Bioresource Technology 110 (2012) 496–502

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium

Zechen Wu [a,1], Yi Zhu [a,1], Weiya Huang [b], Chengwu Zhang [b,*], Tao Li [b], Yuanming Zhang [a,*], Aifen Li [b]

[a] Department of Chemistry, Jinan University, Guangzhou 510070, PR China
[b] Research Center of Hydrobiology, Jinan University, Guangzhou 510070, PR China

ARTICLE INFO

Article history:
Received 31 October 2011
Received in revised form 17 January 2012
Accepted 19 January 2012
Available online 28 January 2012

Keywords:
Flocculation
Microalgae
pH increase
Reuse

ABSTRACT

Flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium were evaluated. Increasing the medium pH value induced the highest flocculation efficiency of up to 90% for freshwater microalgae (*Chlorella vulgaris*, *Scenedesmus* sp., *Chlorococcum* sp.) with low/medium biomass concentrations and marine microalgae (*Nannochloropsis oculata*, *Phaeodactylum tricornutum*). The mechanism may be explained that Mg^{2+} in the growth medium hydrolyzed to form magnesium hydroxide precipitate, which coagulated microalgal cells by sweeping flocculation and charge neutralization. Additionally, this study revealed that the microalgal biomass concentrations and released polysaccharide (RPS) from microalgae could influence the flocculation efficiencies. Furthermore, neutralizing pH and then supplementing nutrients allowed the flocculated medium to maintain an approximate growth yield to that of the fresh medium in algal cultivation. These results suggest that the method presented here is effective, and allows the reuse of the flocculated medium, thereby contributing to the economic production from algae to biodiesel.

1. Introduction

Today about 80% of global energy demand is produced from fossil fuels. However, with the depletion of fossil fuels, governments and research institutions are making a great effort to develop new fuels (Brennan and Owende, 2010). In this regard, biofuels are rapidly being developed. Unfortunately, biodiesel from sources such as plantation oil crops, waste vegetable oil and animal fat cannot realistically satisfy the existing demand for fuels (Clarens et al., 2010). Some species of microalgae, with high growth rate and high lipid content, appear to be attractive alternatives as a feedstock for biodiesel production (Chisti, 2007; Hu et al., 2008; Halim et al., 2011).

Microalgal cells, with a size range between 5 and 50 μm, always form stable suspensions in growth medium due to their negative surface charge. The separation and recovery of microalgal biomass from growth medium is a critical step in the microalgal biomass production process, which accounts for about 20–30% of the total production cost (Gudin and Therpenier, 1986). So it is necessary to develop cost-effective technologies that would permit efficient harvesting. There are several methods that have been developed for harvesting microalgae (Chen et al., 2011): centrifugation (Knuckey et al., 2006), foam fractionation (Gsordas and Wang, 2004), filtration (Zhang et al., 2010a,b), and flocculation (Rossingol et al., 1999).

Among the above methods, flocculation is considered to be an effective and convenient process, which allows rapid treatment of large quantities of microalgae cultures (Oh et al., 2001). Flocculation is the coalescence of separate suspended microalgal cells into larger loosely attached conglomerates. Firstly, the suspended cells aggregate into larger particles *via* the interaction of the flocculant with the surface charge of the cells. Then, the aggregates coalesce into large flocs that settle out of suspension (Knuckey et al., 2006). A large number of chemical products have been tested as flocculants including various inorganic multivalent metal salts (Duan and Gregory, 2003) and organic polymer/polyelectrolyte (Vandamme et al., 2010). In addition, some microbes have been applied to flocculating certain microalgae recently (Lee et al., 2009; Salim et al., 2011; Kim et al., 2011). Furthermore, electrolytic flocculation, another flocculation technique in which the electrolysis of the microalgal suspension leads to the flocculation of microalgae, has also been developed (Gao et al., 2010; Ilhan et al., 2008).

It is well known that flocculation of microalgal biomass is particularly sensitive to pH of culture suspension. pH increase enhances the flocculation efficiency substantially by promoting the precipitation of added flocculants (McCausland et al., 1999). In fact, pH increase may also influence the charge of microalgal cells (Danquah et al., 2009) and change the existing forms of metal cations in culture suspension due to their hydrolysis (Gregory and Duan,

* Corresponding authors. Tel./fax: +86 20 85225036.
E-mail addresses: tzhangcw@jnu.edu.cn (C. Zhang), Tzhangym@gmail.com (Y. Zhang).
[1] These authors contributed equally to this paper.

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2012.01.101

2001). In this respect, flocculation simply by pH increase could be an attractive alternative because it is low-cost, low energy consumption, non-toxic to microalgal cells and it also eliminates the use of flocculants. Another outstanding advantage of this method is that the growth medium can be recycled after the microalgal flocculation since no flocculants are used and the medium is not contaminated. However, this method was only tested to a very small number of microalgal strains and has rarely been reported to date (Harith et al., 2009; Lee et al., 1998). Especially, few researches investigated the effects of the key factors, such as pH value, microalgal biomass concentration and the concentration of RPS on the flocculation by pH increase; and the detailed flocculation mechanism was also not fully understood yet.

In the present study, the effectiveness of flocculation by pH increase for three freshwater microalgae, namely *Chlorella vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp. and two marine microalgae, namely *Phaeodactylum tricornutum* and *Nannochloropsis oculata* were evaluated. The detailed flocculation mechanism of this method was studied and the influences of the key parameters were systematically examined. Furthermore, the reuse of the flocculated growth medium for cultivation was also investigated.

