , which was relatively high in an autotrophic cultivation system. In actual applications, therefore, the optimum dosage for 4 mM ( $1432 \text{ mg}\cdot\text{L}^{-1}$ ) CPC

was probably the maximum dosage required. With regard to a more dilute system, the dosage can be significantly reduced as long as the ratio between PAA and CPC is kept around 90-100 mg/mmol.

## **4. Discussion**

### **4.1 Mechanism of decreasing the water content in the harvested microalgal biomass by the polymer-surfactant aggregate process**

The difference between the PSA process and conventional flocculation is shown in Fig. 7. In the conventional flocculation process, the main mechanisms for the recovery of microalgae include electric double-layer compression, adsorption and charge neutralization, adsorption and inter-particle bridging and enmeshment in a precipitate. In the PSA process, electrostatic and hydrophobic interactions are supposed to be the dominant forces in recovering microalgae. In the vicinity of the polymer chain, the oppositely charged surfactant monomers enrich and form aggregates onto the polymer chains. Once the PSA forms in the solution, each surfactant aggregate can electrostatically bind to negatively charged microalgal cells. PSA-cells and PSA-PSA binding results in the formation of large flocs. In addition, the surfactant monomers can also electrostatically bind individually to the surface of the microalgal cell. The cell surface becomes somewhat less negatively charged and more hydrophobic, and therefore has a strong tendency to aggregate and flocculate. Therefore, the PSA process achieves a much higher recovery efficiency than the conventional methods.

As mentioned above, lots of hydrophilic species present on the microalgal cell membrane, such as membrane proteins and polysaccharides, forming a hydration layer. During the conventional flocculation process, the removal of the hydration layer is difficult to achieve by a hydrophilic-based treatment technique. Furthermore, a large amount of water is trapped in the flocs due to the intercellular capillary effect. The bonding force between microalgal cells and flocculants as well as the bonding force between the microalgal cells themselves is

weakened by the relatively thick intercellular water layer. Therefore, the flocs formed by conventional flocculation are more fragile and thus more difficult to harvest and process.

On the contrary, during the PSA process, the surfactants can either form bilayer or single layer on the microalgal cell membrane via electrostatic and hydrophobic attractions with the head groups facing towards the culture. Both mechanisms displace the water from the vicinity of microalgal cells, which significantly reduces the amount of intercellular water present in the harvested biomass. As the thickness of the intercellular water layer decreases, the van der Waals' forces between individual microalgal cell and flocs becomes much stronger. Meanwhile, with careful control of the dosage between oppositely charged polymer and surfactant, the surface charge of microalgal cells and small flocs can be minimised and allow the dominance of the van der Waals's force. As a result, the flocs formed by the PSA process have a low water content and a strong mechanical strength. These two characteristics are beneficial for the further handling and conversion of the harvested microalgal biomass for biofuel production.

#### **4.2 The advantages of the polymer-surfactant aggregate process in harvesting microalgal biomass for bioenergy production**

Due to the fact that the water content of the biomass harvested by the PSA process is lower than 70% and the flocs can be recovered by coarse filtration, the whole downstream harvesting and dewatering process of microalgal biomass can be simplified significantly by this technology. Currently, the most feasible process to harvest microalgal biomass for bioenergy production is flocculation and sedimentation followed by a further dewatering process [8]. The whole process is complex and has to include some energy-intensive processes such as centrifugation and filter pressing, which may lead to negative energy production from the point of view of life-cycle assessment [4]. On the other hand, if the PSA process was applied to recover microalgal biomass, neither a sedimentation tank nor a further



dewatering process would be needed. The flocs of the biomass could be easily recovered from the coarse filter, and directly used for further conversion. Compared with micro-filtration, the pore size of a coarse filter is two orders of magnitude larger, which means coarse filtration is a much faster and more energy-saving choice when the flocs are large enough to be recovered. In addition, cleaning of the cake layer is easier and fouling is more reversible for coarse filtration than for micro-filtration [22].

## 5. Conclusions

A novel microalgal biomass harvesting technique was developed using the polymer surfactant aggregate (PSA) process. The technique was applicable to 6 different strains of microalgae and has three main advantages: a high recovery efficiency, a high concentration ratio and a short flocculation time.

