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Microalgae harvesting by flocculation method

Lenin Thiruvarasn[1], Papa jeba Sangeetha[2], P. Sampathkumar[3]* and B. Sundarapandiyan[2]

[1,2,3,] Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annaamalai University, Parangipettai- 608502. Tamilnadu, India.

Corresponding Author: Dr. P. Sampathkumar, Associate professor, CAS in Marine biology, Faculty of Marine Sciences, Annamalai University, Parangipettai- 608502.

. E-mail: *sampathcas@gmail.com*

Abstract

Microalgae as a feedstock for biofuels or bulk protein or wastewater treatment. Realizing these applications will require the development of a cost efficient harvesting technology. In this experiment, it is explained that the potential of flocculation induced by high pH level for harvesting *Chlorella marina*. Here, flocculation can be induced by increasing the pH of the medium from pH 8.5 to pH 11.5. Although different flocculating agents such as calcium hydroxide, sodium hydroxide, potassium hydroxide and magnesium hydroxide were used in this experiment. When the pH was increased, sodium hydroxide effectively induces flocculation. The high pH induced flocculation, therefore, a potentially useful method for harvesting of microalgal biomass. The objective of the present study was developed to assay the viability of microalgae and was tested in three strains of *Chlorella marina*. This dye only penetrates damaged cell membranes and auto fluorescence was observed, allowing the discrimination of live and dead cells. It is also concluded that this method provides a simple and rapid technique for assessing the flocculation and viability of microalgae.

Keywords: Microalgae, *Chlorella marina*, Flocculation and Cell viability.

Introduction

Microalgae are photoautotrophic organisms that need light as their main energy source. They are the primary food source for a large number of aquatic organisms and play a key role in aquaculture development. There are certain elements which are most important for the growth of microalgae, for example N, P and K elements are added in the form of salts (Michael and Navid, 2013). The selection of a best medium is very much essential for growing algae and to obtain a biomass, different nutrient components influence the growth of algae. Before going to modern techniques involved in biofuel production from microbial lipid, it is important to optimize the microalgae growth under different physiological condition to obtain greater biomass (Kaushik, 1987). Intensive cultivation for production of large quantities of microalgae biomass requires a proper harvesting technique.

The microalgae concentration is very low for a large-scale culture method and very few micrometres in size. The microalgae biomass harvesting technology is very crucial to economic production. The harvesting technique is based on the properties of microalgae, such as density, size, and the value of the desired products (Brennan and Owende, 2010). Currently used microalgae harvesting methods include sedimentation, filtration, flocculation and centrifugation. The use of sedimentation is technically simplest option, but this method reported to have low biomass recovery rate and requires additional settling spaces.

Centrifugation is also too costly and energy intensive for large-scale biofuel production although it has high biomass recovery rate. Filtration is a slow and species dependent process, so a very large capacity system would be required to keep up with the production of a large algal form considering the features of the four approaches. Flocculation is an optimistic harvesting or pre-harvesting method due to its relative high biomass recovery rate achieved at lower capital and energy cost.

Flocculation is a process in which dispersed particles are aggregated together to form large particles for settling (Chen *et al*., 2011). Flocculation can be achieved in several ways and wide ranges of approaches for microalgae have been explored in the past years. These approaches range from traditional flocculation methods that are widely used in emerging technologies and other fields of industry. Microalgae can also be flocculated by inorganic flocculants at sufficiently low pH (Uduman *et al*., 2010). That is however too expensive and energy-intensive if biomass is to be used for low value products such as biofuels due to the large volumes of culture medium that need to be processed. Autoflocculation occurs as a result of precipitation of carbonate salts with algal cells in elevated pH, a consequence of photosynthetic CO2 consumption with algae (Sukenik and Shelef, 1984) which can be simulated by adding NaOH to achieve certain pH values. Alkaline flocculants neutralized the repelling surface charge of algae cells, allowing them of flocculation. It was discovered long ago that Mg $(OH)_2$ and $Ca(OH)_2$ flocculate algal suspensions (Folkman and Wachc, 1973).

In inorganic flocculation microalgae are negatively charged (Uduman *et al*., 2010), as a result of adsorption of ions such as iron chloride and alum are efficient but are required in high dose and result in contamination of the biomass with aluminium or ion (Becker 1994). To find an alternative technology it is possible to reduce the cost and increase the scale of microalgal biomass production and processing large volumes of culture medium at a minimal coast.

