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Full Length Research Paper

Effect of different flocculants on the flocculation performance of microalgae, *Chaetoceros calcitrans*, cells

Zuharlida Tuan Harith[1], Fatimah Mohd Yusoff[1], Mohd Shamzi Mohamed[2], Mohamed Shariff Mohamed Din[3] and Arbakariya B. Ariff[1,2]*

[1]Laboratory of Industrial Biotechnology, Institute of Bioscience, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3]Faculty of Veterinary Medicine, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

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The possibility of using flocculation technique for the separation of microalgae, *Chaetoceros calcitrans,* biomass from the culture broth was investigated. The flocculation experiments were conducted in 500 mL beaker using culture broth obtained from 10 L photobioreactor. The harvesting efficiency of 90 and 60% was obtained in flocculation without flocculants conducted for 10 days at 27ºC (in light and dark) and 4ºC (dark), respectively. Harvesting efficiency higher than 90% with short settling time was achieved by adjusting the culture pH to 10.2 using either sodium hydroxide (NaOH) or potassium hydroxide (KOH). Improved cell viability (> 80%) and settling time with a slight improvement of flocculation efficiency was achieved by the addition of polyelectrolytes flocculant (Magnafloc® LT 27 and LT 25). However, the flocculants were only functioned when the pH of the microalgae culture was pre-adjusted to a certain value that promotes cells entrapment and surface charge neutralization prior to flocculation process. The flocculation efficiency and cell viability obtained in flocculation with Magnafloc® (LT 25 and LT 27) was comparable to that obtained in flocculation with chitosan. When chitosan and Magnafloc® (LT 25 and LT 27) were used as flocculants, the highest flocculation efficiency of *C. calcitrans* cells was observed at pH 8 and 10.2, respectively. Substantial increased in sedimentation rate was observed with increasing flocculants dosage though the flocculation efficiency and cell viability were not significantly varied.

Key words: Flocculation, microalgae, *Chaetoceros calcitrans*, polyelectrolyte, chitosan.

INTRODUCTION

Microalgae are widely used in aquaculture as live feeds for several aquaculture species such as molluscs, fish, penaeid prawn larvae and rotifers (Brown, 2002). Intensive cultivation for production of large quantities of microalgae biomass requires a proper harvesting technique. One of the problems in large scale productions of microalgae is the development of efficient downstream processing to enable efficient separations of cells from culture broth as well as to maintain their viability and bioactivity prior to use in the field. The process options and economics of the different methods for the recovery of microalgal biomass have been reviewed by Molina Grima et al. (2003). Different production systems require a proper selection and optimization of the harvesting method and these must accommodate to the requirement of the final form of the algal cells and the subsequent processing steps of the harvesting and formulation methods.

Flocculation or sedimentation occurs when particles in liquid settle to the bottom of the tank due to gravitational force and fluid drag force. This method of solid-liquid separation is preferred for harvesting large cells like

*Corresponding author. E-mail: arbarif@biotech.upm.edu.my. Tel.: 603 8946 7591. Fax: 603 89467510.

microalgae due to its low costs compared to other methods such as centrifugation and filtration (Bilanovic et al., 1988). The flocculation efficiency of microalgae can be improved by the use of flocculants (Tilton et al., 1972; Bilanovic et al., 1988). 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 clarification of waste water treatment (Mahvi and Razavi, 2005), clarification of drinking water, colour removal in paper making industry and mineral processing (Yoon and Deng, 2004).

