J Appl Phycol (2014) 26:399–406
DOI 10.1007/s10811-013-0114-4

# Comparative evaluation of inorganic and organic amendments for their flocculation efficiency of selected microalgae

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Received: 5 June 2013 / Revised and accepted: 13 August 2013 / Published online: 5 September 2013

**Abstract** Cost-efficient harvesting of microalgae is a major challenge due to their small size and often low concentration in the culture medium. The flocculation efficacy of different inorganic and organic amendments was evaluated on various microalgae genera—one strain each belonging to *Chlamydomonas*, *Chlorococcum*, two of *Botryococcus*, and of *Chlorella*. An improvised medium comprising of commercial grade urea, single super phosphate, and muriate of potash was used to grow the microalgae for flocculation experiments. High pH induced increased flocculation efficiency (72–76 %) in selected microalgal strains. Ferric chloride was found to be the most efficient for most of the microalgal strains, while maize starch and rice starch proved superior for *Chlorella* sp. MCC6 and *Botryococcus* sp. MCC32. Although the highest flocculation efficiency was obtained with inorganic flocculant, i.e., ferric chloride (87.3 %) with *Botryococcus* MCC31, this was comparable with rice starch (86.8 %) for *Botryococcus* MCC32. This study showed that widely available cheaper biopolymers such as rice starch, maize, and potato starch can be promising flocculants due to their better harvesting efficiency (>80 %) and low price, thereby contributing to economical production of biodiesel from algae.

**Keywords** Flocculation · Harvesting · Microalgae · Inorganic · Organic amendments

## Introduction

Microalgae have potential as feedstocks for biodiesel (Ratha and Prasanna 2012; Singh and Dhar 2011), besides their value as a source of bioactive molecules, pigments, and nutraceuticals (Gupta et al. 2013); however, most of the technologies currently available are not economically feasible. Intensive cultivation for production of large quantities of microalgal biomass requires an efficient harvesting technique, especially for minimizing the energy consumption of harvesting microalgae (Benemann 1997). One of the major hurdles in large-scale cost-effective production of microalgae is the development of effective downstream process to enable efficient separation of cells from culture broth. Additionally, maintaining their viability and bioactivity prior to use as biofuels or as sources of value added products becomes integral to their utility. Low cell densities, small size of cells, and the electronegative surface of cells makes recovery of microalgal biomass difficult (Brennan and Owende 2010), and efficient biomass harvesting is one of the bottlenecks to develop a cost-effective process (Chen et al. 2011; Georgianna and Mayfield 2012; Larkum et al. 2012). The major techniques presently applied in the harvesting of microalgae include centrifugation, flocculation, filtration, gravity sedimentation, flotation, and electrolytic methods (Chen et al. 2011; Christenson and Sims 2011). The process options and economics of the different methods for the recovery of microalgal biomass have been reviewed by Molina Grima et al. (2003).

Flocculation is a widely used process for microalgae harvesting, which can be induced by inorganic or polymeric flocculants. Despite versatile applications of such flocculants, there are some limitations with their use. Inorganic flocculants (e.g., alum and ferric chloride) are toxic and produce large amount of sludge, while polymeric flocculants are expensive to use. Biopolymers, on the other hand, are cheap, efficient, and environmentally friendly. An efficient flocculation process that

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can maintain high cell viability could be a method of choice, as it is rapid, inexpensive, and a more simple method for harvesting large quantity of microalgae cells from culture broth, prior to utilization of microalgal biomass for extraction of biochemicals or biofuels. The flocculation efficiency of microalgae for separation of cells from the culture broth was found to be greatly influenced by pH, NaOH, and varying concentrations of inorganic (ferric chloride, aluminium sulphate, and calcium chloride) and organic amendments.

Evaluation of several harvesting methods has shown that flocculation combined with flotation or sedimentation and subsequent further dewatering by centrifugation or filtration is the most promising cost- and energy-efficient alternative (Schenk et al. 2008). Flocculation induced in different ways and chemical flocculation using $Zn^{2+}$, $Al^{3+}$, $Fe^{3+}$, or other flocculants has been studied extensively (e.g., McGarry 1970; Lee et al. 1998; Papazi et al. 2010; Wyatt et al. 2012). Inorganic flocculants such as alum and iron chloride are efficient, but these are required in high doses and result in contamination of the biomass with aluminium or iron (Becker 1994; Wyatt et al. 2012).

