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Harvesting of microalgal biomass: Efficient method for flocculation through pH modulation

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HIGHLIGHTS

- Demonstration of the auto-flocculation capability of *Chlorococcum* sp. R-AP13.
- Demonstration of the use of chitosan for flocculating *Chlorococcum* sp.
- 94% efficiency in cell harvesting achieved through flocculation by modulation of medium pH.
- Medium after flocculation re-used for cultivation without significant reduction in cell densities.
- No significant change in fatty acid profiles for cells flocculated by pH change, chitosan or by auto-flocculation.

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ABSTRACT

Harvesting of the micro alga *Chlorococcum* sp. R-AP13 through autoflocculation, chemical flocculants or by change in medium pH was evaluated. Surface charge of algal cells changed in response to the method used and affected flocculation efficiency. While aluminum sulfate and $FeCl_3$ supported 87% and 92% efficiency, auto flocculation could recover 75% of biomass in 10 min. Maximum efficiency (94%) was obtained with change in medium pH from 8.5 to 12.0 achieved through addition of 40 mg l^{-1} of NaOH. Since high concentrations of $FeCl_3$ and $AlSO_4$ were toxic to the cells, flocculation induced by pH change may be considered the most effective strategy. Residual medium after flocculation could be reused efficiently for algal cultivation, minimizing the demand for fresh water.

1. Introduction

Microalgal biomass production systems generally involve cultivating them in an environment that stimulates the accumulation of target metabolites and the recovery of the biomass for the downstream processing (Cheng et al., 2011). However major bottleneck in the algal biomass based product development is the recovery of biomass from the production medium, mainly due to the smaller size (5–50 μm), presence of negative surface charge, low biomass concentrations, and similarity of the density of algal cells to the growth medium (Garzon-Sanabria et al., 2012; Milledge and Heaven, 2013). Key factor limiting the commercial use of microalgal biomass is cost effective harvesting, which is considered to be the most challenging area in algal based biofuels (Georgiana and Mayfield, 2012). It has been suggested that 20–30% of the cost of algal biomass is the cost of harvesting (Mata et al., 2010). Harvesting technology is an important factor in the production of algal based biofuels, and an effective, convenient and economical method of microalgal harvesting is yet to evolve. The high costs involved in harvesting are acceptable only in cases where the target microalgal products are of high value. For low-value bulk products, both the investment as well as the operational costs should be drastically reduced to make commercial production feasible (Wijffels and Barbosa, 2010). So it is necessary to develop cost effective techniques that can permit efficient harvesting of microalgal biomass from culture systems.

Several methods have been tested for the harvesting of algal biomass, which includes centrifugation, filtration, flotation and flocculation (Uduman et al., 2010; Milledge and Heaven, 2013). Flocculation is a chemical based separation process that needs less energy than centrifugation and filtration, and thus it is regarded as one of the most promising means of dewatering algal biomass

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(Wan et al., 2015). A large number of chemical products have been tested as flocculants including various inorganic multivalent metal salts (Duan and Gregory, 2003) and organic polymers/polyelectrolytes (Vandamme et al., 2010). A variety of flocculation strategies, such as physical, chemical and biological methods have been developed for microalgal harvesting as summarized in recent reviews (Vandamme et al., 2013; Wan et al., 2015).

The mechanism of flocculation depends on the interaction of cell surface charge and flocculent charges. Metal salts such as aluminum sulfate, ferric chloride, ferric sulfate, etc. are generally employed in flocculation processes, since they lead to improved harvesting efficiencies. One of the disadvantages of these inorganic flocculants is that they are required in high doses and results in contamination of the biomass with aluminum or iron (Wyatt et al., 2012). Chitosan has recently emerged as a favorable organic flocculating agent for harvesting of microalgae. Compared with other flocculants, it presents various advantages, including formation of larger flocs, resulting in faster sedimentation of biomass and providing a clearer residual solution. Chitosan is also non toxic and biodegradable which makes it possible to reuse the flocculated medium for algal cultivation (Chen et al., 2014). Another strategy being actively investigated is the use of auto-flocculation for harvesting of algal cells. Auto-flocculation can occur naturally in some microalgae and they flocculate in response to certain environmental stresses such as change in nitrogen concentrations, pH, dissolved oxygen and the amount of some metal ions in the medium (Uduman et al., 2010).