2. Methods

2.1. Microalgal strains and culture conditions

All microalgal strains were obtained from the Laboratory of Microalgal Bioenergy & Biotechnology, Research Center of Hydrobiology at Jinan University. *C. vulgaris*, *Scenedesmus* sp. and *Chlorococcum* sp. were grown in a BG-11 medium containing the following components: $NaNO_3$ (1.5 g L^{-1}); $K_2HPO_4{\cdot}3H_2O$ (40 mg L^{-1}); $MgSO_4{\cdot}7H_2O$ (75 mg L^{-1}); $CaCl_2{\cdot}2H_2O$ (36 mg L^{-1}); $NaCO_3$ (20 mg L^{-1}) $FeCl_3{\cdot}6H_2O$ (3.15 mg L^{-1}); citric acid (6 mg L^{-1}) and 1 mL of microelements composed of H_3BO_3 (2.86 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (1.81 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.22 mg L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.39 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.08 mg L^{-1}), $Co(NO_3)_2{\cdot}6H_2O$ (0.05 mg L^{-1}) in 1000 mL acidified water which included 1 mL concentrated H_2SO_4 in 1 L distilled water. *N. oculata* was grown in a modified Artificial Seawater (ASW) medium containing the following components: NaCl (27.00 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (6.60 g L^{-1}), $MgCl_2{\cdot}6H_2O$ (5.60 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (1.50 g L^{-1}), KNO_3 (1.45 g L^{-1}), K_2HPO_4 (0.12 g L^{-1}) $NaHCO_3$ (0.04 g L^{-1}), $FeCl_3{\cdot}6H_2O$ (2.76 μg L^{-1}), EDTA–Na_2 (18.6 μg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (22 μg L^{-1}), $CoCl_2{\cdot}6H_2O$ (10 μg L^{-1}), $MnCl_2{\cdot}4H_2O$ (180 μg L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (6 μg L^{-1}) and $CuSO_4{\cdot}5H_2O$ (10 μg L^{-1}) dissolved in demineralized water. *P. tricornutum*, was grown in the similar modified artificial seawater (ASW) medium except the addition of $Na_2SiO_3{\cdot}9H_2O$ (30 mg L^{-1}).

All the microalgal strains were grown in a glass column photobioreactor (Φ3 × 60 cm) at 25 °C, and exposed to a continuous illumination at a light intensity of 300 μmol m^{-2} s^{-1} by cool-white fluorescent lamps. The cultures were continuously aerated by gentle bubbling air containing 1% CO_2 (v/v).

2.2. Determination of flocculation efficiency

After the flocculation of microalgal cells, an aliquot of culture was withdrawn and used to measure OD_{750} (optical density at the wavelength of 750 nm) (Buelna et al., 1990; Makridis and Vadstein, 1999). The flocculation efficiency was calculated according to the following equation: flocculation efficiency (%) = $(1-A/B) \times 100$ (A: OD_{750} of sample, B: OD_{750} of reference).

2.3. The effect of RPS from the microalgal cells on the flocculation

In order to prepare corresponding media containing various concentrations of RPS, microalgal cells were removed by centrifugation. The supernatant was filtered through a 0.45 μm porous membrane, followed by a 0.22 μm porous membrane. The filtered supernatant was dialysed against distilled water for 72 h and the dialysed RPS solution was used as stock solution. RPS solution was measured by the phenol–sulfuric acid method (Dubois et al., 1956), using glucose as a standard. To prepare microalgal culture in corresponding media containing various concentrations of RPS, microalgal cells were collected via centrifugation and washed five times with deionized water, and then the cells were resuspended in the media containing various concentrations of RPS which was prepared by diluting the above RPS stock solution with growth medium.

In order to investigate the effect of RPS on flocculation of microalgal cells, comparative flocculation tests were performed using microalgal culture in corresponding media containing various concentrations of RPS. The influence of RPS on flocculation of the microalgae was assessed by reduction of flocculation efficiency in the presence of various concentrations of RPS compared to that in their absence.

2.4. Equipments

Environmental scanning electron microscopy (ESEM) experiments and energy-dispersive X-ray (EDX) analysis were performed at 60 Pa using a FEI QUANTA 200. Zeta potential measurements were obtained using a Malvern Zetasizer 2000HSA (Malvern, UK). OD_{750} was measured using a Lambda 45 UV–vis spectrometer (Perkin-Elmer Instruments). Microscopic pictures were taken on an optical microscope (OLYMPUS CX41RF). For ESEM experiments, the reference microalgae samples without being flocculated were first filtered through a 0.45 μm nylon membrane filter (MFS, Japan). The membrane filters were air-dried and mounted on an Aluminum alloy sample stage. The microalgae samples flocculated were withdrawn from the medium, placed on a glass substrate for air-drying and then mounted on an Aluminum alloy sample stage. The concentrations of the metal contents in the growth media were determined using inductively coupled-atomic emission spectrometry (ICP-AES).

2.5. Flocculation by pH increase

Flocculation experiments were all run with small volumes of medium (20 mL) distributed in cylindrical glass tubes (40 mL). For freshwater microalgae with low/medium biomass concentrations (dry weight $\leqslant$ 1 g/L) and marine microalgae, effective flocculation was achieved simply by adjusting the pH with 1 M NaOH. After the pH had been adjusted, the glass tube was vortexed thoroughly for 30 s and allowed to stand at room temperature for 10 min. Then an aliquot of medium was withdrawn and used to measure OD_{750}. For freshwater microalgae with high biomass concentrations (dry weight > 1 g/L), minim magnesium were added to the medium prior to pH adjustment.