Firstly, the recovery efficiency of *Chlorella* sp. ZTY4 can be as high as 99.9% using 360 mg·L<sup>-1</sup> PAA and 4 mM (1432 mg·L<sup>-1</sup>) CPC. The reasons for such a high recovery efficiency are that the individual nano-sized PSAs form in-situ in the culture and contain both positive and negative charges. These characteristics allow the PSAs to effectively bond to negatively charged microalgal cells and form large flocs via the bridging effect between polymers and microalgae. In addition, the surfactant can also displace the associated water in the vicinity of the microalgal cell membrane. Consequently, the flocs contains a smaller amount of intercellular water and this leads to a low water content below 70%. This can obviate the need for subsequent dewatering processes, and the harvested biomass could be directly used for bioenergy production. Finally, the flocculation time for harvesting microalgae is less than 20 minutes which significantly increases the operational speed and reduces the footprint of recovery capacity. The optimum dosage ratio for PAA to CPC ranges from 90 to 100 mg/mmol. The recovery efficiency of *Chlorella* sp. ZTY4 remains above 90% for biomass concentrations below 2.5 g·L<sup>-1</sup> using 360 mg·L<sup>-1</sup> PAA and 4 mM (1432 mg·L<sup>-1</sup>) CPC. A

lower biomass concentration requires a lower total dosage as long as the PAA and CPC ratio remains in the optimum ratio.

In conclusion, this novel microalgal harvesting process has shown great potential to simplify the whole downstream harvesting and dewatering process of microalgal biomass.

### **Authors' contribution**

Y. H. Wu and L. C. Shen developed the conception and design of the study, acquired the data, drafted the article and participated in final approval of the version to be submitted; H. Y. Hu participated in the analysis and interpretation of data, revising the article critically for important intellectual content and final approval of the version to be submitted; N. P. Hankins participated in the design of the study, the analysis and interpretation of data, revising the article critically for important intellectual content and final approval of the version to be submitted; W. E. Huang participated in the design of the study, the analysis and interpretation of data, revising the article critically for important intellectual content and final approval of the version to be submitted, and will take responsibility for the integrity of the work as a whole, from inception to finished article.

### **Acknowledgement**

This study is supported by the International Postdoctoral Exchange Fellowship Program of China (【2015】38). The authors thank Tianyuan Zhang, Xiaoxiong Wang and Guangyu Chen for providing the microalgal strains.

### **References**

- [1] Y. Chisti, Biodiesel from microalgae, *Biotechnol. Adv.*, 25 (2007) 294-306.
- [2] R.H. Wijffels, M.J. Barbosa, An Outlook on Microalgal Biofuels, *Science*, 329 (2010) 796-799.

- [3] S.R. Medipally, F.M. Yusoff, S. Banerjee, M. Shariff, Microalgae as Sustainable Renewable Energy Feedstock for Biofuel Production, *BioMed Research International*, 2015 (2015) 13.
- [4] L. Lardon, A. Helias, B. Sialve, J.-P. Stayer, O. Bernard, Life-Cycle Assessment of Biodiesel Production from Microalgae, *Environ. Sci. Technol.*, 43 (2009) 6475-6481.
- [5] Y. Zhou, L. Schideman, G. Yu, Y. Zhang, A synergistic combination of algal wastewater treatment and hydrothermal biofuel production maximized by nutrient and carbon recycling, *Energy & Environmental Science*, 6 (2013) 3765-3779.
- [6] D.R. Georgianna, S.P. Mayfield, Exploiting diversity and synthetic biology for the production of algal biofuels, *Nature*, 488 (2012) 329-335.
- [7] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: A review, *Renew. Sust. Energ. Rev.*, 14 (2010) 217-232.
- [8] J.J. Milledge, S. Heaven, A review of the harvesting of micro-algae for biofuel production, *Reviews in Environmental Science and Bio/Technology*, 12 (2013) 165-178.
- [9] E.M. Grima, E.H. Belarbi, F.G.A. Fernandez, A.R. Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics, *Biotechnol. Adv.*, 20 (2003) 491-515.
- [10] R. Harun, M. Singh, G.M. Forde, M.K. Danquah, Bioprocess engineering of microalgae to produce a variety of consumer products, *Renew. Sust. Energ. Rev.*, 14 (2010) 1037-1047.
- [11] L.-C. Shen, X.-T. Nguyen, N.P. Hankins, Removal of heavy metal ions from dilute aqueous solutions by polymer–surfactant aggregates: A novel effluent treatment process, *Sep. Purif. Technol.*, 152 (2015) 101-107.
- [12] L.C. Shen, A. Lo, X.T. Nguyen, N.P. Hankins, Recovery of heavy metal ions and recycle of removal agent in the polymer–surfactant aggregate process, *Sep. Purif. Technol.*, 159 (2016) 169-176.