Flocculation process has been applied in the harvesting of microalgal biomass (Gualteri et al., 1988; D'Souza et al., 2002; Knuckey et al., 2006). Algae flocculated using aluminium sulphate has been fed to common carp, *Cyprinus carpia* since 1970 (Sandbank and Hepher, 1978). Substantially high flocculation efficiency (> 80%) of marine microalgae was obtained when ferric chloride was used as flocculant (Sukenik et al., 1988). In addition, alum and lime can also be used as flocculants to concentrate *C. calcitrans*, *Skeletonema costatum* and *Tetraselmis chuii* but not *Isochrysis* sp. (Millamena et al., 1990). Mineral coagulants such as alum and ferric chloride might be toxic to animals when consumed due to high concentration of residual aluminum and iron in the biomass harvested (Buelna et al., 1990). Therefore, the choices of flocculants are crucial and several alternatives have been studied as replacement to mineral coagulants. Polyelectrolytes are known as effective flocculants and have been used in the improvement of flocculation. They are usually high in molecular weight and water soluble organic compound, which can be anionic, cationic or nonionic (Baraniak et al., 2004; Voisin and Vincent, 2003; Kim et al., 2001). Magnafloc® is one of the well known brand of polyelectrolytes flocculants. Chitosan, which is an organic cationic polymer, has also been used as a flocculating agent in the treatment of wastewater and food industry. It is a β-N-acetyl-D-glucosamine polycationic polymer extracted from crustaceans exoskeletons (Gualteri et al., 1988). Chitosan remains insoluble in water, alkali solution and alcohol but easily dissolved in dilute acids. Reduced flocculation efficiency of microalgae cells in salt water system has been reported (Heasman et al., 2001). However, the assessment of chitosan as a potential flocculant in concentrating algae need to be explored due to its low toxicity, ease of manufacturing and low working dosage. Recently, Knuckey et al. (2006) claimed that the adjustment of the microalgae culture pH by adding base to promote precipitation and entrapment of cells increased the harvesting efficiency substantially. This simple method could be an attractive choice because it is non-toxic to the cells and it also eliminates the use of mineral coagulants. However, this method was only tested to small number of microalgal strain.

The objective of this study was to compare the flocculation efficiencies of different types of flocculants in harvesting microalgae, *C. calcitrans*, from culture broth obtained from the cultivation in the laboratory scale photobioreactor (10 L working volume). The effects of culture pH, flocculation conditions and flocculant dosage on the flocculation efficiency were also investigated.

MATERIALS AND METHODS

Microalgae and cultivation method

Microalgae, *C. calcitrans,* obtained from Aquatic Animal Health Unit, Faculty of Veterinary, Universitiy Putra Malaysia was used throughout this study. The microalgae was cultivated in 10 L photobioreactor using Conway medium (Walne, 1966) at 29 ppt salinity and with the addition of 0.02 g/L of silica. The temperature within the photobioreactor was regulated at 20 ± 2ºC by air-conditioning and aeration was provided by air bubbling through sparger. The pH of the medium was maintained at 8 ± 0.2 by sparging with a mixture of air and carbon dioxide (CO_2) at a ratio of 97:3. Cultures were grown under continuous illumination by white fluorescent (4500-5000 lux) during day and night time. Cells were harvested at late logarithmatic growth phase (after 6 days of cultivation) for subsequent use in the flocculation experiments.

Flocculation experiments

All flocculation or sedimentation experiments of *C. calcitrans* cells were carried out using 500 mL beaker (diameter = 85 mm and height = 120 mm). Initially, the microalgae cultures (pH 8) were allowed to settle at 3 different conditions;

(i) 27°C (in the dark),
(ii) 27°C (with light) and
(iii) 4ºC (in the dark) without flocculants.

Samples were taken everyday for a period of 15 days at 4 cm of above the base of the beaker for evaluation of flocculation efficiency. The viability *of C. calcitrans* cells was determined after 15 days of flocculation.

Subsequently, the effect of culture pH on the flocculation efficiency was carried out by adjusting the culture pH ranging from pH 10 to pH 10.6 using either 5 M sodium hydroxide (NaOH) or 5 M potassium hydroxide (KOH). The bases were added to the culture at high mixing rate provided by agitation using magnetic bar stirrer (38 mm), agitated at 200 rpm to allow for steady increase and homogeneity in pH. When the required pH was reached, slower mixing (50 rpm) was applied for 2 min, followed by quiet settling under gravity for flocculation process. At the end of flocculation (4 h), surface water was siphoned off and the flocs were collected for analysis.

In the subsequent experiments, the effect of polyelectrolytes flocculants was studied by adding different dosages of Magnafloc®LT 25, Magnafloc®LT 27 (Ciba Specialty Chemicals, Switzerland) and chitosan (SIGMA, United States) to the culture where the pH was previously adjusted to the required value according to method as described earlier. Magnafloc® LT 25 and LT 27) evaluated in this study have been approved for waste water treatment process in United Kingdom by the drinking water inspectorate and the Scottish office as proved by the manufacturer. It is recommended for use as coagulants aid in clarification and filtration process (Mahvi and Razavi, 2005) suggesting that these polyelectrolyes are safe to be used in microalgae cells separation.

