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Study on Flocculation Efficiency for Harvesting Nannochloropsis oculata for Biodiesel Production

D. Surendhiran and M. Vijay*

Department of Chemical Engineering, Faculty of Engineering and Technology, Annamalai University, Annamalainagar, Tamilnadu-608002, India

***Corres.author:drmvijay2009@gmail.com**
Contact: +91-9443227891

Abstract: Flocculation is one of the principal steps in biodiesel production from microalgae. Eight different flocculants were analysed for their efficiency of flocculation of marine microalga, *Nannochloropsis oculata*. Maximum flocculation was observed as 93.80 and 87.33% with $FeCl_3$ and $Fe_2(SO_4)_3$ at a concentration of 0.4 and 0.6g/L respectively at 180min. Zinc salts achieved second maximum efficient flocculation with $ZnCl_2$ (0.6g/L) as 89.12% and $ZnSO_4$ (0.8g/L) as 84.17% at 210 and 240 min respectively. Whereas salts of Aluminium showed flocculation efficiency of 85.46% with $AlCl_3$ (0.6g/L) and 82.27% with $Al_2(SO_4)_3$ (0.4g/L) at 210 and 240 min respectively. Magnesium salts showed less effect on flocculation though the incubation period was prolonged. As Iron salts exhibited complete cell lysis, even at lower concentrations, they were excluded from further study for flocculation. Effect of temperature, light and darkness were studied for Al salts and Zn salts, which showed that optimum temperature of 35^0C and light, are required for better flocculation. $ZnCl_2$ showed $ZnSO_4$, an efficient flocculation of 92.34 and 90.49% under illumination and under dark it was retarded to 83.14 and 80.14 % respectively; moreover cells remained intact on higher concentration and temperature. $AlCl_3$ and $Al_2(SO_4)_3$ showed efficiency of 88.49 and 85.91% under illumination but reduced to 74.77 and 68.79% respectively under darkness. Since structural instability is apparent with Al salts, Zinc chloride could be used as the efficient flocculant to flocculate *Nannochloropsis oculata.*

Keywords: *Nannochloropsis oculata*, flocculation, biomass, biodiesel, cell viability.

1. Introduction

Adverse environmental consequences of green house gases and forthcoming depletion of fossil fuel reserves has led to an energy deficit and critical need to develop alternative fuels. Biodiesel is a potential substitute for conventional diesel fuel [1, 2] and produced from palm, rapeseed, soybean or jatropha etc. Recent research has proved that oil production from microalgae is clearly superior because biomass doubling time(less than 24 h), growth rate and capable of synthesizing more oil per acre [3, 4, 5] when compared to terrestrial plants. Moreover, microalgal biodiesel will not interfere in the production of food, fodder and other products derived from crops [5].

Because of increasing population and industrialization, there is no much arable land for cultivation of terrestrial crops and also the global annual production of TAGs (triglycerols) from additional oil crops, waste cooking oils and fats, cannot meet current and future demand for biodiesel [6]. Thus, Microbiodiesel represents the only viable and renewable source of oil lipid feedstock that can meet global demand for transport fuels [6, 7].

Producing fresh water microalgal biomass for biodiesel production is generally more expensive than growing crops because growth medium must provide the inorganic elements such as nitrogen (N), phosphorous (P), iron and in some cases silicon [8] along with water. To minimize expense, we can use sea water which is readily available in large for cultivation of marine microalgae which are containing higher lipid content than the fresh water microalgae. *Nannochloropsis oculata* one of the important marine microalgae, that contains 31-68 % oil (by dry weight) [7, 8, 9].

Intensive cultivation for production of large quantities of microalgae biomass requires a proper harvesting technique. One of the major problems in large scale productions of microalgae is the development of efficient separations of cells from culture broth and also to maintain their viability and bioactivity prior to use in the field [10]. Harvesting biomass represent one of the significant cost factors in the production of biomass. Efficient harvesting of biomass from cultivation broth is essential for mass production of biodiesel from microalgae [11]. The potential of microalgae for biodiesel production is based on the microalgal biomass concentrate. Concentration of microalgal biomass from an extremely dilute culture environment is one of the most challenging processing steps [12], because of the small size of the algal cells (3–30 µm in diameter) and concentration of microalgal biomass in cultures is typically only about 0.5 to 5 gL^{-1} or 0.05 to 0.5 %. Moreover, their density is similar to water and the large volumes of water that must be handled to recover the biomass [13, 14]. Therefore, microalgae harvesting is one of the difficult process thus obstructing to develop algae biodiesel.