2.6. Reuse of flocculated medium

After flocculation, the flocs and the growth medium were separated immediately. The pH of the growth medium was adjusted to the original value by adding the necessary amount of inorganic acid (HNO_3 for BG-11 medium and HCl for modified ASW medium). Nutrients were also supplemented to the flocculated medium. Some microalgal cells flocculated were cultivated in the

medium and the biomass concentration as a function of growth phase was investigated.

3. Results and discussion

3.1. Flocculation of microalgal cells by pH increase

The flocculation efficiency was investigated as a function of pH variations in Fig. 1a and b. An alkaline flocculation zone was observed for each microalgal species. The freshwater microalgal cells began to agglomerate when the pH was adjusted up to about 8.6. As pH increased to 10.6, the efficiencies were greatly raised to more than 90% and reached plateaus. The flocculation for the two species of marine microalgae was initiated at pH 8.0 and 8.2 respectively. And the highest efficiencies above 90% were obtained at pH 9.0 and 9.3. The above results demonstrated that effective flocculation for the five species could be attained by pH increase.

3.2. Mechanism of flocculation formation

As is well known, some multivalent metal salts were employed as flocculants to harvest microalgae from culture suspensions and the metal cations were confirmed to be vital in the flocculation (Molina Grima et al., 2003). In light of such reports, the concentrations of the multivalent metal cations in the growth medium before and after flocculation were detected. It was found that Mg^{2+} concentration decreased considerably, indicating Mg^{2+} played an important part in the flocculation process (Table 1).

In order to further investigate the mechanism, ESEM coupled with EDX analysis was used to examine the surface morphology and elemental composition of the microalgal flocs. It could be observed that the microalgal flocs were mainly composed of algae cells, which were flocculated together by some agglutinant (Fig. S1a). The elemental compositions of two sites (one on the algae cell and the other on the agglutinant) were shown in Fig. 2a and b. The EDX analysis revealed that high content of magnesium was detected in the agglutinant. As is reported, the alkaline pH range led to magnesium precipitation to form magnesium hydroxide (Semerjian and Ayoub, 2003). Hence, in our experiments, high pH values, decrease of Mg^{2+} concentration in the growth medium after flocculation and high content of magnesium in the agglutinant, strongly suggested that Mg^{2+} in the medium hydrolyzed to form magnesium hydroxide precipitate at high pH values. The magnesium hydroxide precipitate then interacted with the microalgal cells and caused the flocculation.

However, how the magnesium hydroxide precipitate interacted with the microalgal cells was vague. For further revealing the detailed flocculation mechanism, Zeta potential, an important parameter, was measured. The results in Fig. 3a and b showed zeta potentials as functions of pH. The zeta potentials were pH dependant and negative at pH values of practical interest. For freshwater microalgal systems, the trends of zeta potentials firstly went downwards and then upwards. On gradual increase of the pH by adding NaOH solution, the potentials gradually declined and reached the lowest values at about pH 10.3. pH 10.3 was the inflexion and as pH increased the potentials gradually rose. However, for marine microalgal systems, *N. oculata* and *P. tricornutum*, there was no pH inflexion and the trend went downwards within pH values arranging from 7.4 to 10.3.

The downward trend with pH increase demonstrated the decrease of surface charge of microalgae cells, indicating that the charge neutralization occurred in this region. Mg^{2+} in the growth medium could form $Mg(OH)_2$ precipitate with a large adsorptive surface area and a positive superficial charge as pH increased (Parks, 1967). The precipitate attracted the negatively charged microalgal cells, thus resulting in the compression of the electrical double-layer and causing them to become destabilized and hence to flocculate. For freshwater microalgae, the trend went upwards as pH further increased, which was attributable to dissociation of carboxylic acid groups on the surface of microalgal cells (Kam and Gregory, 2001; Henderson et al., 2010). However, the flocculation efficiency was significantly higher. Hence, it was likely that in this region sweep flocculation was responsible. Sweep flocculation, defined as a process in which particles were enmeshed in the growing precipitate and then formed sediment. $Mg(OH)_2$ precipitates tended to have a rather open structure, so that even a small mass could give a large effective volume concentration and hence a high probability of capturing microalgal cells (Duan and Gregory, 2003). The flocculation efficiency was therefore considerably improved than when particles were destabilized just by charge neutralization.

3.3. The effect of microalgae biomass concentration on flocculation

Actually, different microalgae biomass concentrations might occur during the flocculation process. The effectiveness of flocculation by pH increase for microalgae with different biomass concentrations was therefore evaluated. Fig. 4a and b showed the