- [13] L.C. Shen, J. Wu, S. Singh, N.P. Hankins, Removal of metallic anions from dilute aqueous solutions by polymer–surfactant aggregates, *Desalination*, 406 (2017) 109-118.
- [14] L.-C. Shen, N.P. Hankins, R. Singh, Chapter 10 - Surfactant and Polymer-Based Technologies for Water Treatment, *Emerging Membrane Technology for Sustainable Water Treatment*, Elsevier, Boston, 2016, pp. 249-276.
- [15] X.-X. Wang, Y.-H. Wu, T.-Y. Zhang, X.-Q. Xu, G.-H. Dao, H.-Y. Hu, Simultaneous nitrogen, phosphorous, and hardness removal from reverse osmosis concentrate by microalgae cultivation, *Water Res.*, 94 (2016) 215-224.
- [16] T.-Y. Zhang, Y.-H. Wu, S.-f. Zhu, F.-M. Li, H.-Y. Hu, Isolation and heterotrophic cultivation of mixotrophic microalgae strains for domestic wastewater treatment and lipid production under dark condition, *Bioresour. Technol.*, 149 (2013) 586-589.
- [17] J.-Q. Jiang, N.J.D. Graham, C. Harward, Comparison of Polyferric Sulphate with Other Coagulants for the Removal of Algae and Algae-Derived Organic Matter, *Water Sci. Technol.*, 27 (1993) 221-230.
- [18] M.R. Granados, F.G. Acién, C. Gómez, J.M. Fernández-Sevilla, E. Molina Grima, Evaluation of flocculants for the recovery of freshwater microalgae, *Bioresour. Technol.*, 118 (2012) 102-110.
- [19] N. Uduman, Y. Qi, M.K. Danquah, G.M. Forde, A. Hoadley, Dewatering of microalgal cultures: A major bottleneck to algae-based fuels, *Journal of Renewable and Sustainable Energy*, 2 (2010) 012701.
- [20] R.M. Knuckey, M.R. Brown, R. Robert, D.M.F. Frampton, Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds, *Aquac. Eng.*, 35 (2006) 300-313.

- [21] A.K. Lee, D.M. Lewis, P.J. Ashman, Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel, *J. Appl. Phycol.*, 21 (2009) 559-567.
- [22] M. Rickman, J. Pellegrino, R. Davis, Fouling phenomena during membrane filtration of microalgae, *J. Membr. Sci.*, 423–424 (2012) 33-42.
- [23] X. Li, H.-Y. Hu, J. Yang, Lipid accumulation and nutrient removal properties of a newly isolated freshwater microalga, *Scenedesmus* sp LX1, growing in secondary effluent, *New Biotechnol.*, 27 (2010) 59-63.

## Figure Captions

Figure 1 Recovery of different microalgal strains using polymer-surfactant aggregates. Data presented are of means  $\pm$  SD of three biological replicates.

Figure 2 Comparison in recovery efficiency between the polymer-surfactant aggregate process and conventional flocculation methods in recovering *Chlorella* sp. ZTY4 (initial biomass concentration was  $1.43 \text{ g}\cdot\text{L}^{-1}$ ). Data presented are of means  $\pm$  SD of three biological replicates.

Fig. 3 Comparison in the water content of harvested biomass (*a*) and concentration ratio (*b*) between the polymer-surfactant aggregate process and other conventional methods via sedimentation or filtration methods. Data presented are of means  $\pm$  SD of three biological replicates.

Figure 4 Dosage optimisation for recovering *Chlorella* sp. ZTY4 (initial biomass concentration was  $1.43 \text{ g}\cdot\text{L}^{-1}$ ) at (*a*)  $1 \text{ mM}$  ( $358 \text{ mg}\cdot\text{L}^{-1}$ ) CPC and (*b*)  $4 \text{ mM}$  ( $1432 \text{ mg}\cdot\text{L}^{-1}$ ) CPC with varying polymer concentrations. Data presented are of means  $\pm$  SD of three biological replicates.