By using the autofluorescence of chlorophyll and an unspecific green autofluorescence of phototroph organisms, the proposed method allows a very easy to determine the number of viable cells in a culture. In viable cells, the red fluorescent signal of the chlorophyll showed a stronger intensity than the green signal from non-viable cells. Nevertheless a good detection of all signals was possible. The phase contrast image and overlay of the fluorescent confirms that the green (non-viable) and red (viable) fluorescent signal originate from Synechocystis cells.

Additionally, one can clearly observe a decrease number of red fluorescent cells and an increase number of green fluorescent cells for a decreased number of viable cells in different samples. For all samples, fluorescent images of a defined volume were taken in a Helber counting chamber in an automated procedure, with the subsequent analysis via Image. The expected numbers of red (viable) and green (non-viable) cells could be determined with great accuracy. In cell viability, fluorescent microcopy is much more sensitive than the light microscopical ones. The mechanism of the penetration of the fluorochromes RB and NR in the cell is based on the fact that walls of dead cells lose their semipermeability and these fluorochromes accumulate in cells. Trypan blue exclusion is widely used as an objective method of determining viable cell count.

The objective of this study was to compare the flocculation efficiencies of different types of flocculants in harvesting microalgae, *C. marina* from culture broth. The effects of culture pH, flocculation conditions and flocculants dosage on the flocculation efficiency were also investigated.

Materials and Methods

Culture Collection and maintenance

Chlorella marina species was obtained from the Rajiv Gandhi Centre of Aquaculture (RGCA) in Sirkazhi, Tamil Nadu, India. The microalgae were cultured in laboratory condition (Conway medium-prepared in 30 psu seawater). The temperature ranges between 25-30 ± 2 °C and light intensity between 2500 ± 500 lux with a 12 ± 12 light and dark diurnal cycle and pH was 7.8 – 8.0 were maintained. The cultures were re inoculated in fresh media at a week interval for maintaining growth phase culture. The species mixed culture were identified by Marine Phytoplankton manual of Hassle and Syvertsen (1996).

Culture cultivation

The marine microalgae, *Chlorella marina* were grown in Conway medium, an enriched seawater medium designed for growing marine algae. It is cultivated under batch culture consists of a single inoculation of cells into a container of sterilized seawater followed by growing period of 15 days and finally the total biomass was harvested. When the algal population

reaches its maximum or near maximum density nutrient level, contamination with predators and other competing algae. The pH was maintained from7.8 to 8.0 throughout the growth study.

The microalgae were grown in 1 L volumes of 2 L Erlenmeyer flasks. The inoculums for the strain were 5 ml, containing microalgae density about $5x10^3$cells /ml. The strains were cultivated at temperature ranges between 25-30 ± 2 °C and light intensity between 2500 ± 500 lux with a 12 ± 12 light and dark diurnal cycles. The pH was maintained at 7.8 – 8.0 and were continuously stirred for aeration.

Flocculants

Four different alkalis (Ca(OH), NaOH, KOH, Mg(OH) were used (for) as a flocculants for this experiment. The onset of flocculation was visually determined by a "grainy" appearance of the algae cells. During flocculation, the pH was continuously measured by pH electrode (Ecotest pH2: Accuracy ± 0.1 pH). Once flocculatation was visually observed, pH and quantity of base were added and recorded. Flocculation were allowed to settle and supernatants were decanted after 15 min. The flocculation experiment was determined using the optical density data measured at 750 nm wavelength by Spectrophotometer. The algal biomass removal efficiency (RE) was calculated using Eq. (1)

$$\text{Flocculation efficiency (\%)} = \frac{OD_i - OD_f}{OD_i} X\ 100$$

where the initial (OD_i = before adding flocculation agent) and final (OD_f = after 1 h settling) optical density data was replaced. All experiments were performed with three replicates and the mean value was taken for quantification.

Cells viability

In cell viability, the non-fluorescent stains such us trypan blue, neutral red and rodamine b were commonly used for the evaluation of cell viability. For staining procedure, 10 µl of samples were treated with 0.01µl of each dyes. The samples were allowed to stand at room temperature for 30 min. and cells were observed microscopically under fluorescent microscope. The dead cells were stained blue, red, and pink colour due to the penetration of the stains through the cell wall whereas the viable cells would retain their natural colour due to intact cell wall. Cell numbers were counted by Haemocytometer. The percentage of viable cells was calculated by the following equation.

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

Results and Discussion

The flocculation efficiency was tested in different pH ranged from 8.5 to 11.5 (Fig. 1 -pH treatment). No flocculation occurred up to pH 10.3. At pH 10.8, a flocculation efficiency of 83 % was observed. At pH more than 11.1, the flocculation efficiency was exceeded to 95 %. This indicates that *Chlorella marina* can be flocculated efficiently by increasing the pH of the culture medium to 11.5. This observation is in agreement with previous studies of Blanchemain *et al*. (1994) and Yahi *et al*., 1994).