It is well known that flocculation of algal biomass is sensitive to pH of the culture medium and enhancement in flocculation efficiency with increase in pH increase has been reported (Wu et al., 2012). Recently, several studies have revealed that microalgae can be successfully flocculated by adjusting the pH. The pH threshold for flocculation may vary with several parameters, such as properties of cell surface, biomass concentration, medium composition, and flocculation time (Yang et al., 2015). When the pH increased from 8.5 to 10.5, the flocculation efficiency of *Phaeodactylum tricornutum* was higher than 90% (Sirin et al., 2012). In this context, flocculation by simple increase of the medium pH could be an attractive alternative because it is low cost, low energy and non toxic to microalgal cells and the use of flocculants can be avoided. Another advantage of this strategy is that the growth medium can be recycled after flocculation, since no flocculants are used and medium is not contaminated by toxic chemicals. However, this method was tested only in a few number of microalgal strains (Castrillo et al., 2013; Yang et al., 2015). The method was also successfully demonstrated in *Chlorococcum* sp. (Wu et al., 2012; Liu et al., 2013).

Present investigation highlights the potential of physical conditions/features like pH change of medium in flocculating the algal cells. Different chemical flocculants including aluminum sulfate, ferric chloride, chitosan and auto-flocculation and pH change of medium was compared for harvesting of *Chlorococcum* sp. R-AP13 biomass. Flocculation efficiency, dose, and zeta potential of algal biomass during flocculation were studied and fatty acids profiling was conducted in the optimized flocculated biomass. Recycling of the residual medium after flocculation for the cultivation of alga was also investigated. For the first time, we demonstrate that the residual medium after flocculation using pH change, autoflocculation or using chitosan as flocculent supports similar algal growth as fresh medium and there are no significant differences in the fatty acid profiles of algal cells grown in fresh or re-used medium. On the other hand, the fatty acid profiles of algal cells grown in residual medium from chemically flocculated cultures were considerably different from those of their counter parts grown in fresh medium.

2. Materials and methods

2.1. Microalga and culture conditions

Chlorococcum sp. RAP-13 (Ummalyma and Sukumaran, 2014) was used for flocculation studies. The alga was maintained in MA medium, with a composition in mg l^{-1}: Ca $(NO_3)_2{\cdot}4H_2O$-50, KNO_3-100, $NaNO_3$-50, Na_2SO_4-50, $MgCl_2{\cdot}6H_2O$-50, Na-β-glycerophosphate$\cdot 5H_2O$-100, $Na_2EDTA{\cdot}2H_2O$-5, $MnCl_2$-5, $FeCl_2{\cdot}6H_2O$-5, $Na_2MoO_4{\cdot}2H_2O$-0.8, H_3Bo_3-20. pH of the medium was adjusted to 6.8. Cells for the study were grown in 5 L flasks containing 3 L medium and incubated in a climate controlled chamber at 30 °C with diurnal cycle of 14/10 h. Flocculation studies were performed after the cells reached stationary phase of the growth. Flocculants tested were procured either from Merck, India or Sigma–Aldrich, India.

2.2. Flocculation experiment

The effect of flocculent type and concentration on the flocculation efficiency was determined using a jar test (Vandamme et al., 2010; Gerde et al., 2014). Briefly, the algal suspension (100 ml) was stirred at 250 rpm in a 100 ml beaker, while the flocculent was added slowly. After this, the stirring was continued for 2 min, then stopped and allowed to settle for 10 min. Then an aliquot of the supernatant was taken at a depth of ~2.0 cm from the surface of the liquid and its absorbance was measured at 680 nm in UV visible spectrophotometer. Absorbance of the original suspension was also taken before addition of the flocculent. The absorbance values were extrapolated to cell numbers based on a standard curve constructed with algal cell suspensions having different cell densities. Flocculation efficiency of *Chlorococcum* sp. was calculated as below (Eq. (1))

$$\frac{(\text{Initial Cell Conc.} - \text{Cell Conc. in Supernatant})}{\text{Initial Cell Conc.}} \times 100 \quad (1)$$

2.3. Zeta potential measurement

Zeta potential of the *Chlorococcum* sp. R-AP13 was measured before and after the addition of various flocculants into the medium using Malvern Zetasizer 90 (Malvern Instruments Ltd., USA). Zeta potential was analyzed in triplicates at room temperature and the mean values were taken.

2.4. Cell viability

The viability of flocculated cells was tested by dye exclusion method using 1.0% Trypan blue, which is excluded by viable cells. One milliliter samples of each experiment were centrifuged at 6000 rpm for 5 min and the supernatant was discarded. Then 100 µl of the 1.0% Trypan blue solution was added, and the cells were incubated for 3 h at room temperature. Next, the cells were washed twice using deionized water to remove excess of unbound dye. Finally, the fresh preparations of cells were examined for dye exclusion under a Phase contrast Microscope (Leica DMLS2000, Germany). Cells with intact cell wall (live cells) exclude Trypan Blue, while the dead cells take up the dye, differentiating viable and non-viable cells.