Figure 5 The effects of mixing time on the recovery efficiency of *Chlorella* sp. ZTY4 (initial biomass concentration was  $1.43 \text{ g}\cdot\text{L}^{-1}$ ) and polymer and surfactant usage efficiency at  $360 \text{ mg}\cdot\text{L}^{-1}$  PAA and  $4 \text{ mM}$  ( $1432 \text{ mg}\cdot\text{L}^{-1}$ ) CPC. Data presented are of means  $\pm$  SD of three biological replicates.

Figure 6 Recovery efficiency of *Chlorella* sp. ZTY4 at varying biomass concentration using  $360 \text{ mg}\cdot\text{L}^{-1}$  PAA and  $4 \text{ mM}$  ( $1432 \text{ mg}\cdot\text{L}^{-1}$ ) CPC. Data presented are of means  $\pm$  SD of three biological replicates.

Figure 7 The difference between the PSA process and conventional flocculation.



## Tables

Table 1 Microalgal strains used in this study

Strain	Description	Reference
<i>Synechococcus</i> sp. PCC 7002	Cyanobacteria	*
<i>Microcystis aeruginosa</i>	Cyanobacteria	*
<i>Scenedesmus</i> sp. LX1	Originally isolated by Li et al. from tap water	[23]
<i>Chlorella</i> sp. ZTY4	Isolated from the primary sedimentation tank of a wastewater treatment plant in Beijing, and now is identified by the 18S-rRNA method, named as <i>Chlorella pyrenoidosa</i> THUZTY1304, and preserved in China General Microbiological Culture Collection Center, CGMCC (No. 12521)	[16]
<i>Selenastrum capricornutum</i>	Microalgae	*
<i>Chlamydomonas reinhardtii</i>	Microalgae	*

\* Freshwater Algae Culture Collection of the Institute of Hydrobiology in Chinese Academy of Science (Wuhan, China).



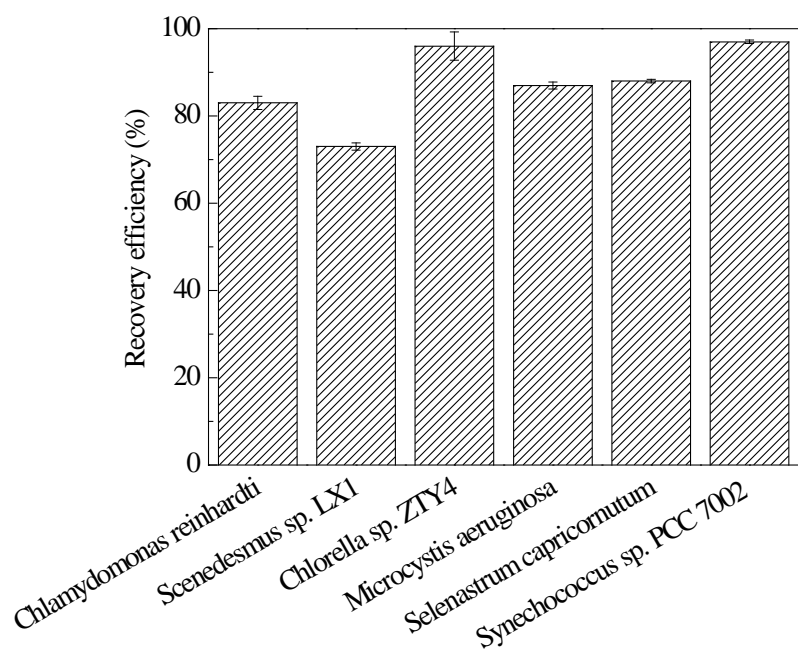


Figure 1 Recovery of different microalgal strains using polymer-surfactant aggregates. Data presented are of means  $\pm$  SD of three biological replicates.