Biodegradable organic flocculants may not contaminate the algal biomass and are often required in lower doses (Singh et al. 2000; Rashid et al. 2013). They are based on biopolymers like chitosan, guar gum, alginic acid, or starch. Among these, chitosan has been shown to be an effective flocculant for microalgae (Divakaran and Pillai 2002), which has no apparent toxic effects on the harvested algae (Knuckey et al. 2006). During flocculation, the dispersed microalgal cells aggregate and form larger particles with higher sedimentation rate. Flocculation occurs when solid particles aggregate into large but loose particles resulting from the interaction of the flocculants with the surface charge of the suspended solid and subsequent coalescing of these aggregates into large flocs that settle out of suspension (Knuckey et al. 2006). This process has been extensively used in the industry to remove suspended solids such as in wastewater treatment (Mahvi and Razavi 2005), clarification of drinking water, color removal in paper making industry, and mineral processing (Yoon and Deng 2004).

Several factors such as the flocculant type and its dose, settling time, and culture pH affect the harvesting efficiency of microalgae (Xu et al. 2011). Scattered information is available in published literature regarding the use of different organic/inorganic chemicals or pH or comparative analyses of different unicellular microalgal strains. In the present investigation, we have compared the potential of promising organic and inorganic chemicals (based on available literature) and pH as a flocculant for harvesting of biomass of seven unicellular microalgae strains.

## Materials and methods

Seven microalgae, namely *Chlamydomonas* sp. MCC28, *Chlorella* sp. MCC29, *Chlorococcum* sp. MCC30, *Botryococcus* sp. MCC31, *Botryococcus* sp. MCC32, *Chlorella* sp. MCC6, and *Chlorella sorokiniana* MIC-G5 were obtained from the culture collection of Division of Microbiology and CCUBGA, IARI, New Delhi. These cultures were grown in trays under greenhouse conditions of 25 °C and natural sunlight (61–68 μmol photons $m^{-2}$ $s^{-1}$) in a commercial medium containing NPK fertilizers (i.e., urea, single super phosphate, and muriate of potash in appropriate ratio). Batch cultures were maintained by inoculating a known volume of fresh medium every 2 weeks for use as seed culture.

*Determination of flocculation efficiency* Inorganic (pH, aluminium sulphate, calcium chloride, and ferric chloride) as well as organic (chitosan, carboxymethyl cellulose, maize starch, cationic starch, potato starch, tapioca starch, yellow dextrin, rice starch, oxidized starch, and pregelatinized starch) amendments were added to the 2-week-old microalgae cultures.

Microalgal suspension (50 mL) in a 250 mL conical flask either alone as reference or with inorganic/organic amendments were kept on shaker at 50 rpm for 10 min at room temperature and allowed to settle for 30 min in an Imhoff cone. After the flocculation of microalgal cells, an aliquot of culture was withdrawn at a level two-thirds from the bottom of the Imhoff cone. The absorbance of remaining suspension of the clear region was measured at 680 nm. The flocculation efficiency was evaluated by comparing this with the absorbance before treatment. The flocculation or harvesting efficiency (%) was calculated using Eq (1) (Harith et al. 2009).

$$Flocculation/harvest\,efficiency(\%) = \frac{\mathrm{C_i - C_f}}{\mathrm{C_i}} \times 100 \qquad (1)$$

Where $C_i$ is the absorbance of cells in suspension before treatment and $C_f$ is the final absorbance of cells in suspension after flocculation.

### Cost economics of organic and inorganic amendments used for harvesting microalgae

The economics of use of all the inorganic as well as organic amendments for harvesting of microalgae was calculated based upon the costs incurred for procurement of the chemicals and related requirements.

## Results

*Effect of pH on flocculation efficiency* Alkaline pH values of 10 and 11 were found to be most effective in enhancing the flocculation efficiency of the microalgal cultures. pH 10 was optimum for all cultures except for *Chlorella* sp. MCC29 and *Botryococcus* sp. MCC32. Modulation of pH did not bring about any significant change in the efficiency of flocculation of *Chlorella* sp. MCC6 and *C. sorokiniana* MIC-G5 (Fig. 1a).

### Effect of inorganics on flocculation efficiency

Inorganic chemicals viz, aluminium sulphate, calcium chloride, and ferric chloride were more effective than pH adjustment for the recovery of algae. *Botryococcus* sp. MCC32 formed dense flocs and quickly precipitated during the settling phase of the test at 200 mg $L^{-1}$. The supernatant was almost clear and the removal rate reached up to 81.9 % with aluminium sulphate. Low concentration (50 mg $L^{-1}$) of aluminium sulphate was more effective in enhancing flocculation of most of the microalgal cultures except for *Botryococcus* sp. MCC31 and MCC32, which showed enhanced flocculation at 150 and 200 mg $L^{-1}$ concentration (Fig. 1b).

Calcium chloride concentrations had a variable effect on flocculation efficiency of microalgae, but flocculation of *Chlorella* sp. MCC6 and *C. sorokiniana* MIC-G5 was not influenced significantly (Fig. 1c). The change in ferric chloride concentrations showed a differential effect on the flocculation efficiency of microalgae. Flocculation efficiency of above 80 % was achieved for most of the microalgae except for *Botryococcus* sp. MCC32 and *Chlorella* sp. MCC6 (Fig. 1d).