2.5. Recycling of flocculated medium

Flocculated biomass and medium were separated by aspirating the medium. pH of the residual medium was adjusted to 6.8–7.0 using 1N NaOH or HCl. After that, components of MA medium were added and used for cultivation of the next batch of cells. Fresh MA

medium was used as control. The control and recycled media were inoculated with 10% v/v of an inoculum containing 3×10^6 cells ml^{-1}. Biomass production was monitored as cell density at two days interval.

2.6. Fatty acids profiling

Fatty acid profile of oil from different flocculated biomass were done by acid mediated trans-esterification for FAME generation followed by gas chromatography methods as described in Ummalyma and Sukumaran (2014). FAME was identified by comparing their fragmentation pattern with internal standards (Sigma Aldrich, India).

3. Results and discussion

3.1. Evaluation of inorganic flocculants for harvesting microalgal cells

Among the inorganic flocculants-aluminum sulfate and ferric chloride tested for flocculation of *Chlorococcum* sp. R-AP13 cells, $FeCl_3$ was found to be more effective than aluminum sulfate. $FeCl_3$ supported a flocculation efficiency of 92% at concentrations of 70–80 mM while aluminum sulfate had an efficiency of 87% at 120 mM concentration (Fig. 1A and B). Initial zeta potential of the algal cells was found to be −20 mV. The surface charge of the cell changed after the addition of flocculants. Flocculation efficiency was increased near to the neutralization point. Higher concentrations of aluminum sulfate and ferric chloride increased the positive charges in the medium which affected the flocculation efficiencies

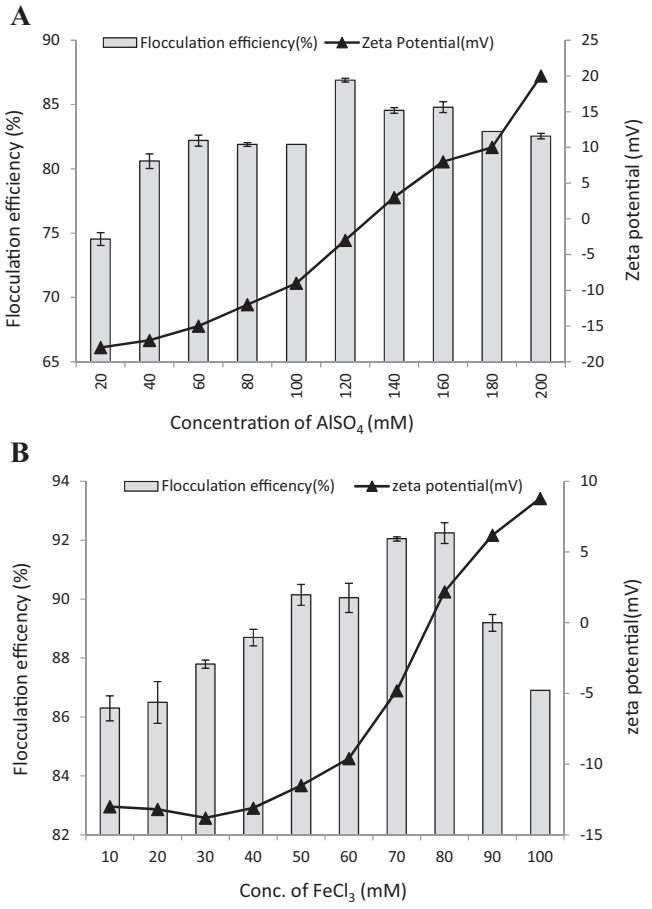

Fig. 1. Flocculation of *Chlorococcum* sp. RAP-13 cells using inorganic flocculants. A: Aluminum sulfate, B: Ferric chloride.

of cells (Fig. 1A and B). Possible explanation for this could be that the amount of flocculent that exceeded the optimum concentration could contribute to excess of positive charges, thus stabilizing the cell particles in suspension by charge repelling, as well as by stearic hindrance (Vandamme et al., 2010).