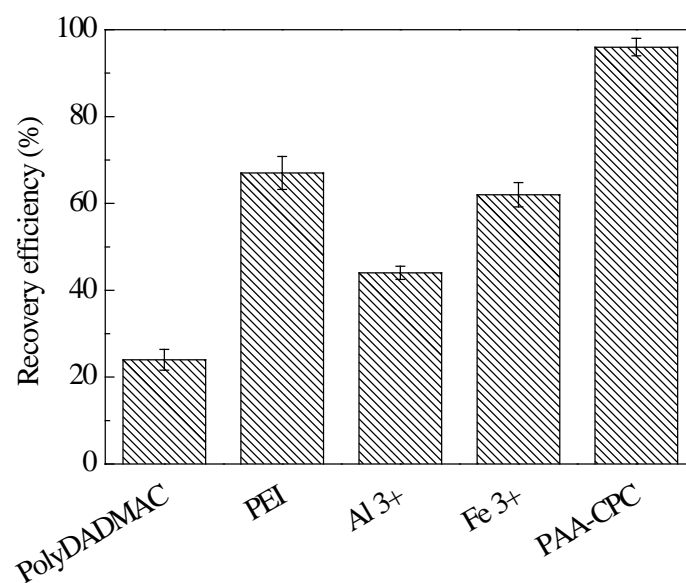


Figure 2 Comparison in recovery efficiency between the polymer-surfactant aggregate process and conventional flocculation methods in recovering *Chlorella* sp. ZTY4 (initial biomass concentration was  $1.43 \text{ g} \cdot \text{L}^{-1}$ ). Data presented are of means  $\pm$  SD of three biological replicates.

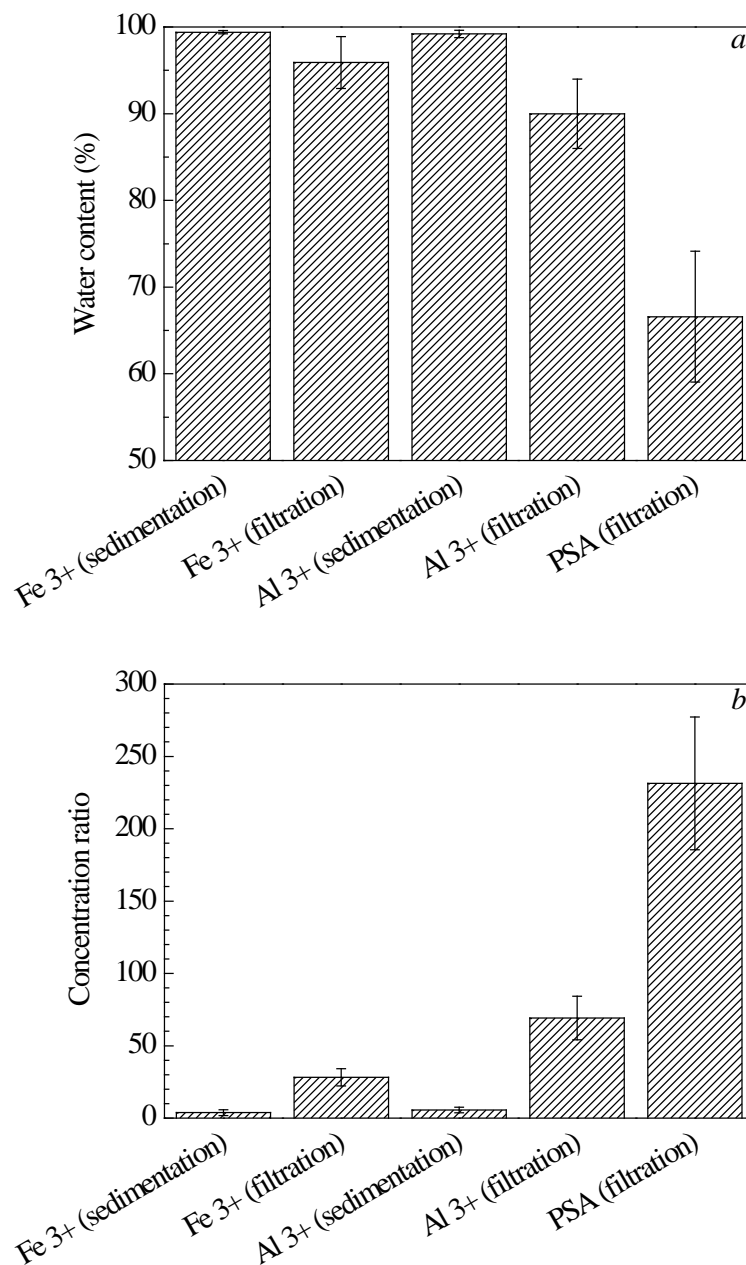


Fig. 3 Comparison in the water content of harvested biomass (*a*) and concentration ratio (*b*) between the polymer-surfactant aggregate process and other conventional methods via sedimentation or filtration methods. Data presented are of means  $\pm$  SD of three biological replicates.