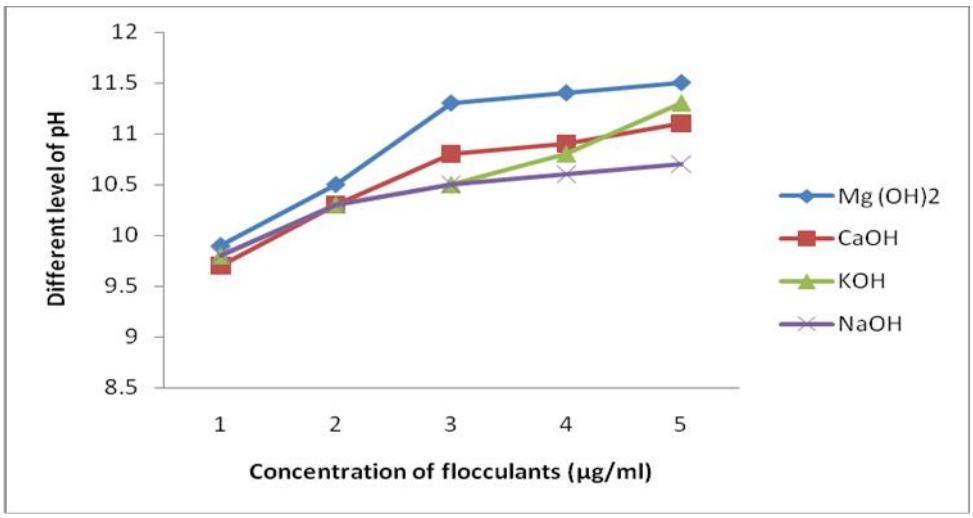


Fig. 1 Flocculation efficiency in different flocculants

Harvesting of microalgae induced by high pH requires the addition of base to raise the pH. It is tested whether other bases could be used to induce flocculation or not. In this experiment, different types of flocculating agents are widely used such as magnesium hydroxide, calcium hydroxide, potassium hydroxide and sodium hydroxide. Magnesium hydroxide induced flocculation at higher pH (11.5) than the other bases, but large quantity of base was required (0.1g/25ml). The pH was adjusted up to 10.5 using KOH, the flocculation efficiency was observed only at 35%. When NaOH was used, the flocculation efficiency was increased up to 78% and less quantity of NaOH was required to adjust the pH of the microalgae culture, whereas Potassium hydroxide (KOH) and Sodium hydroxide (NaOH) induced flocculation at the same pH of 10.5 only.

The lowest quantity of base was required for sodium hydroxide (0.01g in 25 ml of culture) followed by calcium hydroxide (0.06 g/25 ml) and potassium hydroxide (0.03 g/25 ml). When the pH was adjusted to 11.5, the flocculation efficiency of 95% was obtained. Moreover, increment of pH did not show further improvement in the flocculation efficiency. It is noted that the additional bases tend to increase the precipitation and formation of loose flocculation. Adding additional bases and pH adjustment, the flocculation of some microalgae species did not increase. The flocculation efficiency of *Nannochloropsis oculata* and *Isochrysis* sp. was observed 30% only after adjustment of the microalgae culture pH as evidenced by Knuckey *et al*. (2006).

Several studies have suggested that bivalent cations such as calcium hydroxide and magnesium hydroxide play a major role in the flocculation process at high pH (Shelef *et al*., 1984; Nurdogan and Oswald, 1995).