Stock solution of these polyelectrolytes were prepared by dis-

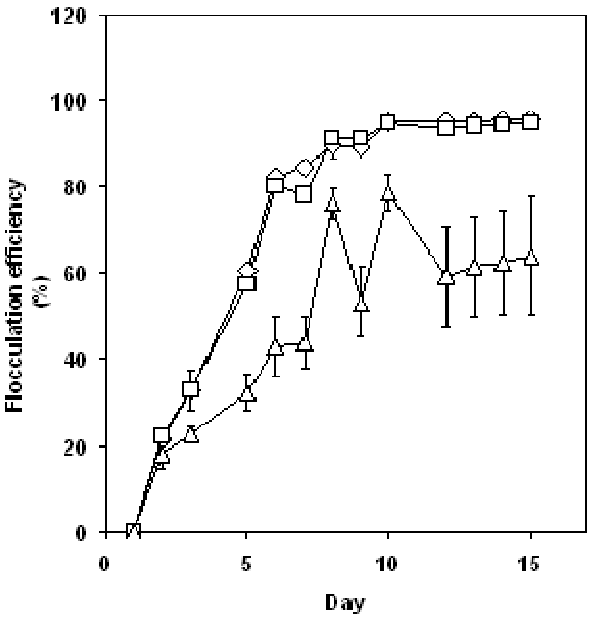

Figure 1. Profiles of flocculation efficiency of C. *calcitrans* during sedimentation without flocculant at three different culture conditions. Initial pH was 8 and not adjusted prior to the flocculation experiment. Symbols represent (▲) 4 ºC (dark); (■) 27 ºC (dark); (♦) 27 ºC with (light). Arrow bars indicate standard deviation of three replicates.

solving 0.5 g/L Magnafloc® LT 25 and Magnafloc® LT 27 in distilled water followed by extensive stirring. Flocculants were added to the culture, followed with vigorous mixing (200 rpm) for 1 min and subsequently with slow mixing (50 rpm) for another 2 min. Then, the stirrer was removed from the culture to allow the flocculation under gravity. At the end of flocculation (4 h), surface water was siphoned off and the flocs were collected for analysis.

For comparison, flocculations using chitosan as a flocculant were also carried at different pH values (pH 5 to pH 10) and dosages (10 mg/L to 150 mg/L). The pH of microalgae culture was dropped from 8 to 3.14 after the addition of chitosan, due to its acidic characteristic. NaOH was used to adjust the culture pH to the required value. Stock solution of chitosan was prepared by dissolving 1 mg/mL of chitosan flakes in 1% (v/v) acetic acid followed by extensive sonication (Model no. Branson 3510, United States) until the flakes was totally dissolved.

Cells viability

For staining procedure, 20 mL of samples were treated with 1 mL of 1% (w/v) stock solution of Evan's blue. The samples were allowed to stand at room temperature for at least 30 min and cells were observed microscopically under light microscope (*Leica* DMLB, Germany). The dead cells were stained blue due to the penetration of the stain through the cell wall whereas the viable cells would retain their natural colour due to intact cell wall. Cell numbers were counted using haemacytometer. The percentage of viable cells was calculated using equation (1);

$$Cell\ viability\ (\%) = \frac{Viable\ cells}{Total\ cells} \times 100 \quad (1)$$

Flocculation or harvesting efficiency

The flocculation efficiency was evaluated by comparing the remaining cell density in the clear region with the concentration before treatment. The flocculation or harvesting efficiency (%) was calculated using equation (2).

$$Flocculation/harvest\ efficiency\ (\%) = \left[\frac{Ci - Cf}{Ci}\right] \times 100 \quad (2)$$

Where *Ci* is the concentration of cell in suspension before treatment and *Cf* is the final concentration of cells in suspension.

Determination of the flocculation or sedimentation rate

During the flocculation experiments, the flocculation or sedimenttation rate was estimated by the observation of the displacement of the upper interface of the cell suspension with time (in sec) through the naked eyes. This means that the movement of the layer between the clear solution with the layer of high density cell suspension toward the bottom of the beaker during the flocculation was monitored on the attached scale (in mm), which was termed as sediment height. The maximum flocculation or sedimentation rate was determined from the slope of the plot of sediment height versus flocculation time. Determination of maximum sedimentation rate is necessary for an appropriate comparison of the sedimentation rate without the effect of cell accumulation at the bottom of the beaker (López et al., 1996).