### Effect of organics on flocculation efficiency

Higher concentrations of 120 and 150 mg $L^{-1}$ were more effective in enhancing flocculation of microalgal cultures, except for *Chlorella* sp. MCC6 and *C. sorokiniana* MIC-G5, which showed enhanced flocculation at 60 and 10 mg $L^{-1}$ concentration. Flocculation efficiency was at par using 10 and 120 mg $L^{-1}$ concentration of chitosan for *Chlamydomonas* sp. MCC28.

The effect of carboxymethyl cellulose (CMC) concentrations on flocculation efficiency of microalgae was variable. CMC was not effective for most of the microalgal cultures, except for *Botryococcus* sp. MCC32 and *Chlorella* sp. MCC6, which showed enhanced flocculation of 82 and 62 % at 10 mg $L^{-1}$ concentration. Low concentrations of maize starch were not very effective in enhancing flocculation, whereas concentration of 120 mg $L^{-1}$ showed higher flocculation efficiency for most of the microalgal cultures. Cationic starch concentrations had a variable influence on the flocculation efficiency of microalgae (Fig. 2a).

A higher concentration (150 mg $L^{-1}$) of potato starch was more effective in enhancing the flocculation of the microalgal cultures except for *Chlamydomonas* sp. MCC28, *Botryococcus* sp. MCC31, and *C. sorokiniana* MIC-G5, which showed enhanced flocculation at 120 mg $L^{-1}$ potato starch. Tapioca starch concentrations showed a variable effect on flocculation efficiency.

Yellow dextrin at a concentration of 120 mg $L^{-1}$ was more effective in enhancing flocculation of *Chlamydomonas* sp. MCC28, *Chlorella* sp. MCC29, *Botryococcus* sp. MCC32, *Chlorella* sp. MCC6, and C. *sorokiniana* MIC-G5. However, *Chlorococcum* sp. MCC30 and *Botryococcus* sp. MCC31 showed enhanced flocculation at 60 and 30 mg $L^{-1}$ concentration (Fig. 2b).

Addition of rice starch into microalgal suspension at various concentrations showed a differential effect on the flocculation efficiency. A concentration of 90 mg $L^{-1}$ was more effective in enhancing flocculation of *Chlamydomonas* sp. MCC28, *Botryococcus* sp. MCC31, and *Botryococcus* sp. MCC32. However, *Chlorella* sp. MCC29, *Chlorococcum* sp. MCC30, and *Chlorella* sp. MCC6 showed enhanced flocculation at 120 mg $L^{-1}$ concentration. Oxidized starch at a concentration of 150 mg $L^{-1}$ was most effective in flocculation of the algae, except for *Chlamydomonas* sp. MCC28 and *C. sorokiniana* MIC-G5, which showed enhanced flocculation at 120 and 90 mg $L^{-1}$, respectively. Lower concentration of 10 mg $L^{-1}$ of pregelatinized starch was more effective in enhancing flocculation of *Chlorococcum* sp. MCC30 and *Chlorella* sp. MCC6. In *Botryococcus* sp. MCC31, maximum flocculation was observed at 90 mg $L^{-1}$ concentration; while for the remaining microalgal suspensions, a concentration of 120 mg $L^{-1}$ exhibited enhanced flocculation (Fig. 2c).