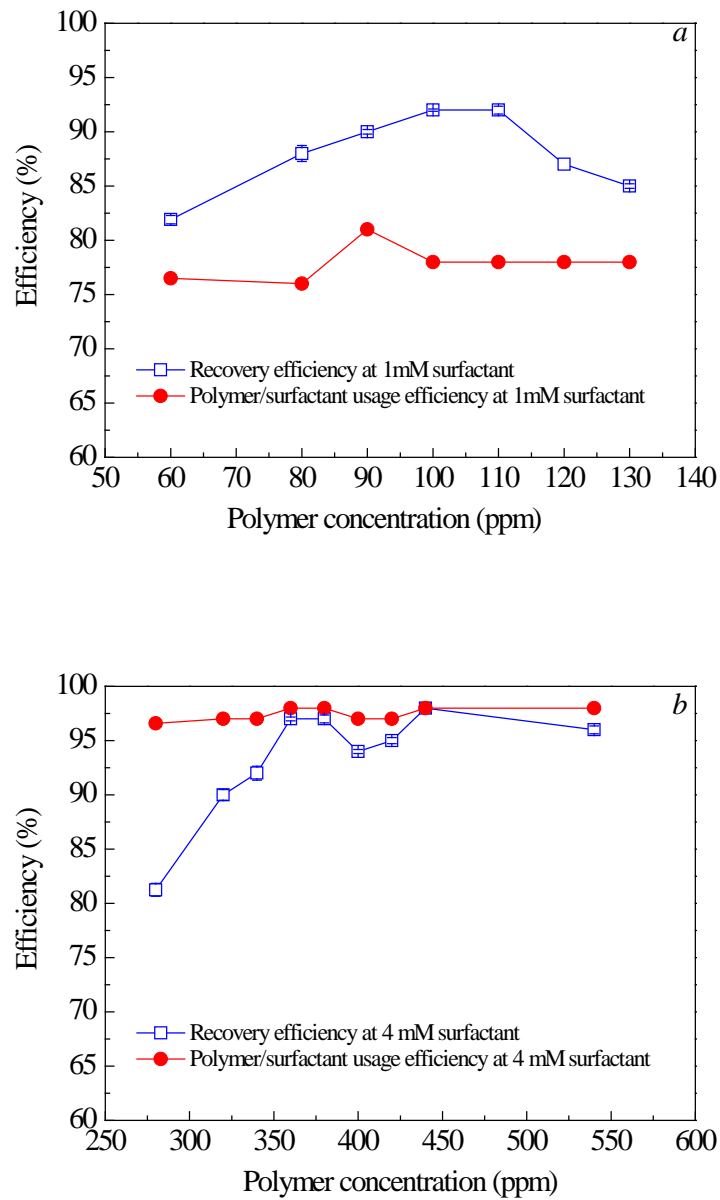


Figure 4 Dosage optimisation for recovering *Chlorella* sp. ZTY4 (initial biomass concentration was  $1.43 \text{ g} \cdot \text{L}^{-1}$ ) at (a) 1 mM ( $358 \text{ mg} \cdot \text{L}^{-1}$ ) CPC and (b) 4 mM ( $1432 \text{ mg} \cdot \text{L}^{-1}$ ) CPC with varying polymer concentrations. Data presented are of means  $\pm$  SD of three biological replicates.

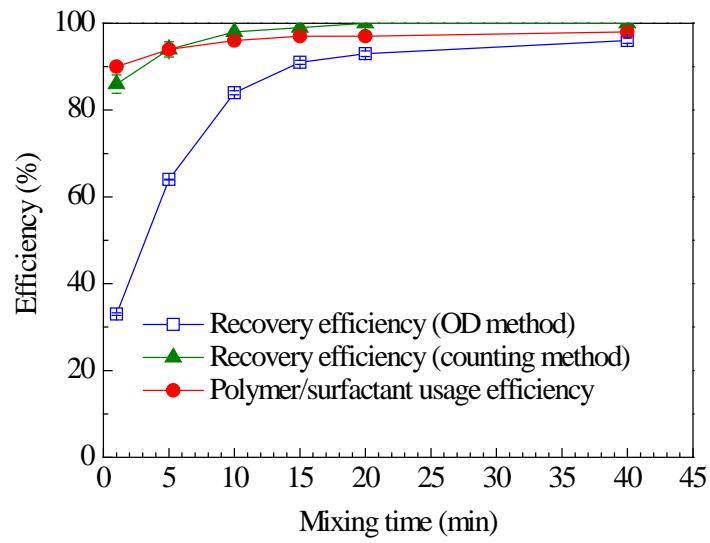


Figure 5 The effects of mixing time on the recovery efficiency of *Chlorella* sp. ZTY4 (initial biomass concentration was  $1.43 \text{ g} \cdot \text{L}^{-1}$ ) and polymer and surfactant usage efficiency at  $360 \text{ mg} \cdot \text{L}^{-1}$  PAA and  $4 \text{ mM}$  ( $1432 \text{ mg} \cdot \text{L}^{-1}$ ) CPC. Data presented are of means  $\pm$  SD of three biological replicates.