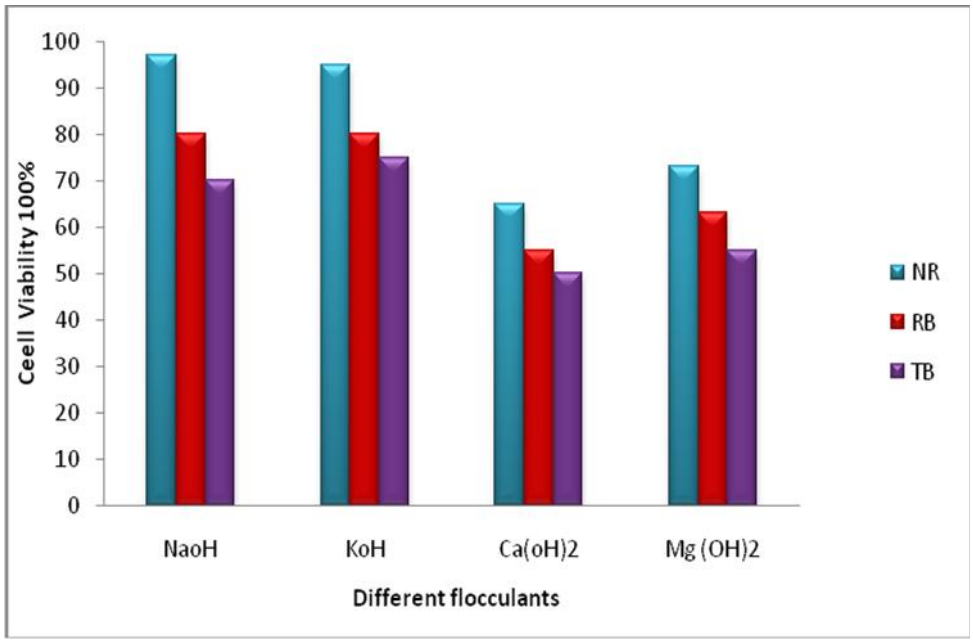
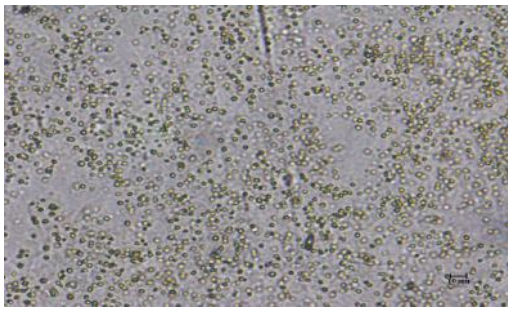


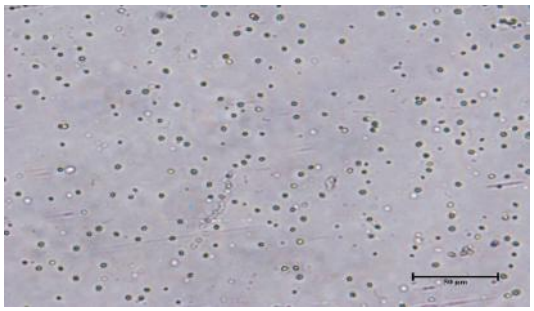
Fig. 2: Cell viability in different dyes with different flocculants

Usually cell viability was determined after staining of different compounds, presented in the normal cells with specific dyes (NR, RB, Trypan Blue) and also used the same flocculants. The percentage of cell viability obtained with the flocculant NaOH with different dyes such as NR 97%, RB 80% and Trypan Blue 70% (Fig. 2, 3, 4 and 5). The result also showed the percentage of fluorescent cells recorded by aid of RB and NR was close to the percentage of cells which had lost the chlorophyll fluorescence. Neutral Red staining has been reported to give ambiguous results for small phytoplankton as evidenced by Reynolds *et al*. (1978).

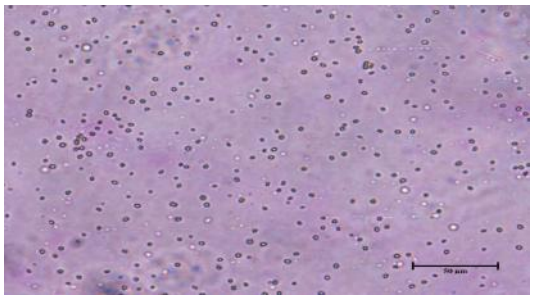
Fig.3: Cell viability in Neutral red stain with flocculants



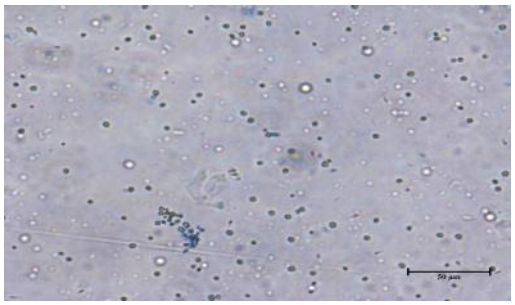
Neutral red Control



Ca (OH)