Different studies showed a contribution of the costs for harvesting to more than 30% of the total cost in case of algal production in open ponds [15]. To minimize the energy consumption of harvesting microalgae, an integrated approach is needed [16]. Many separation methods, such as centrifugation, gravity sedimentation, (ultra)filtration and screening, flocculation, flotation, and ultra sound waves have been developed for microalgae recovery [13, 17-20]. However, each has its disadvantages that affect the overall economics of the process. Centrifugation requires high energy input and initial capital cost [19] and the process involves exposing cells to high gravitational and shear forces which damage the cell structure. Second, the processing of large culture volumes can be time-consuming [21, 22]. Filtration and screening require regular replacement of filters, screens, membranes and can be very time consuming. Gravity sedimentation is a slow process and electroflotation requires replacement of worn electrodes that have high cost of electricity consumption [19, 23].

Evaluation of several harvesting methods showed that flocculation combined with flotation or sedimentation and subsequent dewatering by centrifugation or filtration is the most promising cost and energy efficient alternative. During flocculation, the dispersed microalgal cells aggregate and form larger particles with higher sedimentation rate [15, 24, 25]. Among them, flocculation, this is widely applied for its high efficiency and low cost [26]. In addition, it allows the handling of large volumes of cultures and cells harvested by flocculation are in better physical condition than those manipulated cellular integrity with preservation of the produced metabolites [23]. Flocculation is the process where a solute particle in a solution forms an aggregate called a floc and it occurs when the solute particles collide and adhere to each other in a suspension [19].

Flocculation of microalgae results from charge neutralization due to the reduction in the electrostatic force of repulsion between charged microalgal cells in suspension and intra-particle bridging. Flocculants that have a high charge density are therefore more effective [19, 27]. The production of biodiesel from marine microalgal species is fast growing and high lipid content when compared to fresh water culture thus becoming sustainable and economically more promising technology.

Since there have been only few studies on the flocculation of marine microalgae, this paper contributes to improve the harvesting of marine microalgae for the production of biodiesel by developing optimum sedimentation process with various concentrations of different flocculating agents.

2. Materials and Methods

2.1 Organism and culture medium

Nannochloropsis oculata, obtained from Central Marine and Fisheries Research Institute (CMFRI), Tuticorin, Tamilnadu (India), was grown in sterile Walne's medium. The filtered sterilized sea water was enriched with required quantity of Walne's medium composition containing (g L^{-1}): $NaH_2PO_4 \cdot 2H_2O$, 20.0; Na_2EDTA, 4.0; H_3BO_3, 33.6; $MnCl_2 \cdot 4H_2O$, 0.36; $FeCl_3 \cdot 6H_2O$, 13.0; vitamin B_{12}, 0.001; vitamin B_1, 0.02; and $NaSiO_3$, 6.6. The trace metal solution contained (g L^{-1}): $ZnSO_4 \cdot 7H_2O$, 4.4; $CoCl_2 \cdot 6H_2O$, 2.0; $(NH_4)_6Mo_7O_{24} \cdot H_2O$, 0.9; and $CuSO_4 \cdot 5H_2O$, 2.0. The medium was adjusted to pH 8 and autoclaved at 121^0 C for 20 min. The filter sterilized

vitamins were added after cooling. The contents were later introduced into a 250-ml Erlenmeyer flask and finally transferred to 25L photobioreactor (PBR). Mixing was provided by sparging air from the bottom of the PBR; lighting was supplied by four cool-white fluorescent tubes with an intensity of 5000 lux.