The flocculation mechanism depends on the nature of the algal cells and the charge of the flocculent. Numerous chemical coagulants or flocculants have been tested for microalgal flocculation (Rakesh et al., 2014). Metal salts (aluminum sulfate, ferric chloride, etc.) are generally preferred because they lead to improved harvesting efficiency. The results of $FeCl_3$ as flocculent showed an almost comparable efficiency with the reported efficiencies for flocculation of *Chlorella zofingiensis* (Wyatt et al., 2012). For any given algae species, effective flocculation with $FeCl_3$ might be obtained if the conditions of negative surface charge and sufficient flocculent concentrations are available in the medium. Since different algal species vary in their concentrations of functional groups on the cell surface, the minimum amount of $FeCl_3$ required for effective flocculation may differ (Wyatt et al., 2012). When compared with aluminum sulfate, ferric chloride is generally required in minimum concentrations to promote coagulation of algal cells. In solution, ferric chloride forms positively charged hydroxide precipitate (at pH < 8) which associates with the negative algal cell surface. The ferric hydroxide precipitates form bridges between algal cells which bind them together into flocs. At low algal concentrations, the amount of $FeCl_3$ required to achieve coagulation increases linearly with algal concentration. However, at higher concentrations, the minimum amount of $FeCl_3$ required for flocculation becomes independent of algal concentration, as the dominant mechanism changes from electrostatic bridging to sweep flocculation by large coagulated algal flocs (Wyatt et al., 2012). Major disadvantage of inorganic flocculants such as alum and iron chloride is that it may lead to contamination of growth medium with aluminum or iron (Oh et al., 2001). Nevertheless, they may be useful in treatment of wastewaters, wherein the spent water after mass multiplication of microalgae can be passed through columns to remove the Fe ions and then reused for algal cultivation. In this present study, ferric chloride was found to be a more effective flocculent for harvesting of microalgae compared to alum.

3.2. Evaluation of chitosan for flocculation

Chitosan is a cationic polysaccharide, which has emerged as a favorable flocculating agent in the harvesting of microalgae (Xu et al., 2013). Compared with other commercial flocculants, it has various advantages, including production of larger flocs (Zeng et al., 2008) resulting in faster sedimentation rates and providing a clearer residual solution after harvesting, and being nontoxic and biodegradable (Knuckey et al., 2006). Use of chitosan as flocculent makes it possible to reuse the residual solution to grow microalgae. Chitosan mediated flocculation of *Chlorococcum* sp. R-AP13 was tested at concentrations of 20–120 mg l^{-1}. Flocculation efficiency of 84% was obtained at a concentration of 40 mg l^{-1} and the zeta potential of algal cell was changed from −20 mV to +5 mV (Fig. 2). Further increase in the concentration of chitosan increased the positive charges on the cells which affected further flocculation. This drastic decrease in performance could have resulted when the chitosan overdose caused an overload of positive charges, which were retained on the surface of the cell causing repulsion between positively charged microalgal cells resulting in re-stabilization.

3.3. Flocculation of algal cells by changing pH of the medium

Recently, flocculation induced by increase in pH has gained more attention for algal flocculation (Wu et al., 2012; Rakesh

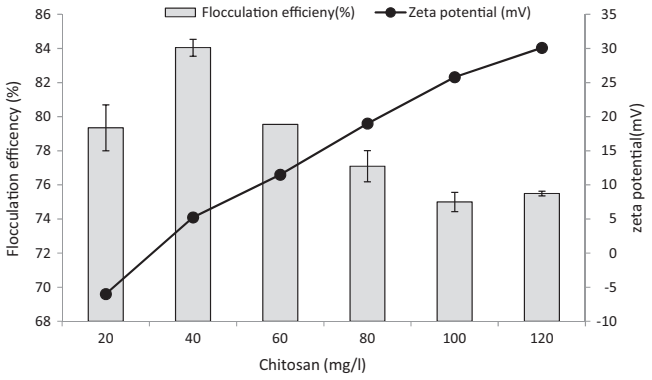

Fig. 2. Flocculation of *Chlorococcum* sp. RAP-13 cells using chitosan.

et al., 2014). In this present study, increase in medium pH as a flocculation agent was evaluated for *Chlorococcum* sp. R-AP13 cells. Flocculation efficiency increased as the medium pH was increased to the alkaline range of 11–12. Maximum efficiency of 94% was obtained with the pH increased to 12. Zeta potential of the algal cell varied with different pH, but the surface charge of the algal cells was negative in the alkaline pH (Fig. 3).