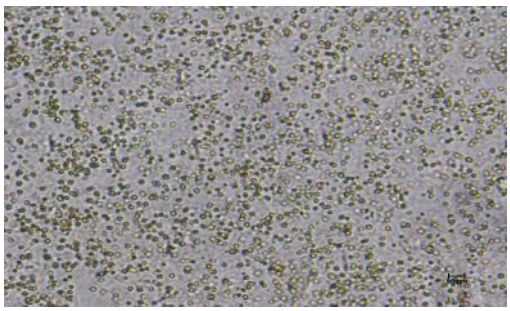


KOH

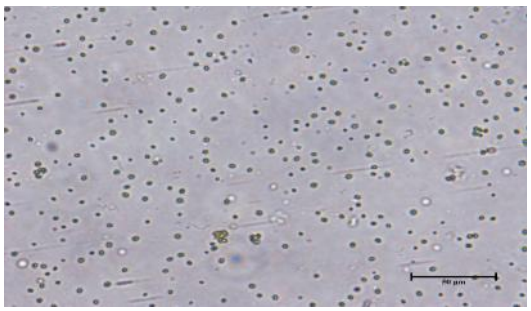


NaOH

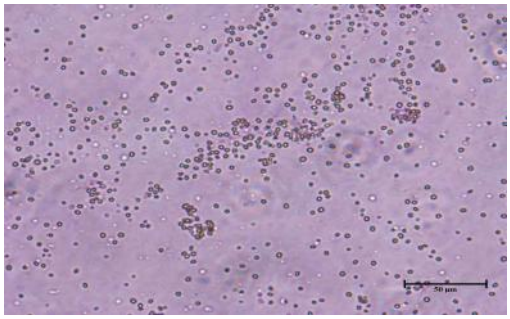
Fig. 4: Cell viability in Rodamine b stain with flocculants



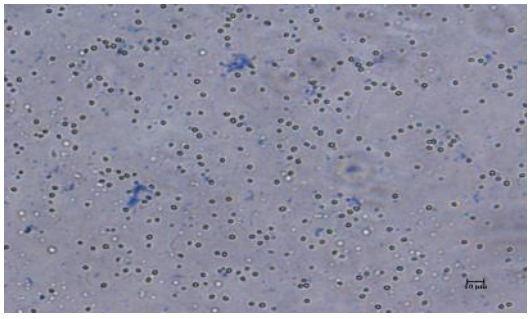
Rodamine b Control



Ca(OH)

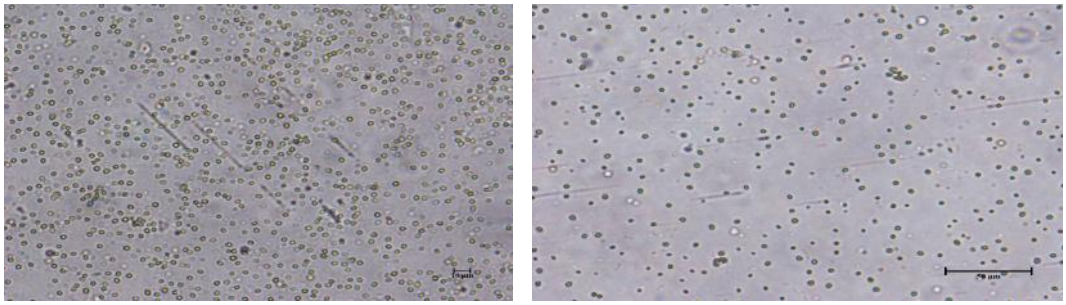


KOH



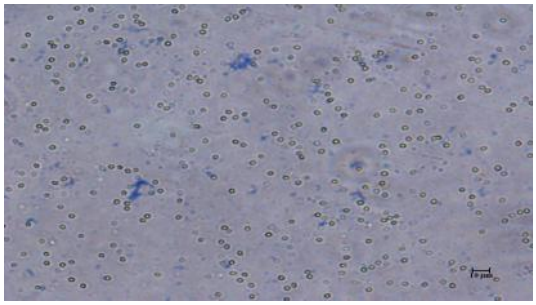
NaOH

Fig. 5: Cell viability in Trypan blue stain with flocculants

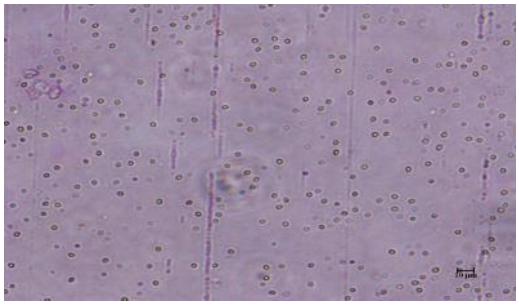


Trypan blue Control

Ca(OH)



KOH



NaOH

Conclusion

The flocculation efficiency of microalgae for separation of cells from the culture was greatly influenced by increasing the culture pH. The flocculation efficiency was compared with different flocculants, more than 95% was obtained in flocculation using NaOH. Although NaOH is more effectively induced the flocculation process compared to other flocculants. 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.

Acknowledgments

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