RESULTS AND DISCUSSION

Flocculation without flocculant at different conditions

The flocculation of *C. calcitrans* culture without flocculant at different flocculation conditions is shown in Figure 1. In all cases, flocculation efficiency was increased gradually with time and reached maximum after about 8 days. However, increased in flocculation efficiency with time was higher for flocculation carried out at 27ºC as compared to 4ºC. The effect of providing light and total darkness gave not significant effect on the flocculation efficiency. The maximum flocculation efficiency obtained at 27ºC (either in the dark or with light) was about 91%, while the value obtained for flocculation at 4ºC was only about 70%. Fluctuation of flocculation efficiency was observed when it reached maximum values for flocculation at 4ºC. On the other hand, very stable flocculate at steady-state was observed for flocculation at 27ºC though a slight increase in flocculation efficiency from day 8 (91%) to 94% at day 15 were observed.

Figure 2 shows the results for the viability of *C. calcitrans* obtained after 15 days of flocculation without flocculant at different conditions. The highest viability (80.8 ± 3.19%) was obtained for sedimentation at 4ºC and significantly reduced for sedimentation at 27ºC. It is also interesting to note that the viability during sedimentation at 27ºC was higher in culture without light (79.98 ± 2.93%) as compared to culture with light (63.53 ± 9.91%).

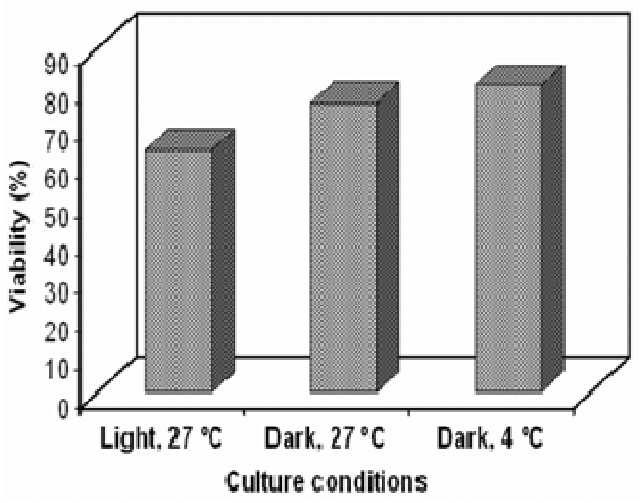

Figure 2. Viability of *C. calcitrans* cells after 15 days of flocculation process without flocculant at three different culture conditions. Initial pH was 8 and not adjusted prior to the flocculation experiment.

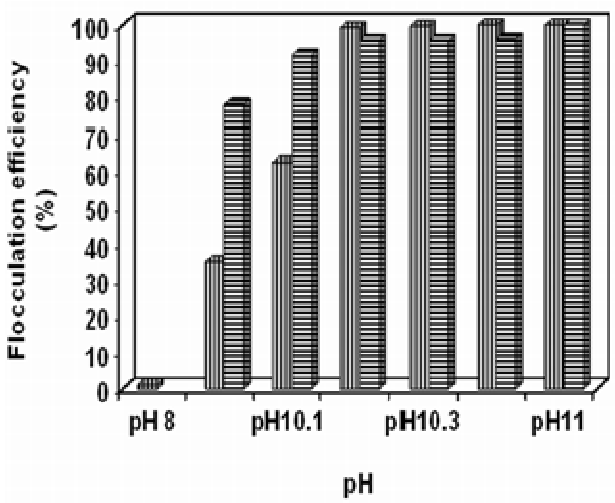

Figure 3. Flocculation efficiency of C. *calcitrans* cells in flocculation with pH adjustment using KOH and NaOH. Symbols represent; (▥) KOH; and (▤) NaOH.

Microscopic examination of sedimented *C. calcitrans* cells after 15 days of sedimentation indicate that the cells were in the singly form without aggregation occurred.