The zeta potentials were pH dependant and negative at different pH values. For freshwater microalgal systems, the zeta potential was shown to initially decrease with increase in pH, but increasing on further increase of pH. The decrease in zeta potential with pH increase indicated the decrease of the cell surface charges, possibly due to charge neutralization in this range. Possible mechanisms of pH mediated flocculation is the formation of $Mg(OH)_2$ precipitate from Mg^{2+} in the growth medium as the pH increased. The $Mg(OH)_2$ precipitate has a large adsorptive surface area and a positive superficial charge (Parks, 1967). This precipitate attracts 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, zeta potential increased after the initial decline, which was attributed to the dissociation of carboxylic acid groups on the surface of microalgal cells (Henderson et al., 2008). However, the flocculation efficiency was significantly higher, indicating that sweep flocculation was active in this pH range (Wu et al., 2012). $Mg(OH)_2$ precipitates tend to have a rather open structure, so that even a small mass could give a large effective volume concentration and hence it has 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. Present results agreed with the previous reports by Wu et al. (2012) and Vandamme et al. (2011).

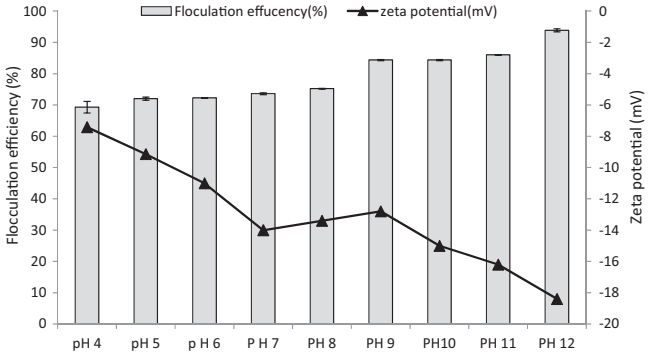

Fig. 3. Flocculation of *Chlorococcum* sp. RAP-13 cells by increase in medium pH.

Flocculation induced by high pH is considered as a potentially useful method to pre-concentrate fresh water microalgal biomass during harvesting (Vandamme et al., 2011). However, as microalgae usually carry a negative surface charge, an increase in pH will cause an increase in surface charge rather than a decrease, which might be the possible cause for flocculation induced by high pH. The use of flocculation induced by high pH for harvesting microalgae may have an additional advantage that the high pH may effectively sterilize the microalgal biomass as well as the process water. This may be advantageous when microalgae are used in wastewater treatment, as the high pH may kill pathogenic microorganisms (Semerjian and Ayoub, 2003). It has been reported that an increase in pH within the range of 8.5–11.0 allows the recovery of microalgae (Horiuchi et al., 2003; Sirin et al., 2012), and has biomass recovery efficiencies higher than 90%.

3.4. Autoflocculation

Autoflocculation of *Chlorococcum* sp. R-AP13 was evaluated by culturing the cells up to 3rd week of incubation under phototrophic condition and flocculation efficiency was tested every week. Flocculation efficiencies increased as the incubation time increased – from 62% in the initial week to maximum efficiency of 75% in the 3rd week of incubation. Zeta potential of cells became more negative with increase in incubation time. Potential of microalgae to autoflocculate depends on their physiological conditions. Autoflocculation may be induced by end of the exponential phase and could be resultant of the pH changes in the culture broth (Wyatt et al., 2012). Algal surface charge and suspension stability is clearly associated to functional groups on the cell wall and zeta-potential is often used as an indicator of cell stability. The decline in zeta-potential from the exponential to stationary phase has been correlated to surface functional groups in *C. zofingiensis* (Zhang et al., 2012). Therefore, micro algal cell instability is presumed to increase in the later growth phase.

Cell flocculation widely occurs in microorganisms and several self-flocculating microalgae have also been discovered, such as *Chlorella vulgaris* JSC-7 (Alam et al., 2014), *Scenedesmus obliquus* AS-6-1 (Guo et al., 2013). Limited reports are available in the literature regarding the auto flocculation of cells and actual mechanism of auto flocculation is still obscure. Guo et al. (2013) and Alam et al. (2014) had studied the biochemical basis of auto flocculation in the micro algae *S. obliquus* AS-6-1 and *C. vulgaris* JSC-7 respectively. They found that the polysaccharides biosynthesized by these two strains were responsible for self-flocculation. Another recent report proposed that glycoproteins are involved in cell flocculation of the green microalga *Ettlia texensis* SAG79.80 (Salim et al., 2014). Therefore, microalgal self-flocculation may occur when the flocculating agents (e.g., polysaccharides and glycoprotein) produced by microalgal cells themselves patch adjacent cells, or it may be due to formation of bridges between the cells via charge neutralization with changes in medium pH, promoting self-flocculation. More research is needed in this area to understand the exact mechanism of self flocculation of microalgal cells. Microalgal self-flocculation, differing from the flocculation induced by pH adjustment, can occur naturally via interaction of adjacent cells without acid, alkaline, or metal ion addition. Moreover, harvesting microalgae using self-flocculation, which requires no extra expenditure in cultivation of microalga or purification of bio-flocculent, is a promising method for low-cost harvesting.