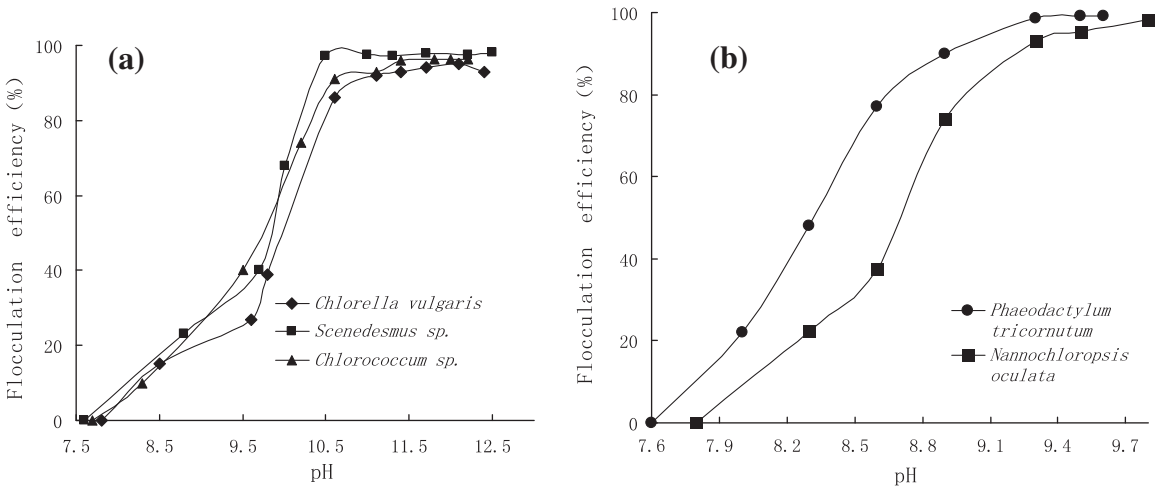

Fig. 1. Flocculation efficiency as a function of pH (a) freshwater algae with respective biomass: *Chlorella vulgaris* (0.68 g/L); *Scenedesmus* sp. (0.75 g/L); *Chlorococcum* sp. (0.77 g/L) (b) marine algae with respective biomass: *Phaeodactylum tricornutum* (1.8 g/L); *Nannochloropsis oculata* (1.6 g/L).

Table 1
Concentrations of Mg^{2+} (mg/L) in the medium before and after flocculation for the five species of algae.

	Chlorella vulgaris (0.9 g/L)	*Scenedesmus* sp. (1.1 g/L)	*Chlorococcum* sp. (1.06 g/L)	*Phaeodactylum tricornutum* (2.2 g/L)	*Nannochloropsis oculata* (2.4 g/L)
Before flocculation	7.37	7.03	7.11	1402.50	1257.25
After flocculation	0.75	0.72	0.68	1216.25	1041.75

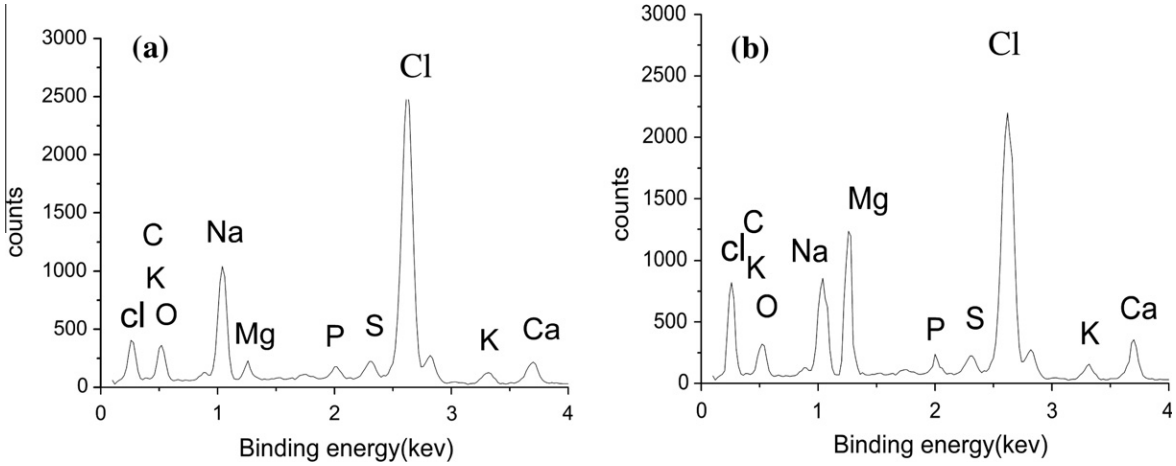

Fig. 2. (a) Spectrum 1: EDX analysis of *Phaeodactylum tricornutum* cells. (b) Spectrum 2: EDX analysis of *Phaeodactylum tricornutum* flocs.

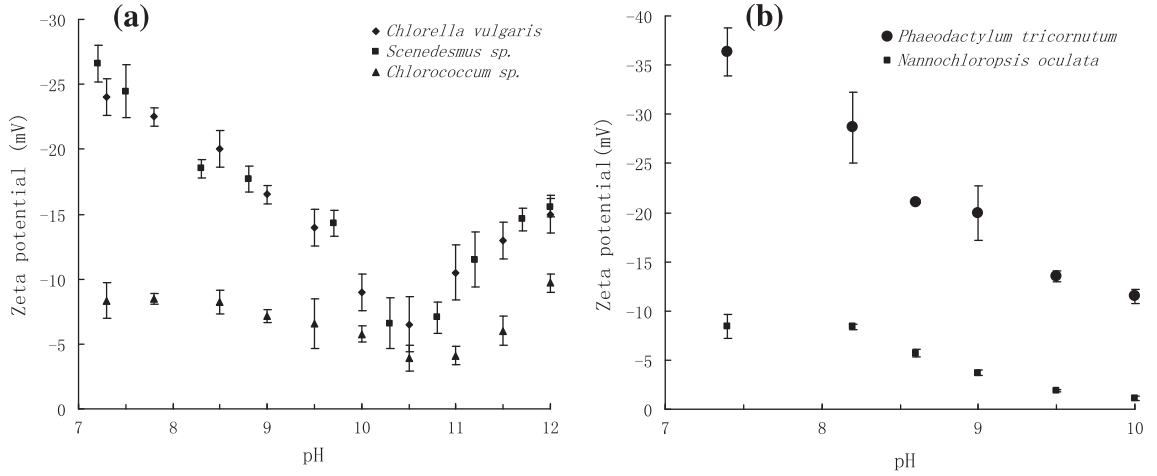

Fig. 3. Zeta potentials as a function of pH (a) for freshwater algal systems (b) for marine algal systems.

effect of biomass concentration on the flocculation efficiency. It was evident that for both freshwater and marine microalgae the efficiency decreased considerably with the increase of biomass concentration. This phenomenon could be attributed to the fact that no sufficient magnesium in the growth medium was available for the flocculation of excessive microalgae cells. Hence for freshwater microalgae with high biomass concentration, extensive flocculation was achieved at high pH values with need of adding minim magnesium (Fig. 4c).