Reduced sedimentation rate of *C. calcitrans* cells is mainly due to it low cell dry weight (15 ρg) and very fine cell size (4.0 x 3.4 µm) (Neil and O'Connor, 1991). Heasman et al. (2001) reported that 100% of harvesting efficiency of *C. calcitrans* cells was only achieved after 162 h of sedimentation time. The morphology of the cell also affect the sedimentation rate due to presence of spike and internal biochemical changes, such as gas and lipid content that promote buoyancy in the absence of light. The presence of spike was also observed on physical observation of *C. calcitrans* cells during sedimentation process, suggesting that the cells were viable with changes in the internal biochemical. In this study, it was found that reduction in temperature and darkness aimed at reduction the metabolic activity of the cells did not enhance the sedimentation process but im-proved the cells viability. Heasman et al. (2001) reported that less than 20% of *Tetraselmis* sp. (T. Iso) cells retained its viability after 14 days of storage. It is important to note that *C. calcitrans* could maintain high viability after 15 days of storage in chilling condition.

Flocculation with pH adjustment

Figure 3 shows the flocculation or harvesting efficiency of *C. calcitrans* cultures with pH adjustments prior to flocculation process. With a slight increase in culture pH from 10 to 10.2, the harvesting efficiency was increased almost 2 times. At pH below 10, very low separation occurs after 4 h of flocculation process. When the pH was adjusted up to 10 using KOH, the efficiency was about 35% and increased to 78% when NaOH was used. Less quantity of NaOH was required to adjust the pH of the microalgae culture to pH above 10 as compared to KOH. However, slightly higher flocculation efficiency was observed when KOH is used compared to NaOH, where the harvesting efficiency of 98% was obtained at pH 10.2 and above. Further increment of pH did not show further improvement in the flocculation efficiency. It is worthy to note that the additional bases tend to increase the precipitation and formation of loose flocs.

The use of pH adjustment did not enhance the flocculation of some microalgae species. For example, the harvesting efficiency of *Nannocloropsis oculata* and *Isochrysis* sp. (T-iso) after pH adjustment was less than 30% (Knuckey et al., 2006). During the initial stage of flocculation process, when the pH of medium was increased, the small particles aggregated and slowly settled due to gravitational force. The cells formed large loose and dense packed aggregates that settled under gravitational force. Once the fine capture achieved equilibrium, further addition of flocculant might lead to the formation of larger aggregates. This in turn might cause higher settling rates with minimal addition of flocculants (Owen et al., 2002). Adding additional bases to the culture medium did not improve the flocculation efficiency but increased in precipitation and formation of loose flocs.

Flocculation with polyelectrolytes and pH adjustment

The changes in flocculation efficiency, cell viability and sedimentation rate of *C. calcitrans* at different pH adjustment with NaOH followed by addition of Magnafloc® LT 25 is given in Figure 4. The flocculation efficiency was increased drastically from 13 to 82% after 4 h when the pH was adjusted from 8 to 10. Further increase in flocculation efficiency to 92% was observed with increasing

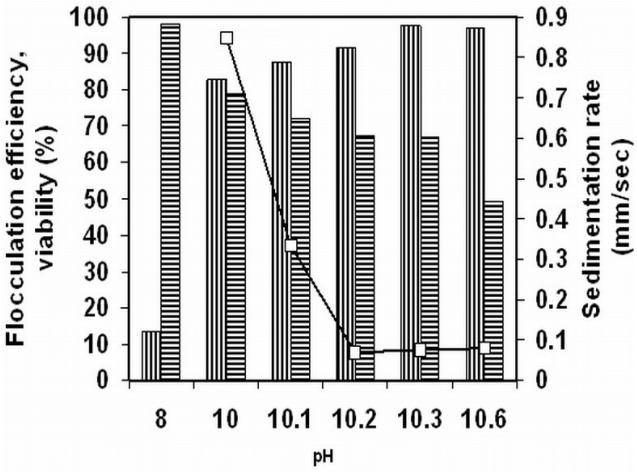

Figure 4. Change in flocculation efficiency, cells viability and sedimentation rate for *C. calcitrans* flocculated at different pH adjustment using NaOH followed by addition of 0.1 mg/L Magnafloc®LT 25. Symbols represent; (▥) flocculation efficiency; (▤) cells viability; and (□) sedimentation rate.