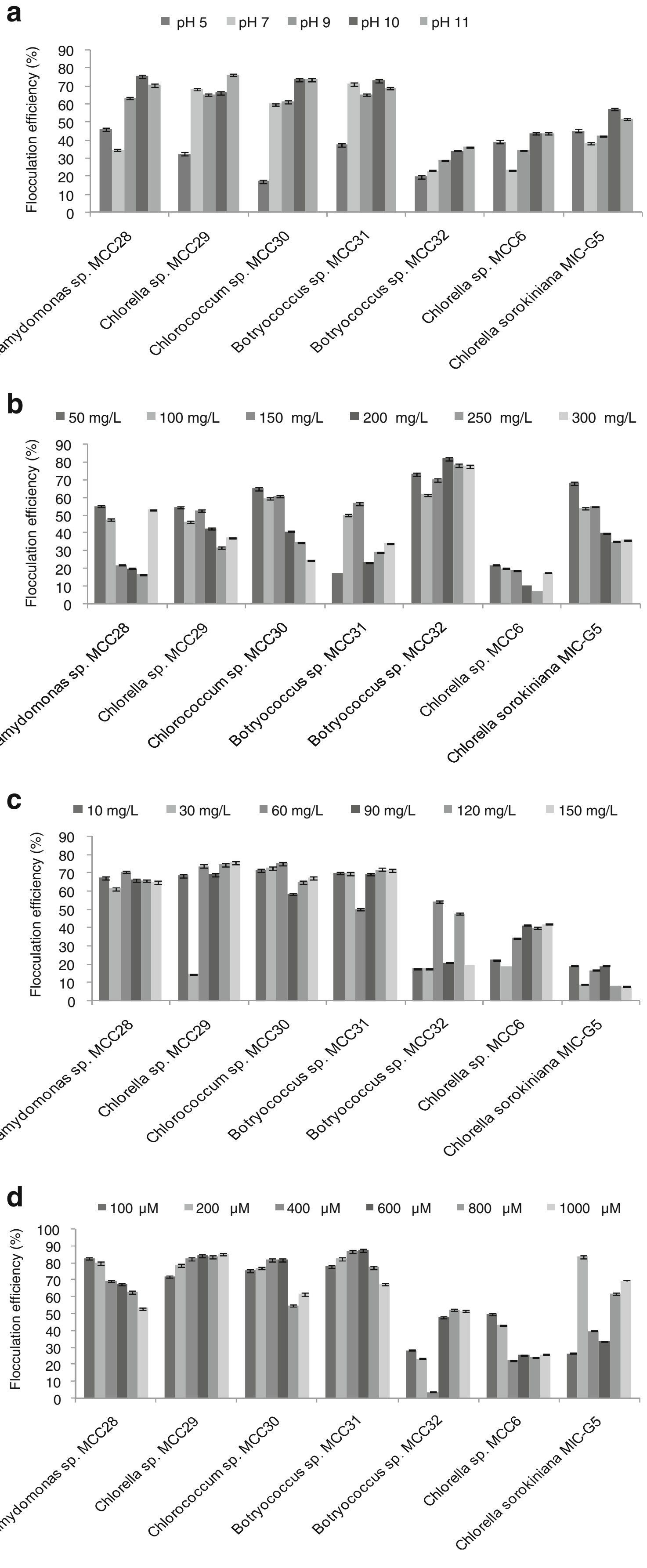
### Cost economics

The cost of all the inorganic and organic amendments was obtained from local suppliers and calculated. Comparison of cost and the efficiency indicated that rice starch was more economically feasible for its utilization as a flocculant for efficient harvesting of microalgae (Table 1). The cost of inorganic chemicals, CMC, and chitosan varied on a per kilogram basis. The price of other organic chemicals like different starches and yellow dextrin was only US$0.7 on a per kilogram basis, while the cost for rice starch was only US$0.1 per 1,000 L culture harvested. The price of ferric chloride was US$0.7 per 1,000 L. In general, the price of inorganic amendments was 20 times more than organic amendments per liter of suspension.

## Discussion

Mass production technologies for microalgae require efficient methods for harvesting, which determine the economics of the available process options (Molina Grima et al. 2003). The nature of flocculants and their concentrations play a critical role in flocculation process, as these can influence the extent as well as the rate of flocculation. Therefore, preliminary experiments were undertaken to determine the effects of pH, salinity, and different concentrations of various organic and

**Fig. 1** Flocculation efficiency of seven microalgal strains (*Chlamydomonas* sp. MCC28, *Chlorella* sp. MCC29, *Chlorococcum* sp. MCC30, *Botryococcus* sp. MCC31, *Botryococcus* sp. MCC32, *Chlorella* sp. MCC6, and *Chlorella sorokiniana* MIC-G5) as influenced by **a** different pH levels, **b** aluminium sulphate concentrations, **c** calcium chloride concentrations, and **d** ferric chloride concentrations. Level of significance was determined at $P < 0.05$. Each value is the mean of three replicates