3.4. The effect of RPS on flocculation

Several previous studies on microalgal removal and harvest have found that microalgal extracellular organic matter (EOM) could interfere with flocculation with multivalent metal salts (Pivokonsky et al., 2006; Henderson et al., 2010; Zhang et al., 2010a,b). Proteins of *A. flos-aqua* and *M. aeruginosa* could form complexes with iron and aluminum, resulting in the increase in doses of the flocculant required and decrease in flocculation efficiency. RPS of *A. halophytica* GR02 could compete for flocculant against microalgal cells by forming complexes with ferrum, thus leading to the reduction of flocculation of the algae with ferric chloride (Chen et al., 2009). The microalgae in this work released few proteins but large amounts of polysaccharides. Thus the influence of RPS should therefore be taken into consideration during flocculation of the microalgae by pH increase. Fig. 5 demonstrated that for each species of alga the increasing concentration of RPS in the culture suspension resulted in higher pH value to achieve the

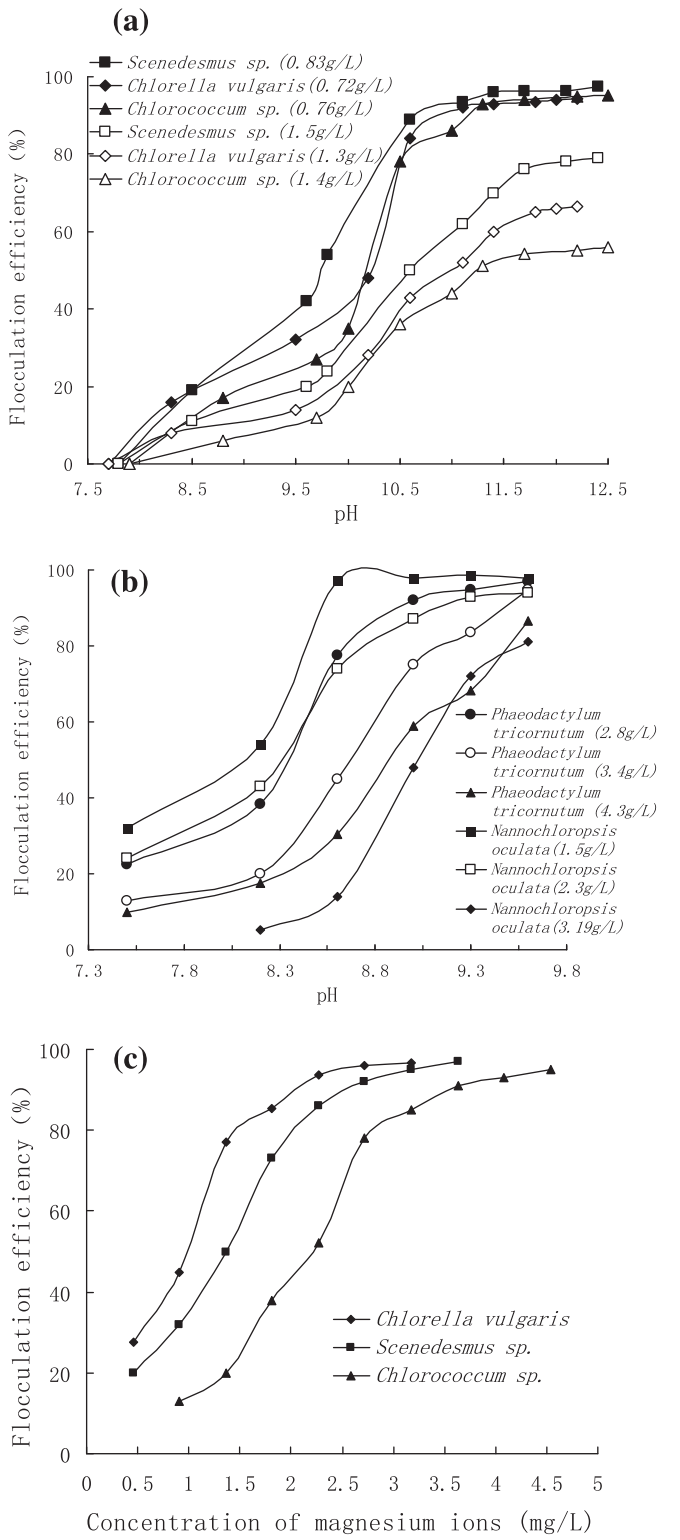

Fig. 4. (a) Flocculation efficiency of freshwater algae as a function of pH with different biomass concentrations. (b) Flocculation efficiency of marine algae as a function of pH with different biomass concentrations. (c) Flocculation efficiency of freshwater algae with high biomass as a function of Mg^{2+} concentration using $MgSO_4$ at pH 11.2: *Chlorella vulgaris* 1.7 g/L; *Scenedesmus* sp. 1.8 g/L; *Chlorococcum* sp. 1.7 g/L.

same flocculation efficiency. For the same pH value, the flocculation efficiency decreased with the increasing RPS concentration. For example, for *C. vulgaris*, the flocculation efficiency at pH 10.5 decreased from approximately 92% to 7% with the increase of RPS concentration from 0 to 70 mg L^{-1}. This indicated that the inhibitory effort of RPS on flocculation of the microalgae was very strong and the increase of RPS concentration was detrimental to the flocculation of the alga. It could be preliminarily inferred that RPS might form complexes with magnesium, bringing about the decrease of magnesium in the medium. Hence more hydroxyl were needed to form $Mg(OH)_2$ precipitate and pH value required to achieve the same flocculation efficiency was thereupon increased.