3.5. Viability of flocculated biomass

Viability assay of flocculated biomass was carried out by Trypan blue staining of the cells. Auto flocculated cells, cells flocculated by chitosan and through change in medium pH were found to be

viable. However, at least some cells flocculated through aluminum sulfate and ferric chloride showed dye uptake indicating the presence of dead cells and the percentage of dead cells were proportionate to the concentration of the flocculent. Inorganic flocculants, including alum and iron chloride, may also lead to contamination of the growth medium with aluminum or iron (Oh et al., 2001). Flocculation by alum or ferric chloride therefore cannot be considered as a preferred method for algal biomass recovery in this case, since it was found to be toxic to the cells besides contaminating the residual medium. Flocculation mediated by chitosan was very effective for harvesting the biomass, with the added advantages of non toxicity and complete clarification of medium after flocculation. However, the cost of chitosan is high making it not a feasible option for large scale usage. Flocculation mediated by auto flocculation or induced by pH increase may be considered as effective strategies for harvesting the microalgal biomass since these are low cost processes and no extra flocculants are required for harvesting of the biomass.

3.6. Recycling of flocculated medium for algal cultivation

Medium recovered from flocculation could preferably be recycled for next round of cultivation. In the flocculation studies performed, the medium was recovered after flocculation and then were supplemented with nutrients (Components of MA medium). The medium pH was adjusted to 6.8–7.0, and was used for algal cultivation so as to evaluate the possibility for medium recycling. *Chlorococcum* sp. R-AP13 cells were cultivated in the recycled medium (Fig. 4). It was observed that the cell densities of *Chlorococcum* sp. R-AP13 cultivated in the recycled growth medium were close to that cultivated in fresh MA medium, indicating that the residual medium after flocculation and separation of cells could be successfully recycled for cultivation of the alga. Previous studies conducted with *P. tricornutum*, *Nannochloropsis oculata* and *Chlorococcum* sp. have also concluded that the biomass recovery from fresh or recycled media were similar (Wu et al., 2012; Liu et al., 2013).

3.7. Fatty acids profiling of flocculated biomass

Fatty acids profiling of flocculated biomass was carried out to check any changes in the lipids profile of biomass after the addition of flocculants in the medium. Results showed that fatty acids profile of auto flocculated biomass, pH induced and chitosan mediated flocculated biomass were not affected, while the biomass from aluminum sulfate and ferric chloride flocculated cultures showed differences in the fatty acids profile (Table 1). Fatty acids profile of biomass from fresh medium and residual medium from pH treatment showed almost similar profile whereas biomass grown in residual medium from chitosan treatment showed longer chain fatty acids with oleic acid as the major fatty acid. Lipids profile of algal cells grown in residual media from aluminum sulfate and ferric chloride treatments also showed elevated oleic acid content which was significantly higher that the levels of this fatty acid from cells grown in fresh media. Oleic acid production might have a protective role to cope up with the toxic chemicals in the medium. Increase in the unsaturated fatty acid content of algae grown in reused media could also be a sign of stress and may be recognized as a mechanism of adaptation to the environmental conditions. It has been suggested that algal TAG serves as a depot of PUFA, which can be mobilized for the construction of chloroplast membranes under certain environmental conditions (Khozin-Goldberg et al., 2005).

Table 1
Fatty acid profile of algal biomass cultivated in fresh medium and residual medium from different flocculation treatments.

Fatty acid type	Fatty acid content in the oil (%)									
	Auto flocculation		Chitosan		pH 12		$FeCl_3$		$AlSO_4$	
	F	R	F	R	F	R	F	R	F	R
C12	3.7	2.8	2.8	1.8	4	3.6	3.0	7.2	1.6	5.6
C14	2.3	3.4	3.2	1.6	3	2.8	–	2.6	–	0.8
C15	1.7	1.8	1.8	–	3.2	3.2	1.8	–	–	–
C16	39.7	39.8	42	22.6	41.2	44.2	44	10.2	38	6
C16:1	3.6	5.2	2.8	2.8	3.7	3.8	–	2.2	–	–
C17	2.1	1.2	3.7	2.6	3.1	4.2	2.1	–	–	–
C18:0	8.1	9.2	6.2	8	3.8	5.8	20	6.4	18	8.2
C18:1	22.0	16.2.	20	34.2	16.5	20.8	24	47.8	26	57.3
C18:2	3.2	4.6	7.8	6.2	7.1	5.8	8	4.4	12	2.8
C18:3	7.5	6	3.1	5.6	8.2	2.4	12	9.8	8	12.7
C22	–	–	–	2.6	–	–	–	1.8	–	2.3
C22:1	5.9	4.6	5.8	5.8	5	2.8	–	5.8	–	2.6
C24	–	–	–	3.4	–	–	–	–	–	–

F – Fresh medium; R – Recycled medium (residual medium after flocculation).