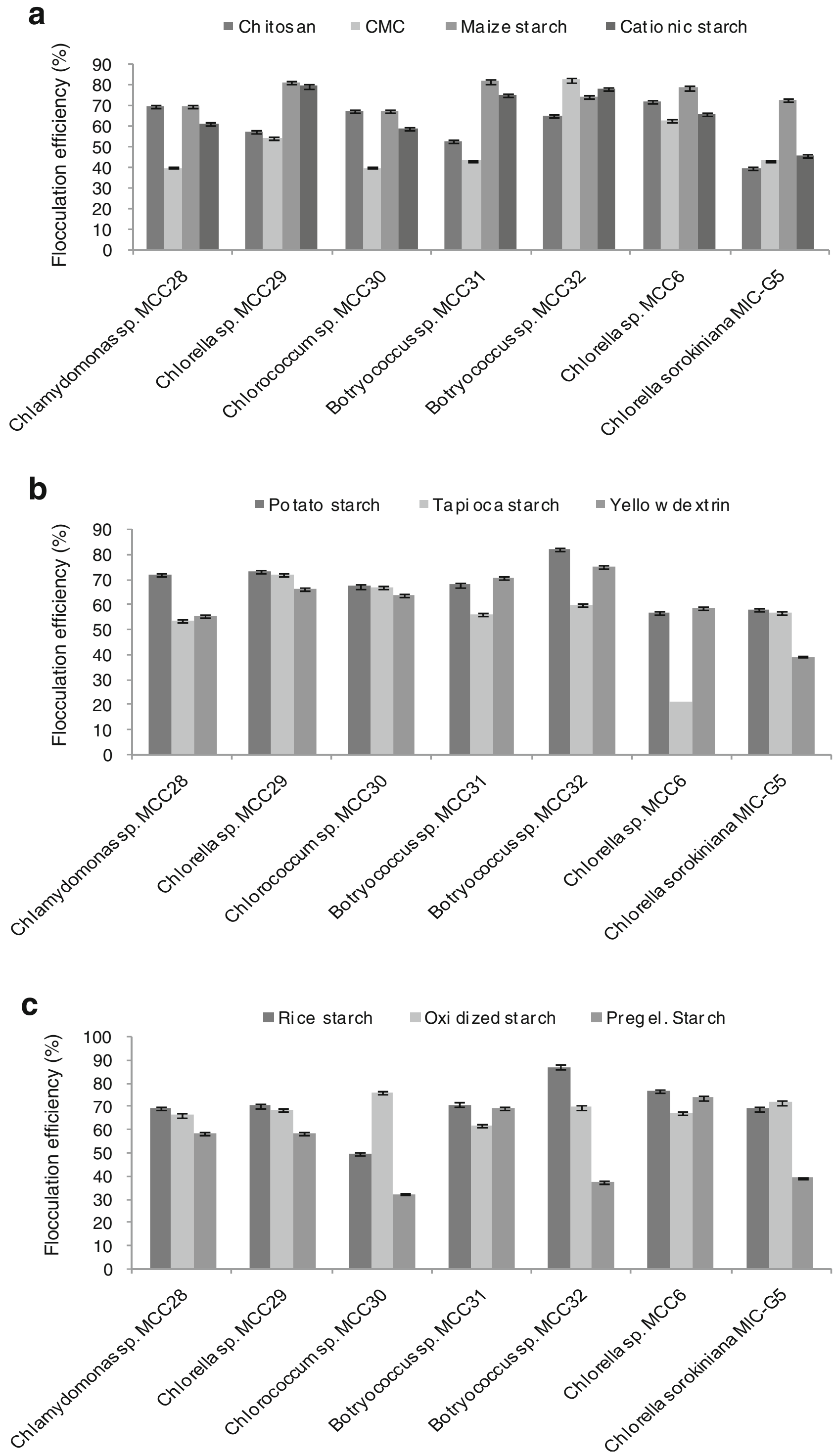


**Fig. 2** Dose-response curves for different flocculation methods in seven microalgal strains (*Chlamydomonas* sp. MCC28, *Chlorella* sp. MCC29, *Chlorococcum* sp. MCC30, *Botryococcus* sp. MCC31, *Botryococcus* sp. MCC32, *Chlorella* sp. MCC6, and *Chlorella sorokiniana* MIC-G5) on flocculation efficiency using **a** chitosan, CMC, maize starch and cationic starch, **b** potato starch, tapioca starch and yellow dextrin, and **c** rice starch, oxidized starch, and pregelitinized starch. Level of significance was determined at $P < 0.05$. Each value is the mean of three replicates

inorganic amendments on the flocculation of 2-week-old cultures of selected microalgae.

Buelna et al. (1990) reported that higher pH levels were effective in algae sedimentation. Millamena et al. (1990) also observed precipitation, when culture pH was raised to 10. Wu et al. (2012) hypothesized that the mechanism underlying flocculation mediated by high pH may be related to the presence of magnesium ions in the growth medium, which is hydrolyzed to form magnesium hydroxide precipitate, which coagulates cells by sweeping flocculation and charge neutralization. The present investigation is in agreement with the previous studies of Blanchemain et al. (1994), Yahi et al. (1994), and Vandamme et al. (2012) on different algae. Flocculation induced by high pH is considered as a potentially useful method to preconcentrate

**Table 1** Cost economics of inorganic and organic amendments used for harvesting microalgae

| Flocculants used | Price/kg (in US$) | Cost involved in flocculation for 1,000 L (in US$)[c] |
|---|---|---|
| Inorganic[b] | | |
| Ferric chloride | 14.1 | 0.7 |
| Aluminium sulphate | 5.6 | 0.3 |
| Calcium chloride | 60.7 | 3.7 |
| Organic[a] | | |
| Chitosan[b] | 207.2 | 31.1 |
| Carboxymethyl cellulose[b] | 18.3 | 2.2 |
| Rice starch | 5.9 (1 kg of each, total 8 kg) US$0.7 approx. per kg | 0.1 |
| Maize starch | | |
| Oxidized starch | | |
| Tapioca starch | | |
| Yellow dextrin | | |
| Potato starch | | |
| Pregelatinized starch | | |
| Cationic starch | | |