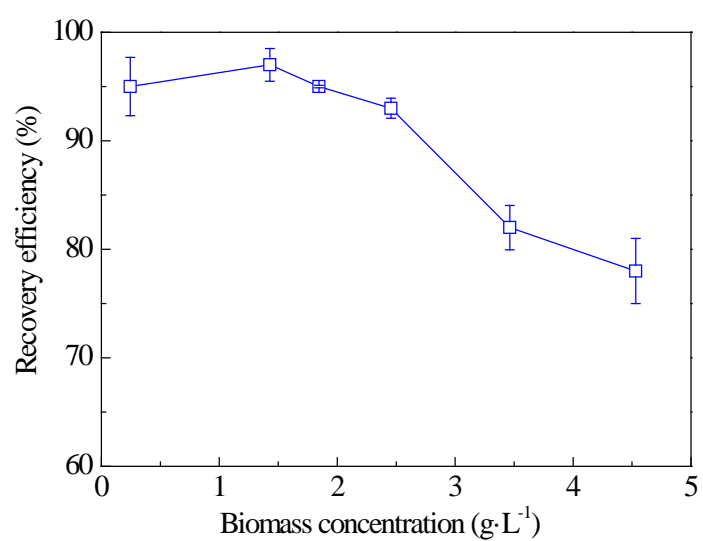


Figure 6 Recovery efficiency of *Chlorella* sp. ZTY4 at varying biomass concentration using 360 mg·L<sup>-1</sup> PAA and 4 mM (1432 mg·L<sup>-1</sup>) CPC. Data presented are of means  $\pm$  SD of three biological replicates.

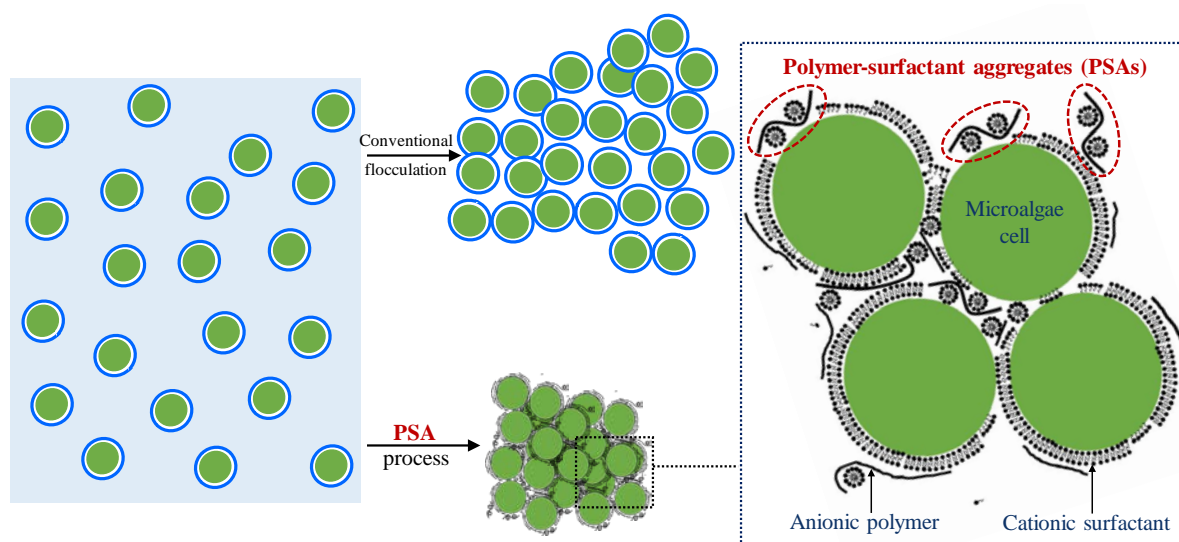


Figure 7 The difference between the PSA process and conventional flocculation.