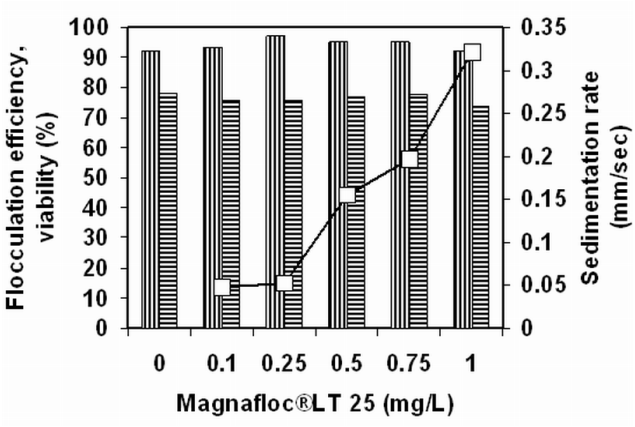

Figure 5. Changes in flocculation efficiency, cells viability and sedimentation rate for *C. calcitrans* flocculated at different dosage of Magnafloc®LT 25 after pH adjustment at 10.2 using NaOH. Symbols represent: (▥) flocculation efficiency; (▤) cells viability; and (□) sedimentation rate. The sedimentation rate for control was too low and not measured.

pH to 10.2 and slightly increases to 98% with increasing pH to 10.3 and 10.6. On the other hand, the sedimentation rate was reduced drastically with increasing pH from 10 to 10.2 and remains at the same rate with further increment in pH to 10.6. At higher pH (10.2 - 10.6) the flocs of the microalgae cells became less dense, thus slowly settled. It is important to note that the viability of the microalgae cells gradually reduced with increment of pH from 8 to 10.6. The viability at pH 8, pH 10 and pH 10.2 was 98, 78 and 68%, respectively. Although reduction in cell viability was observed at pH 10.2, this pH value was chosen as the preferred pH for the flocculation process due to substantial enhancement in sedimentation rate. Higher pH, ranging from 10.6 to 11, was reported in the literature as the preferred pH for the flocculation of microalgae (Heasman et al., 2001).

Effect of polyelectrolyte flocculant dosage on flocculation efficiency

Figure 5 and 6 shows the flocculation/ harvesting efficiency, viability and maximum settling velocity for *C. calcitrans* harvested at optimal pH adjustment (pH 10.2) followed by addition of Magnafloc®LT 25 and Magnafloc® LT 27, respectively. For Magnafloc®LT 25, flocculation efficiency did not show significant difference with increasing dosage. However, the sedimentation rate was increased gradually with increasing dosage of Magnafloc®LT 25. The cells viability was not changed with increasing dosage. Almost similar results were also observed for flocculation with Magnafloc®LT 27. However, the viability of *C. calcitrans* improved with the addition of Magnafloc® LT 27 as compared to culture without flocculant, though the viability was not changed with increasing dosage. The flocculation efficiency was comparable to Magnafloc®LT 25. Interestingly, for both cases, the sedimentation rate was substantially increased with increasing dosage of the flocculants.

If the sedimentation rate shall be considered, higher dosage of both flocculants shall be used. Higher sedimentation rate resulted in shorter flocculation time. When adjusting the pH of cultures, a buffering region was encountered at pH between pH 10.2 and 10.5. Within this region, a precipitate formed entrapped the microalgae cells. The formation of this precipitate was independent of the presence of cells. This precipitate was closely to neutral buoyancy and hence settled slowly (Knuckey et al., 2006). Addition of polyelectrolytes at this stage acts as enhancer by increasing the flocs size and sedimentation rate by promoting the bridging, binding and strengthening of the algal flocs. Besides that, the physical properties of the flocculants such as molecular weight and functionality play an important role in the performance of the flocculation process. Nevertheless, the use of synthetic polymer as flocculants is known to negatively affect the water ecosystem. Therefore, recommendation for maximum permissible concentration was set for flocculants residues. However, Magnafloc®LT 25 and Magnafloc®LT 27 have been approved for use by the drinking water inspectorate in United Kingdom and are classified as non hazardous chemicals.