[a] Tirupati Industrial Chemicals, Pvt. Ltd, Faridabad, Haryana

[b] Sigma-Aldrich, Bangalore

[c] Cost involved in flocculation at a concentration, where maximum flocculation efficiency is achieved

freshwater microalgal biomass during harvesting (Vandamme et al. 2012). However, as microalgae generally 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 in that the high pH effectively sterilizes 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 observed that, irrespective of the method, an increase in pH within the range of 8.5 to 10.5 allows the recovery of microalgae such as *Phaeodactylum tricornutum* (Sirin et al. 2011), *Anabaena marina* (González-López et al. 2009), or *Dunaliella tertiolecta* (Horiuchi et al. 2003) with biomass recovery values higher than 90 %.

Lee et al. (1998) also reported that a high pH of 11 was more effective for the flocculation of the green alga *Botryococcus braunii* than using aluminium sulfate as a flocculant.

Although in the present study, the growth medium was discarded after the harvesting of biomass, since no flocculants were used and medium was not contaminated, the growth medium after flocculation can be reused by neutralizing pH and supplementing with nutrients. The recycling of flocculated medium can minimize the cost of nutrients and the demand for water, besides being useful for growth and value addition purposes (Castrillo et al. 2013). The flocculated microalgal cells exhibit growth which demonstrates that no cell lysis occurs during the flocculation by pH increase, and the function and structure of the photosynthetic apparatus are not affected (Wu et al. 2012).

The flocculation mechanism depends on the nature of the cell and the charge of the flocculant. Numerous chemical coagulants or flocculants have been tested in literature (e.g., McGarry 1970; Papazi et al. 2010). Metal salts (aluminium sulphate, ferric chloride, etc.) are generally preferred because they lead to improved harvesting efficiency.

Alum, which has proven to be an efficient flocculant for other species of algae (Clasen et al. 2000), showed an increase in turbidity of algal cultures at high concentration of 200 mg $L^{-1}$. Avnimelech et al. (1982) reported that the variable flocculation potential of different algae depends on the composition and properties of the cell wall, the extent and type of excretions, physiological conditions, age, and other factors. The biochemical composition of microalgae also changed when culture parameters were modified (Brown et al. 1996).

Granados et al. (2012) observed that polyelectrolytes at low doses of 2–2.5 mg $g^{-1}$ biomass were most efficient for recovery of biomass, but this may not be economically viable, as these products are costly and availability is restricted. Multivalent aluminium and iron metal salts have been widely used to flocculate algal biomass (Bernhardt and Clasen 1994; Papazi et al. 2010), and di- or trivalent cationic flocculants such as $CaCl_2$ and $FeCl_3$ were found to be more effective than the monovalent cationic flocculants.

The results of ferric chloride as flocculant compared well with the reported efficiencies of over 90 % using ferric chloride for the flocculation of marine algae (Sukenik et al. 1988). For any given algae species, effective flocculation with $FeCl_3$ may be obtained, if the conditions of negative surface charge and sufficient ferric chloride concentration are met. Since different algae species vary in their concentrations of functional groups on the algal surface, the minimum amount of $FeCl_3$ required for effective flocculation may differ (Wyatt et al. 2012). Kwon et al. (1996) reported that the flocculating activity of a bioflocculant from *Pestalotiopsis* sp. was highest on addition of cationic solutions, especially 8 mM $CaCl_2$ or $FeCl_3$. It was observed that such concentrations of $Fe^{3+}$ ions contribute to microalgal floc formation and results in a separation efficiency of 62 % after 5 min of sedimentation (Sun et al. 1998).

In general, a minimum amount of ferric chloride is required to promote coagulation of algae cells. In solution, ferric chloride forms a positively charged hydroxide precipitate (at pH <8) which associates with the negative algae cell surface. The ferric hydroxide precipitates form bridges between algae 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). Inorganic flocculants, including alum and iron chloride, also lead to contamination of growth medium with aluminium or iron (Oh et al. 2001); however, 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 growth purposes.

In the last few decades, chitosan has emerged as a favorable flocculating agent as a cationic polysaccharide in the harvesting of microalgae. Compared with other commercial flocculants, it presents various advantages, including producing larger flocs (Zeng et al. 2008), resulting in faster sedimentation rates, providing a clearer residual solution after harvesting for higher flocculation efficiency, and being nontoxic (Knuckey et al. 2006), which makes it possible to reuse the residual solution again to grow microalgae.