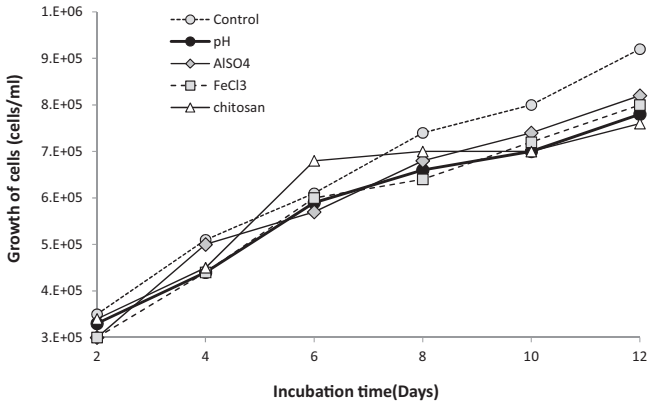

Fig. 4. Growth of *Chlorococcum* sp. R-AP13 in recycled medium.

4. Conclusion

Development of economically feasible flocculation technology can significantly reduce the cost of microalgal biomass production. pH modulation as a flocculation method seems to be a feasible strategy since it attained a flocculation efficiency of 94%, and allowed re-use of the medium. pH-induced flocculation and auto-flocculation therefore can be considered as best possible options for cost effective and efficient harvesting of *Chlorococcum* cells. These methods also allow the re-use of media for further cycles of cultivation thereby minimizing fresh water requirement. The self flocculating micro alga – *Chlorococcum* sp. R-AP13 can be used for various applications such as biofuels and nutraceuticals.

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References

Alam, M.A., Wan, C., Guo, S.L., Zhao, X.Q., Huang, Z.Y., Yang, Y.L., Bai, F.W., 2014. Characterization of the flocculating agent from the spontaneously flocculating microalga *Chlorella vulgaris* JSC-7. J. Biosci. Bioeng. 118 (1), 29–33.

Castrillo, M., Lucas-Salas, L.M., Rodríguez-Gila, C., Martínez, D., 2013. High pH-induced flocculation–sedimentation and effect of supernatant reuse on growth rate and lipid productivity of *Scenedesmus obliquus* and *Chlorella vulgaris*. Bioresour. Technol. 128, 324–329.

Chen, G., Zhao, L., Qi, Y., Cui, Y.L., 2014. Chitosan and its derivatives applied in harvesting microalgae for biodiesel production: an outlook. J. Nanomater. 2014. http://dx.doi.org/10.1155/2014/217537.

Cheng, Y.S., Zheng, Y., Labavitch, J.M., Vander Gheynst, J.S., 2011. The impact of cell wall carbohydrate composition on the chitosan flocculation of *Chlorella*. Process Biochem. 46 (10), 1927–1933.

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

Garzon-Sanabria, A.J., Davis, R.T., Nikolov, Z.L., 2012. Harvesting *Nannochloropsis oculata* by inorganic electrolyte flocculation: effect of initial cell density, ionic strength, coagulant dosage, and media pH. Bioresour. Technol. 118, 418–424.

Georgiana, D.R., Mayfield, S.P., 2012. Exploiting diversity and synthetic biology for the production of algal biofuels. Nature 488 (7411), 329–335.

Gerde, J.A., Yao, L., Lio, J., Wen, Z., Wang, T., 2014. Microalgae flocculation: impact of flocculant type, algae species and cell concentration. Algal Res. 3, 30–35.

Guo, S.L., Zhao, X.Q., Wan, C., Huang, Z.Y., Yang, Y.L., Alam, M.A., Ho, S.H., Chang, J.S., 2013. Characterization of flocculating agent from the self-flocculating microalga *Scenedesmus obliquus* AS-6-1 for efficient biomass harvest. Bioresour. Technol. 145, 285–289.

Henderson, R., Parsons, S., Jefferson, B., 2008. Successful removal of algae through the control of zeta potential. Sep. Sci. Technol. 43 (7), 1653–1666.