Effect of pH adjustment of flocculation efficiency using chitosan

The flocculation of *C. calcitrans* with the addition of 20 mg/L of chitosan into the culture followed by pH adjust-

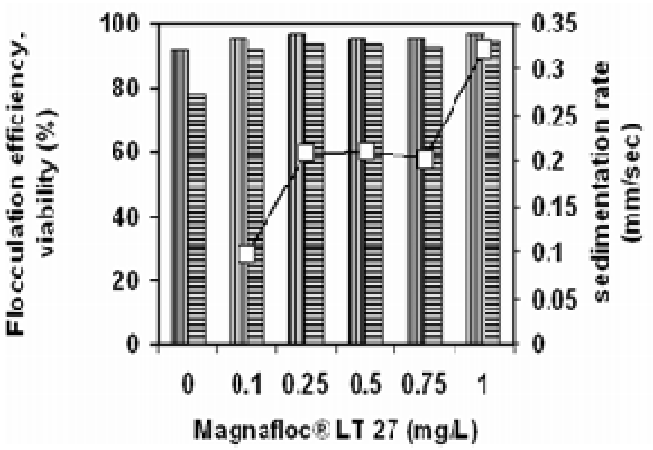

Figure 6. Changes in flocculation efficiency, cells viability and sedimentation rate for *C. calcitrans* flocculated at different dosage of Magnafloc®LT 27 after pH adjustment at 10.2 using NaOH. Symbols represent; (▥) flocculation efficiency; (▤) cells viability; and (□) sedimentation rate. The sedimentation rate for control was too low and not measured.

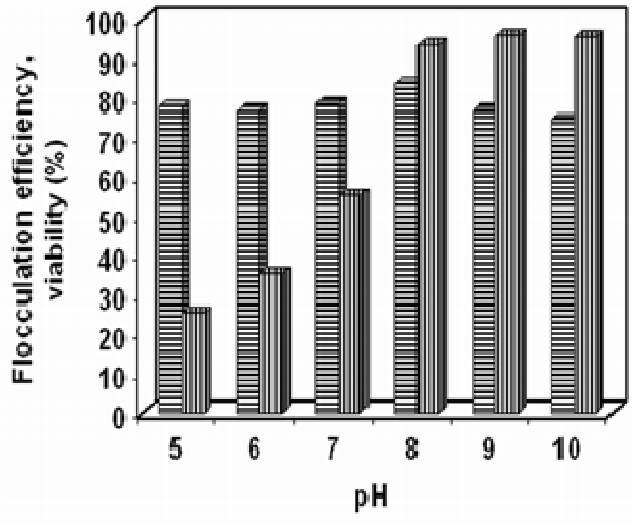

Figure 7. Changes in flocculation efficiency and cells viability of *C. calcitrans* flocculated with 20 mg/L of chitosan followed with pH adjustment at different values. Symbols represent: (▥) cells viability; and (▤) flocculation efficiency.

ment at different values was given in Figure 7. Due to acidic characteristic of chitosan solution, the pH of the culture reduced to around 3.14 after the addition of chitosan. Microscopic examination showed cells in good shape although the colour of cells were slightly greenish due to excretion of pigments from the cells. However, the cells retained their integrity and viability (> 80%). Flocculation efficiency was drastically increased with increasing pH from 5 to 8. Flocculation efficiency obtained at pH 5 was only about 50% and increased to 83% at pH 8. The flocculation efficiency was measured after 4 h of flocculation process. A slight reduction in flocculation efficiency (76-77%) was observed with further increment in pH to 9 and 10. Cells viability was not significantly changed with pH but the highest viability (81%) was obtained at pH 8.

Chitosan's behavior is affected by pH. In acidic condition, it exists as linear chain due to $-NH_2$ groups carrying positive charge and thus closely spaced. The positively charged $-NH_2$ and $-NH^{3+}$ group repel each other and during this condition, chitosan remain dispersed. With an alkaline pH, the positive charge gradually disappear (neutralization point at pH 7.9) and chitosan tends to coil and precipitate (Gualteri et al., 1988). Chitosan and *C. calcitrans* interact with each other through electrostatic interaction. Chitosan attached to the negatively charged algal surface via its positive charged group. Bridges were formed between algal cells when the chain had sufficient length to bind more than one cell. Therefore, during acidic condition the degree of flocculation is very weak. In alkaline pH, the positive charge was neutralized and the highest neutralizing point was approximately achieved at pH 8. At this pH, *C. calcitrans* cells have the highest negative charge, thus the flocculation efficiency was enhanced.