Earlier studies using chitosan revealed similar differential response on flocculation efficiency of microalgae with change in concentrations with an efficiency of 75 % having been reported for Lubian (1989). Rashid and co-workers (2013) found that 120 mg $L^{-1}$ of chitosan showed the highest flocculation efficiency (within 3 min), and pH of 6 was optimal for *Chlorella vulgaris*. Suspensions of unicellular microalgae are known to be stabilized by the negative surface charge of the algal cells and organic flocculants, viz starches which can induce flocculation of negatively charged particles through bridging and/or patch charge neutralization (Sharma et al. 2006; Bratby 2006). Cationic starch can be used as a cheaper and more widely available alternative to chitosan (Vandamme et al. 2010). 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 bulk costs of about US\$2 $kg^{-1}$ for chitosan (Rashid et al. 2013) and US\$1–3 $kg^{-1}$ for cationic starch (Vandamme et al. 2010), and therefore seem to hold promise for their large-scale use.

In conclusion, among the inorganic flocculants, ferric chloride was the most promising, with a flocculation efficiency of 87 % at 600 μM concentration; while among the organics, rice starch was most effective in terms of flocculation, with an efficiency of about 86 % at 90 mg $L^{-1}$ concentration. Enhanced sedimentation rate was the obvious advantage of flocculation, which significantly reduced the sedimentation time. The comparative costs involved revealed that although flocculation efficiency with inorganic flocculant, viz $FeCl_3$, was at par with organic flocculant, viz rice starch (87.4 compared to 86.8 %), organic flocculants are economically more feasible, besides being environmentally safe options. Other organic flocculants such as maize starch (81.7 %) and potato starch (82.2 %) also showed promise in terms of their flocculation efficiency. In our experiments, microalgae which were harvested by high pH induced increased flocculation efficiency. Moreover, pH does not contaminate growth medium, which can be recycled to reduce the cost and the demand for water at larger scale. Thereby, the pH-induced flocculation method can be a suitable option for economic production from algae to biodiesel.

**Acknowledgments** We are grateful to the Director of Indian Agricultural Research Institute, New Delhi for providing funds towards undertaking research under the institute's Challenge Program (Bioprospecting of microorganisms for sustainable production of second generation biofuels). The authors are also grateful to Dr. Rashmi Tyagi from the Institute of Technology and Management, Gurgaon, Haryana, India for supply of *Botryococcus* cultures.

## References

Avnimelech Y, Troeger BW, Reed LW (1982) Mutual flocculation of algae and clay: evidence and implications. Science 216:63–65

Becker E (1994) Microalgae: biotechnology and microbiology. Cambridge University Press, Cambridge

Benemann JR (1997) $CO_2$ mitigation with microalgae systems. Energy Convers Manage 38:475–479

Bernhardt H, Clasen J (1994) Investigations into the flocculation mechanisms of small algal cells. Aqua J Water Supply Res Technol 43: 222–232

Blanchemain A, Grizeau D, Guary JC (1994) Effect of different organic buffers on the growth of *Skeletonema costatum* cultures: further evidence for an autoinhibitory effect. J Plankton Res 16:1433–1440

Bratby J (2006) Coagulation and flocculation in water and wastewater treatment, 2nd edn. IWA Publishing, London

Brown MR, Dunstan GA, Norwood SJ, Miller KA (1996) Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudomona*. J Phycol 32:64–73

Brennan L, Owende P (2010) Biofuels from microalgae: a review of technologies for production, processing, and extractions of biofuels and coproducts. Renew Sust Energ Rev 14:557–577

Buelna G, Bhattarai KK, de la Noue J, Taiganides EP (1990) Evaluation of various flocculants for the recovery of algal biomass grown on pig-waste. Biol Wastes 31:211–222

Castrillo M, Lucas-Salas L, Rodrguez-Gil C, D. Martnez 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 CY, Yeh KL, Aisyah R, Lee DJ, Chang JS (2011) Cultivation, photobioreactor design, and harvesting of microalgae for biodiesel production: A critical review. Bioresour Technol 102:71–81

Christenson L, Sims R (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv 29:686–702

Clasen J, Mischke U, Drikas M, Chow C (2000) An improved method for detecting electrophoretic mobility of algae during the destabilization process of flocculation: flocculant demand of different species and the impact of DOC. J Water Supply Res Technol 49:89–101

Divakaran R, Pillai V (2002) Flocculation of algae using chitosan. J Appl Phycol 14:419–422

Georgianna DR, Mayfield SP (2012) Exploiting diversity and synthetic biology for the production of algal biofuels. Nat Geosci 488:329–335

González-López CV, Acién Fernández FG, Fernández Sevilla JM, Sánchez FJF, Cerón García MC, Molina Grima E (2009) Utilization of the cyanobacteria *Anabaena* sp. ATCC 33047 in $CO_2$ removal processes. Bioresour Technol 100:5904–5910

Granados MR, Acien FG, Gomez C, Fernandez-Sevilla JM, Molina GE (2012) Evaluation of flocculants for the recovery of freshwater microalgae. Bioresour Technol 118:102–110