3.5. Reuse of growth medium for cultivation

For most microalgae harvested by adding flocculants, the used growth medium after flocculation was usually disused as the medium was contaminated by the flocculants, resulting in an environmental problem and a great loss of water. However, for the microalgae harvested by pH increase, since no flocculants were used and the medium was not contaminated, the growth medium after flocculation might be reused by neutralizing pH and then supplementing nutrients. The products of neutralizing pH with HNO_3 for flocculated BG-11 medium and HCl for flocculated ASW medium were $NaNO_3$ and NaCl, which were the necessary nutrients. In this respect, the recycle of flocculated medium could minimise the cost of nutrients and the demand for water. Some microalgal cells flocculated by pH increase were cultivated in the recycled culture solution and the biomass as a function of growth phase was shown in Fig. 6. It was observed that the biomass of each microalgal species cultivated in the reused growth medium was close to that cultivated in the fresh solution, indicating the culture solution could be recycled. Moreover, the fact that the flocculated microalgal cells could be recultivated demonstrated that there was no cell lysis during the flocculating by pH increase, and the molecular function and structure of the photosynthetic apparatus were not affected.

3.6. Comparison with other harvesting methods

For the harvest of microalgae, flocculation induced by pH increase is potentially an improvement over other comment methods. Most commercial organizations use centrifugation, the traditional method for harvesting microalgae, but it is an energy-intensive process as it consumes a great deal of electric power (Chen et al., 2011). Some microalgae can be harvested using filtration, but membranes are rapid fouled by extracellular organic matter (Zhang et al., 2010a,b). Microalgae can also be harvested using foam fractionation, but the energy consumption of large-scale harvesting systems is high (Brennan and Owende, 2010). Another method for harvesting microalgae is by means of flocculation. Inorganic flocculants, including alum and iron chloride, lead to contamination of growth medium with aluminum or iron (Oh et al., 2001). The contaminated growth medium has to be disused, causing an environmental problem and a great loss of water. Organic polymer/polyelectrolyte flocculants, such as cationic starch and chitosan, have no toxic effects and do not contaminate growth medium. They are, however, high-value products with a market value of about $US10 per kilogram for chitosan and $US1–3 per kilogram for cationic starch (Vandamme et al., 2010). In our experiments, microalgae were harvested by flocculation induced by NaOH. By comparison, it is not an energy-consuming process, and NaOH is an inexpensive product about $US 0.13 per kilogram. Moreover, NaOH does not contaminate growth medium, which can be recycled to reduce the cost and the demand for water at larger scales. Thereby the method presented here contributes to the economic production from algae to biodiesel.