Effect of chitosan dosage on flocculation efficiency

The flocculation of *C. calcitrans* with different dosages of chitosan into the culture followed by pH adjustment at 8 is shown in Figure 8, which also include data for flocculation without pH adjustment after addition of chitosan. For both cases, flocculation efficiency was increased almost 2 times with increasing dosage from 10 to 20 mg/L. Flocculation efficiency was not significantly increased with increasing dosage of chitosan up to 150 mg/L. However, the flocculation efficiency for system with pH adjustment to 8 was about 2 times higher than those obtained in flocculation without pH adjustment.

In the flocculation of *Rhodomonas baltica* using chitosan as flocculant, more than 75% flocculation efficiency was obtained at 80 mg/L of chitosan (Lubian et al., 1989). On the other hand, lower dosage of chitosan (40 mg/L) was required for several *Tetraselmis* species and very high dosage (150 mg/L) was required for flocculation of *Chaetoceros muelleri* (Heasman et al., 2001). Gualteri et al. (1988) reported that the flocculation efficiency (96 - 98%) of *Euglena gracilis* was enhanced using 200 mg/L of chitosan followed by pH adjustment at 7.5.

Conclusion

The flocculation efficiency of microalgae for separation of cells from the culture broth was greatly influenced by the pH. Comparable flocculation efficiency (more than 90%) was obtained in flocculation with Magnafloc® (LT 25 and LT 27) to those obtained in flocculation using chitosan. Enhanced sedimentation rate was the obvious advantage of flocculation with flocculant, which significantly reduced

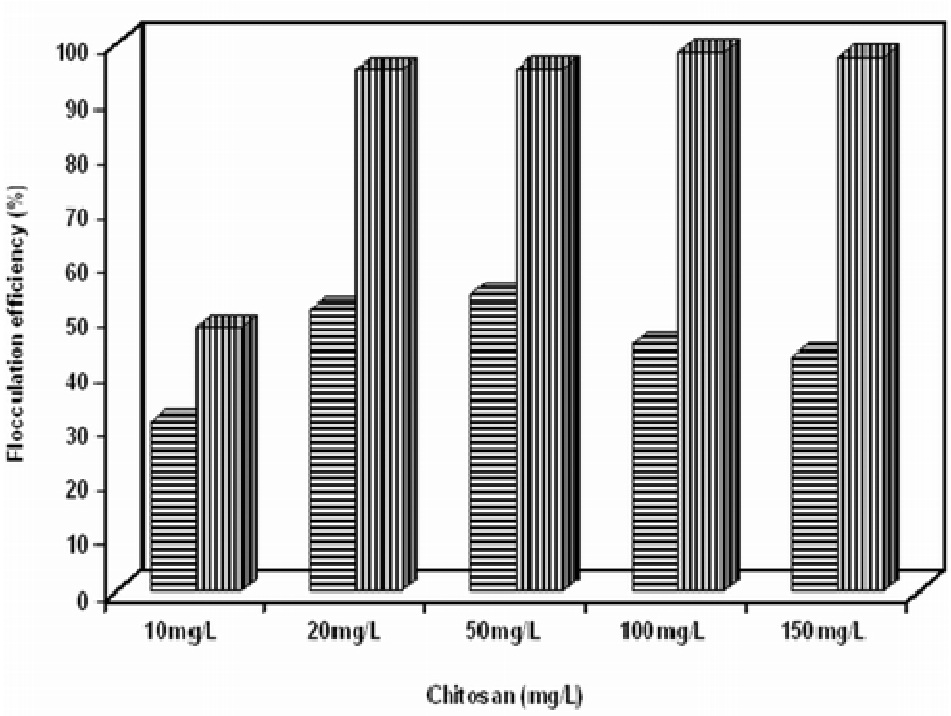

Figure 8. Changes in flocculation efficiency of *C. calcitrans* flocculated at different dosages of chitosan followed with pH adjustment to 8 using NaOH. Symbol represent; (▥) pH was adjusted to 8 after the addition of chitosan; and (▤) pH was not adjusted after the addition of chitosan.

the sedimentation time. Although chitosan is more environmental friendly compared to polyelectrolyte flocculants, it may not be economical for microalgae separation from culture broth due to higher price of chitosan. Efficient flocculation process that can maintain high cell viability could be a method of choice due to rapid, inexpensive and simple method for harvesting large quantity of microalgae cells such as *C. calcitrans* from the culture broth prior to commercial formulation.

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