Gupta V, Ratha SK, Sood A, Chaudhary V, Prasanna R (2013) New insights into the biodiversity and applications of cyanobacteria (blue-green algae)- prospects and challenges. Algal Res 2:69–97

Harith ZT, Yusoff FM, Mohamad MS, Din MSM, Ariff AB (2009) Effect of different flocculants on the flocculation performance of microalgae, *Chaetoceros calcitrans*, cells. J Biotechnol 8:5971–5978

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

Kwon GS, Moon SH, Hong SD (1996) A novel flocculant biopolymer produced by *Pestalotiopsis* sp. KCTC 8637P. Biotechnol Lett 18: 1459–1464

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

Larkum AWD, Ross IL, Kruse O, Hankamer B (2012) Selection, breeding, and engineering of microalgae for bioenergy and biofuel production. Trends Biotechnol 30:198–205

Lee SJ, Kim SB, Kim JE, Kwon GS, Yoon BD, Oh HM (1998) Effects of harvesting method and growth stage on the flocculation of the green alga *Botryococcus braunii*. Lett Appl Microbiol 27:14–18

Lubian LM (1989) Concentrating cultured marine microalgae with chitosan. Aquacult Eng 8:257–265

Mahvi AH, Razavi M (2005) Application of polyelectrolyte in turbidity removal from surface water. Am J Appl Sci 2:397–399

McGarry MG (1970) Algal flocculation with aluminum sulfate and polyelectrolytes. Res J Water Pollut Control Fed 42:191–201

Millamena OM, Aujero EJ, Borlongan IG (1990) Techniques on algae harvesting and preservation for use in culture as larval food. Aquacult Eng 9:295–304

Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20:491–515

Oh HM, Lee S, Park MH, Kim HS, Kim HC, Yoon JH, Kwon GS, Yoon BD (2001) Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM49. Biotechnol Lett 23:1229–1234

Papazi A, Makridis P, Divanach P (2010) Harvesting *Chlorella minutissima* using cell coagulants. J Appl Phycol 22:349–355

Rashid N, Rehman SU, Han JI (2013) Rapid harvesting of freshwater microalgae using chitosan. Process Biochem 48:1107–1110

Ratha SK, Prasanna R (2012) Bioprospecting microalgae as potential sources of “green energy”—challenges and perspectives. Appl Biochem Microbiol 48:109–125

Schenk PM, Thomas-Hall SR, Stephens E, Marx U, Mussgnug JH, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high-efficiency microalgae for biodiesel production. Bioenerg Res 1:20–43

Semerjian L, Ayoub GM (2003) High-pH-magnesium coagulation–flocculation in wastewater treatment. Adv Environ Res 7:389–403

Sharma B, Dhuldhoya N, Merchant U (2006) Flocculants—an ecofriendly approach. J Polymers Env 14:195–202

Singh R, Tripathy T, Karmakar G, Rath S, Karmakar N, Pandey S, Kannan K, Jain S, Lan N (2000) Novel biodegradable flocculants based on polysaccharides. Curr Sci 78:798–803

Singh NK, Dhar DW (2011) Microalgae as second generation biofuel. A review. Agron Sustain Dev 31:605–629

Sirin S, Trobajo R, Ibanez C, Salvadó J (2011) Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan, and alkalinity-induced flocculation. J Appl Phycol 24:1067–1080

Sun ZX, Su FW, Forsling W, Samskog PO (1998) Surface characteristics of magnetite in aqueous suspension. J Colloid Interf Sci 197:151–159

Sukenik A, Bilanovic D, Shelef G (1988) Flocculation of microalgae in brackish and seawaters. Biomass 15:187–199

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

Vandamme D, Foubert I, Fraeye I, Muylaert K (2012) Influence of organic matter generated by *Chlorella vulgaris* on five different modes of flocculation. Bioresour Technol 124:508–511

Wu Z, Zhu Y, Haung 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 NB, Gloe LM, Brady PV, Hewson JC, Grillet AM, Hankins MG, Pohl PI (2012) Critical conditions for ferric chloride-induced flocculation of freshwater algae. Biotech Bioeng 109:493–501

Xu L, Chen G, Feng W, Sen Z, Chun-Zhao L (2011) A simple and rapid harvesting method for microalgae by in situ magnetic separation. Bioresour Technol 102:10047–10051

Yahi H, Elmaleh S, Coma J (1994) Algal flocculation–sedimentation by pH increase in a continuous reactor. Wat Sci Technol 30:259–267

Yoon SY, Deng Y (2004) Flocculation and reflocculation of clay suspension by different polymer systems under turbulent conditions. Adv Colloid Interface Sci 278:139–145

Zeng D, Wu J, Kennedy JF (2008) Application of a chitosan flocculant to water treatment. Carbohydr Polymer 71:135–139