Horiuchi, J.I., Ohba, I., Tada, K., Kobayashi, M., Kanno, T., Kishimoto, M., 2003. Effective cell harvesting of the halotolerant microalga *Dunaliella tertiolecta* with pH control. J. Biosci. Bioeng. 95 (4), 412–415.

Khozin-Goldberg, I., Shrestha, P., Cohen, Z., 2005. Mobilization of arachidonyl moieties from triacylglycerols into chloroplastic lipids following recovery from nitrogen starvation of the microalga *Parietochloris incisa*. Biochim. Biophys. Acta 1738, 63–71.

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

Liu, J.X., Zhu, Y., Tao, Y.J., Zhang, Y.M., Li, A.F., Li, T., Sang, M., Zhang, C.W., 2013. Freshwater microalgae harvested via flocculation induced by pH decrease. Biotechnol. Biofuels 6, 98.

Mata, T.M., Martins, A.A., Caetano, N.S., 2010. Microalgae for biodiesel production and other applications: a review. Renewable Sustainable Energy Rev. 14 (1), 217–232.

Milledge, J.J., Heaven, S., 2013. A review of the harvesting of micro-algae for biofuel production. Rev. Environ. Sci. Bio. 12 (2), 165–178.

Oh, H.M., Lee, S.J., 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 (15), 1229–1234.

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

Rakesh, S., Saxena, S., Dhar, D.W., Prasanna, R., Saxena, A.K., 2014. Comparative evaluation of inorganic and organic amendments for their flocculation efficiency of selected microalgae. J. Appl. Phycol. 26 (1), 399–406.

Salim, S., Kosterink, N.R., Tchetkoua Wacka, N.D., Vermue, M.H., Wijffels, R.H., 2014. Mechanism behind autoflocculation of unicellular green microalgae *Ettlia texensis*. J. Biotechnol. 174, 34–38.

Semerjian, L., Ayoub, G.M., 2003. High-pH–magnesium coagulation–flocculation in waste water treatment. Adv. Environ. Res. 7 (2), 389–403.

Sirin, S., Trobajo, R., Ibanez, C., Salvado, J., 2012. Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. J. Appl. Phycol. 24 (5), 1067–1080.

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. J. Renewable Sustainable Energy 2 (1), 012701.

Ummalyma, S.B., Sukumaran, R.K., 2014. Cultivation of microalgae in dairy effluent for oil production and removal of organic pollution load. Bioresour. Technol. 165, 295–301.

Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K., 2010. Flocculation of microalgae using cationic starch. J. Appl. Phycol. 22 (4), 525–530.

Vandamme, D., Pontes, S., Goiris, C.V., Foubert, I., Pinoy, L.J.J., Muylaert, K., 2011. Evaluation of electro coagulation–flocculation for harvesting marine and freshwater microalgae. Biotechnol. Bioeng. 108 (10), 2320–2329.

Vandamme, D., Foubert, I., Muylaert, K., 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends Biotechnol. 31 (4), 233–239.

Wan, C., Alam, M.A., Zhao, X.Q., Zhang, X.Y., Guo, S.L., Ho, S.H., Chang, J.S., Bai, F.W., 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. Bioresour. Technol. 184, 251–257.

Wijffels, R.H., Barbosa, M.J., 2010. An outlook on microalgal biofuels. Science 329 (5993), 796–799 (Washington).

Wu, Z., Zhu, Y., Huang, W., Zhang, C., Li, T., Zhang, Y., Li, A., 2012. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. Bioresour. Technol. 110, 496–502.

Wyatt, N.B., Gloe, L.M., Brady, P.V., Hewson, J.C., Grillet, A.M., Hankins, M.G., Pohl, P. I., 2012. Critical conditions for ferric chloride induced flocculation of freshwater algae. Biotechnol. Bioeng. 109 (2), 493–501.

Xu, Y., Purton, S., Baganz, F., 2013. Chitosan flocculation to aid the harvesting of the microalga *Chlorella sorokiniana*. Bioresour. Technol. 129, 296–301.

Yang, F., Xiang, W., Fan, J., Wu, H., Li, T., Long, L., 2015. High pH-induced flocculation of marine *Chlorella* sp. for biofuel production. J. Appl. Phycol., 1–10

Zeng, D., Wu, J., Kennedy, J.F., 2008. Application of a chitosan flocculent to water treatment. Carbohydr. Polym. 71 (1), 135–139.

Zhang, X., Amendola, P., Hewson, J.C., Sommerfeld, M., Hu, Q., 2012. Influence of growth phase on harvesting of *Chlorella zofingiensis* by dissolved air flotation. Bioresour. Technol. 116, 477–484.