2.2 Flocculation experiment

Flocculation experiments were carried out in stationary growth phase of microalgae. All experiments were conducted in glass tubes (size 25x150, capacity 50 ml) with 50 ml testing volume containing microalgae and flocculant. Eight different flocculants were used ($AlCl_3$, $Al_2(SO_4)_3$, $FeCl_3$, $Fe_2(SO_4)_3$, $ZnSO_4$, $ZnCl_2$, $MgSO_4$ and $MgCl_2$), at varying dosages which ranged from 0.2 g L^{-1} to 1.0 g L^{-1} with the step of 0.2 g L^{-1}. The flocculation efficiency was measured for each parameter at different time intervals like 0, 30, 60, 90, 120, 150, 180, 210, 240, 360 and 480 min respectively. All experiments were done in triplicates each time.

2.3 Flocculation efficiency

After addition of flocculants, each tube was kept in orbital shaker (Model-Technico, Honeywell Ltd, India) and stirring speed was maintained at 250 rpm. The initial microalgal biomass concentration in the tubes was estimated from the optical density of 750 nm (OD_{750}) [15, 22], in UV-VIS Spectrophotometer (Model- SL 159, ELICO Ltd, India). At every 30 minutes, the optical density of the supernatant was measured at half the height of the clarified culture [13]. Culture broth containing no flocculant was used as control and culture medium with appropriate quantity of each salt were used for blank to respective Flocculants. Flocculation efficiency was calculated by [22, 29]:

$$\text{Flocculation Efficiency (\%)} = \left(1 - \frac{A}{B}\right) \times 100,$$

where, A= OD_{750} value of sample and B=OD_{750} value of control

2.4 Effect of light and temperature on flocculation

The effective flocculants were chosen from the cell viability test to study the effect of temperature on flocculation. Such flocculants were added to the microalgal cells and flocculant test was carried out at different temperature (15, 25, 35, 45^0 C) in orbital shaker incubator in light and under dark condition separately with 250 rpm for 30 min. The optical density of the supernatant was measured at half the height of the clarified culture.

2.5 Cell viability test

Cell viability test was determined with Evans blue staining method [22]. One ml of sample of each culture was centrifuged at 3000rpm for 5 min, the supernatant was discarded, 100µl of 1% of Evans blue solution was added to pellet, and incubated for 10 min at room temperature. The cells were then washed twice in deionized water. The cell pellets were examined for the viability by light microscope (Model-Olympus CH20i BIMF, Olympus India Pvt., India) at 100X magnification. Broken cells appeared blue as Evans blue solution diffused in the protoplasm region and stained the cells blue.

3. Results and Discussion

3.1 Effect of flocculants on microalgal cells

Ferric salts exhibited maximum flocculation efficiency, among all other flocculants used in this study (Figure1). $MgSO_4$ and $MgCl_2$ showed an efficiency of 33% and 45.71 % respectively even after 240 min at their highest concentration, indicating that both the salts were not suitable for the microalgal flocculation. Efficient flocculation of the microalgae cells were observed with other six salts ($FeCl_3$, $Fe_2(SO_4)_3$, $ZnCl_2$, $ZnSO_4$, $AlCl_3$, $Al_2(SO_4)_3$). $FeCl_3$ and $Fe_2(SO_4)_3$ showed complete flocculation of 93.80 % (0.4g L^{-1}) and 87.33 % (0.6g L^{-1}) at 180^{th} min respectively, shown in Figure 2. Zinc salts exhibited maximum efficiency of 89.12 % for $ZnCl_2$ at 0.6 g L^{-1}at 210^{th} min and $ZnSO_4$ resulted in 84.17 % at the concentration of 0.6g L^{-1} at 240^{th} min whereas $AlCl_3$ showed 85.46 % efficiency at a concentration of 0.6 gL^{-1} and $Al_2(SO_4)_3$ gave 82.27 % at 0.8g L^{-1} at 240^{th} min respectively.