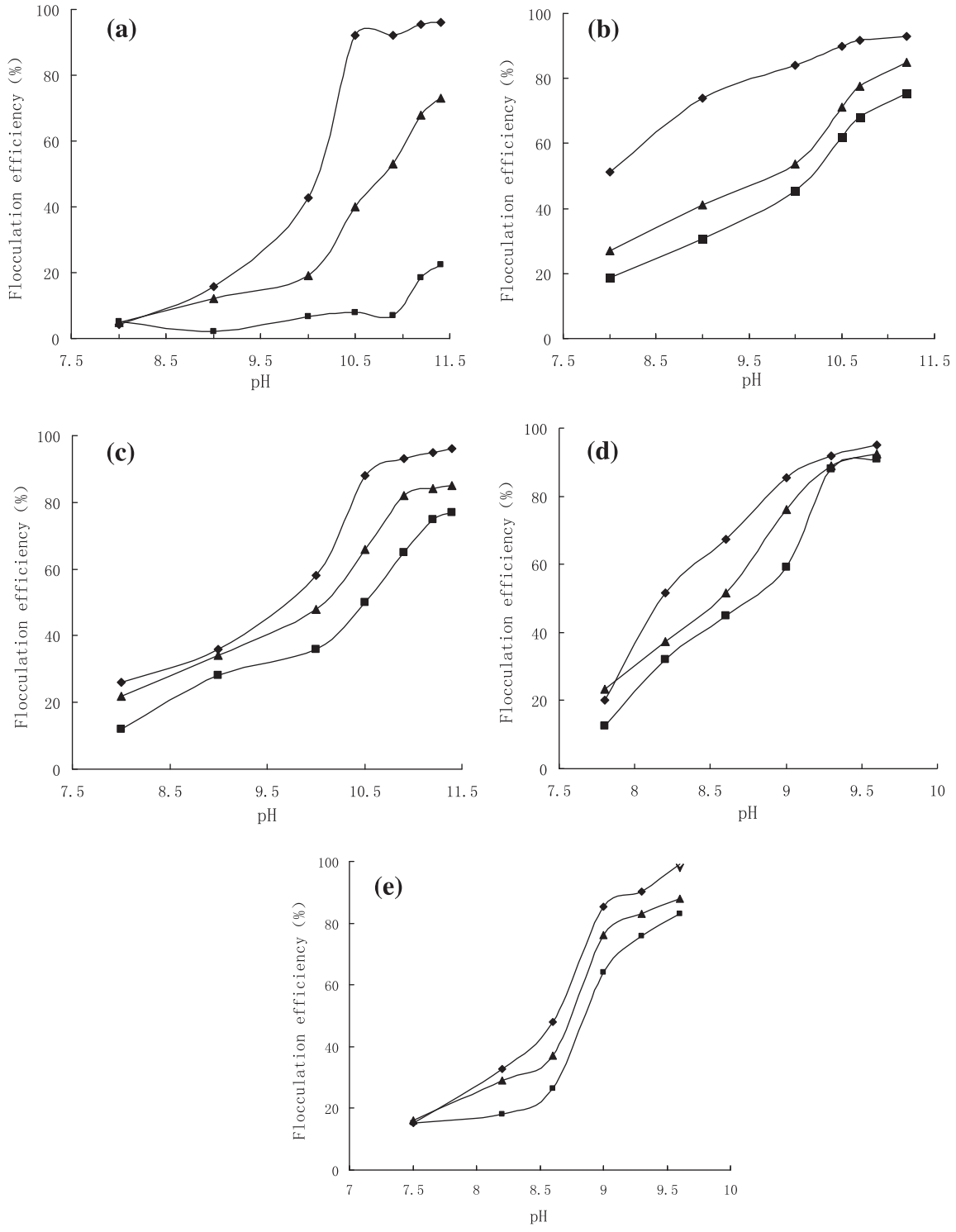

Fig. 5. Flocculation tests of algae by pH increase in algal cultures containing various concentrations of RPS: (a) *Chlorella vulgaris* (♦) in growth medium, (▲) in 25 mg L^{-1} RPS medium, (■)in70 mg L^{-1} RPS medium; (b) *Scenedesmus* sp. (♦) in growth medium, (▲) in 8.1 mg L^{-1} RPS medium, (■) in 12.2 mg L^{-1} RPS medium; (c) *Chlorococcum* sp. (♦) in growth medium, (▲) in 15.2 mg L^{-1} RPS medium, (■) in30.4 mg L^{-1} RPS medium; (d) *Phaeodactylum tricornutum* (♦) in growth medium, (▲) in 67.8 mg L^{-1} RPS medium, (■) in 101 mg L^{-1} RPS medium; (e) *Nannochloropsis oculata* (♦) in growth medium, (▲) in 21 mg L^{-1} RPS medium, (■) in 43 mg L^{-1} RPS medium.

4. Conclusions

A flocculation method induced by pH increase was found effective for harvesting microalgae. The mechanism involved the formation of magnesium hydroxide precipitate, sweeping flocculation and charge neutralization. The flocculation efficiencies decreased considerably with the increase of biomass concentration and RPS concentration. The flocculated medium could be reused, thereby minimizing the demand for water and reducing the cost of biodiesel production from algae. Finally, the method was only conducted at laboratory scales and tested to a very small number of microalgal strains. Therefore, further research would have to look for application development at larger scales and for other algae groups.

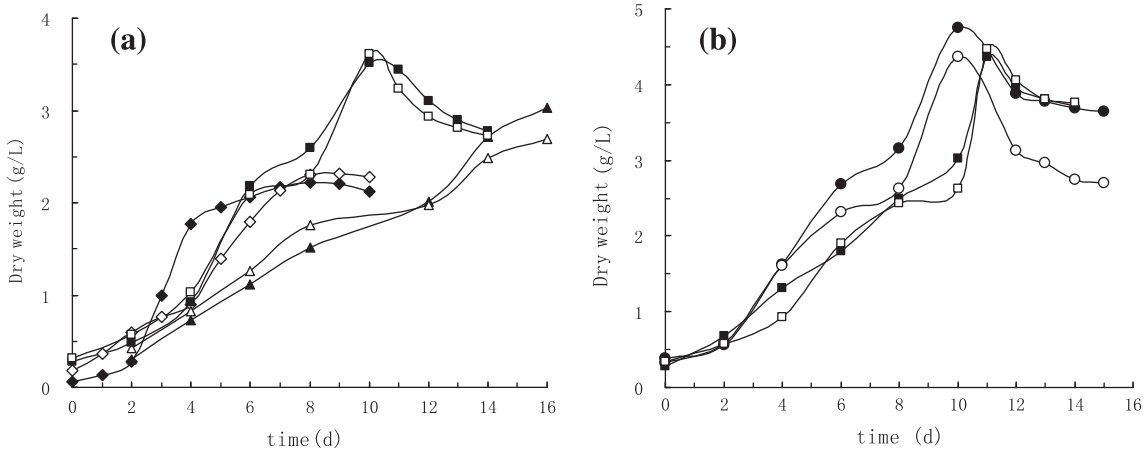

Fig. 6. Comparion of biomass as a function of time in fresh and reused growth medium: (a) *Chlorella vulgaris* in fresh (♦) and reused (◇) growth medium; *Scenedesmus* sp. in fresh (■) and reused (□) growth medium; *Chlorococcum* sp. in fresh (▲) and reused (△) growth medium; (b) *Phaeodactylum tricornutum* in fresh (●) and reused (○) growth medium; *Nannochloropsis oculata* in fresh (■) and reused (□) growth medium.

Acknowledgements

This study was sponsored by Natural Science Foundation of Guangdong (No. S2011040001667), the Fundamental Research Funds for the Central Universities (No. 21611310), and the National High Technology Research and Development Program of China (863 Program) (No. 2009AA064401).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2012.01.101.

References

Brennan, L., Owende, P., 2010. Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew. Sust. Energ. Rev. 14, 557–577.

Buelna, G., Bhattarai, K.K., de la Noue, J., Taiganides, E.P., 1990. Evaluation of various flocculants for the recovery of algal biomass growth on pig-waste. Biol. Wastes 31, 211–222.

Chen, L., Li, P., Liu, Z., Jiao, Q., 2009. The released polysaccharide of the cyanobacterium *Aphanothece halophytica* inhibits flocculation of the alga with ferric chloride. J. Appl. Phycol. 21, 327–331.

Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J., Chang, J.-S., 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour. Technol. 102 (1), 71–81.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25, 294–306.

Clarens, A.F., Resurreccion, E.P., White, M.A., Colosi, L.M., 2010. Environmental life cycle comparison of algae to other bioenergy feedstocks. Environ. Sci. Technol. 44, 1813–1819.

Danquah, M.K., Gladmanb, B., Moheimani, N., Forde, G.M., 2009. Microalgal growth characteristics and subsequent influence on dewatering efficiency. Chem. Eng. J. 151, 73–78.

Duan, J., Gregory, J., 2003. Coagulation by hydrolysing metal salts. Adv. Colloid Interface Sci. 100–102, 475–502.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method of determination of sugars and related substances. Anal. Chem. 28, 350–356.

Gao, S., Yang, J.X., Tian, J.Y., Ma, F., Tu, G., Du, M., 2010. Electro-coagulation–flotation process for algae removal. J. Hazard. Mater. 177, 336–343.

Gregory, J., Duan, J., 2001. Hydrolyzing metal salts as coagulants. Pure Appl. Chem. 73, 2017–2026.

Gsordas, A., Wang, J.K., 2004. An integrated photobioreactor and foam fractionation unit for the growth and harvest of *Chaetoceros* spp. in open systems. Aquacult. Eng. 30, 15–30.

Gudin, C., Therpenier, C., 1986. Bioconversion of solar energy into organic chemicals by microalgae. Adv. Biotechnol. Proc. 6, 73–110.

Halim, R., Gladman, B., Danquah, M.K., Webley, P.A., 2011. Oil extraction from microalgae for biodiesel production. Bioresour. Technol. 102, 178–185.

Harith, Z.T., Yusoff, F.M., Mohamed, M.S., Din, M.S.M., Ariff, A.B., 2009. Effect of different flocculants on the flocculation performance of microalgae, *Chaetoceros calcitrans*, cells. J. Biotechnol. 8 (21), 5971–5978.

Henderson, R.K., Parsons, S.A., Jefferson, B., 2010. The impact of differing cell and algogenic organic matter (AOM) characteristics on the coagulation and flotation of algae. Water Res. 44, 3617–3624.

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., Darzins, A., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54, 621–639.

Ilhan, F., Kurt, U., Apaydin, O., Gonullu, M.T., 2008. Treatment of leachate by electrocoagulation using aluminum and iron electrodes. J. Hazard. Mater. 154, 381–389.

Kam, S.K., Gregory, J., 2001. The interaction of humic substances with cationic polyelectrolytes. Water Res. 35 (15), 3557–3566.

Kim, D.G., La, H.J., Ahn, C.Y., Park, Y.H., Oh, H.M., 2011. Harvest of *Scenedesmus* sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. Bioresour. Technol. 102, 3163–3168.

Knuckey, R.M., Brown, M.R., Robert, R., Frampton, D.M.F., 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacult. Eng. 35 (3), 300–313.

Lee, A.K., Lewis, D.M., Ashman, J.P., 2009. Microbial flocculation, a potentially low-cost harvesting technique for marine microalgae for the production of biodiesel. J. Appl. Phycol. 21, 559–567.

Lee, S.J., Kim, S.B., Kim, J.E., Kwon, G.S., Yoon, B.D., Oh, H.M., 1998. Effects of harvesting method and growth stage on the flocculation of the green alga *Botryococcus braunii*. Lett. Appl. Microbiol. 27, 14–18.

Makridis, P., Vadstein, O., 1999. Food size selectivity of *Artemia franciscana* at three developmental stages. J. Plankt. Res. 21, 2191–2201.

McCausland, M.A., Brown, M.R., Barrett, S.M., Diemar, J.A., Heasman, M.P., 1999. Evaluation of live microalgae and microbial pastes as supplementary food for juvenile Pacific oyster (*Crassostrea gigas*). Pure Appl. Chem. 73, 2017–2026.

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., RoblesMedina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491–515.

Oh, H.M., Lee, S., Park, M.H., Kim, H.S., Kim, H.C., Yoon, J.H., Kwon, G.S., Yoon, B.D., 2001. Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49. Biotechnol. Lett. 23, 1229–1234.

Parks, G.A., 1967. Equilibrium concepts in natural watersystems. Advances in Chemistry Series, vol. 67. American Chemistry Society, Washington, DC.

Pivokonsky, M., Kloucek, O., Pivokonska, L., 2006. Evaluation of the production, composition and aluminum and iron complexation of algogenic organic matter. Water Res. 40, 3045–3052.

Rossingol, N., Vandajon, L., Jaouen, L., Quemeneur, F., 1999. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and unfiltration. Aquaculture 20, 191–208.

Salim, S., Bosma, P., Vermuë, M.H., Wijffels, R.H., 2011. Harvesting of microalgae by bio-flocculation. J. Appl. Phycol. 23, 849–855.

Semerjian, L., Ayoub, G.M., 2003. High-pH–magnesium coagulation–flocculation in wastewater treatment. Adv. Environ. Res. 7, 389–403.

Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K., 2010. Flocculation of microalgae using cationic starch. J. Appl. Phycol. 22, 525–530.

Zhang, Y., Tian, J.Y., Nan, J., Gao, S.S., Liang, H., Wang, M.L., Li, G.B., 2010a. Effect of PAC addition on immersed ultrafiltration for the treatment of algal-rich water. J. Hazard. Mater. 186, 1415–1424.

Zhang, X.Z., Hu, Q., Sommerfeld, M., Puruhito, E., Chen, Y.S., 2010b. Harvesting algal biomass for biofuels using ultrafiltration membranes. Bioresour. Technol. 101, 5297–5304.