Figure 1: Settling of microalgae using flocculants at varying concentrations

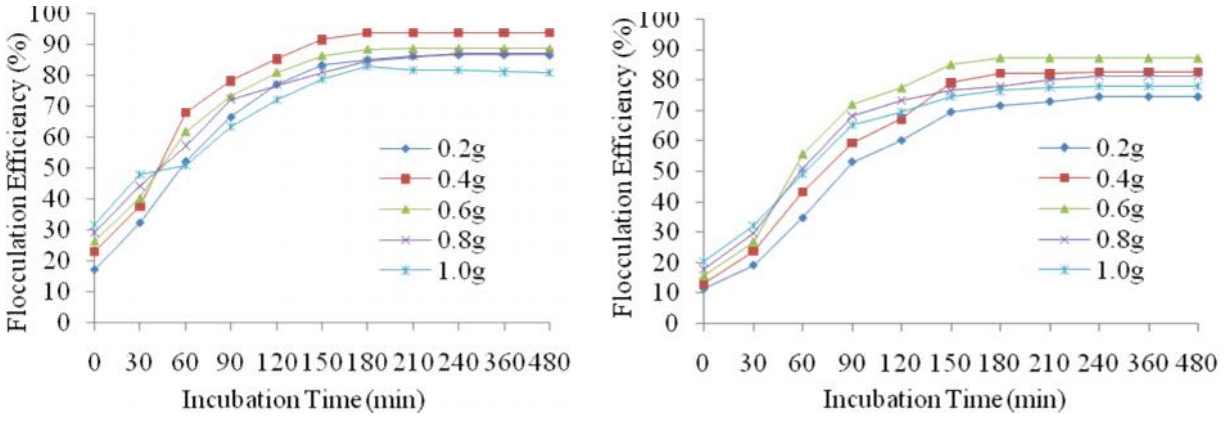
0.2, 0.4, 0.6, 0.8, 1.0g Ferric Chloride | 2, 0.4, 0.6, 0.8, 1.0g Ferric Sulphate | 0.2, 0.4, 0.6, 0.8, 1.0g Zinc Chloride | 0.2, 0.4, 0.6, 0.8, 1.0g Zinc Sulphate

0.2, 0.4, 0.6, 0.8, 1.0g Aluminium Chloride | 0.2, 0.4, 0.6, 0.8, 1.0g Aluminium Sulphate | 0.2, 0.4, 0.6, 0.8, 1.0g Magnesium Chloride | 0.2, 0.4, 0.6, 0.8, 1.0g Magnesium Sulphate

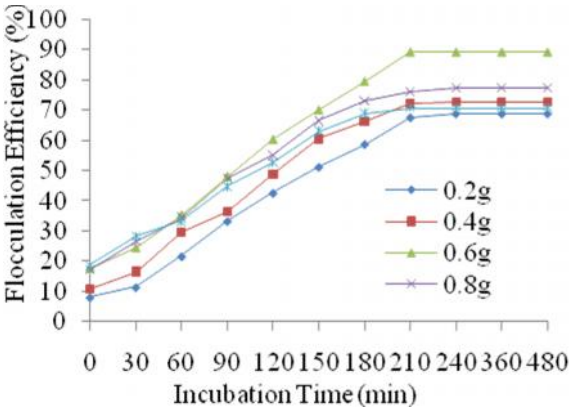
During the process of flocculation, the positively charged flocculants are attracted to negatively charged walls of microalgal cells, thereby particle collision and neutralization of charge occurs followed by deteriorating intraparticle repulsion and thus formation of flocs is achieved. In this study, higher concentration retarded the aggregation of cells, because of the excessive positive charges around the negatively charged cells leading to restabilisation of the cells due to electrostatic repulsion.

Our report showed that ferric salts are the most efficient flocculant than the other salts taken up for the study. Though ferric ions showed maximum efficiency, the colour change or pigmentation of culture due to the addition of these salts, showed that they cannot be used for the flocculation purpose.

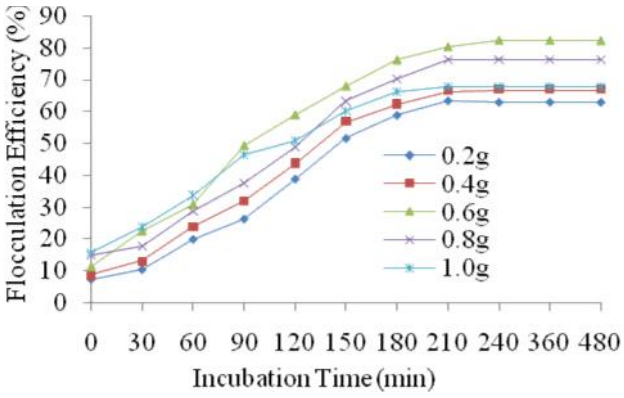
Figure 2: Effect of flocculants at varying concentrations and incubation periods



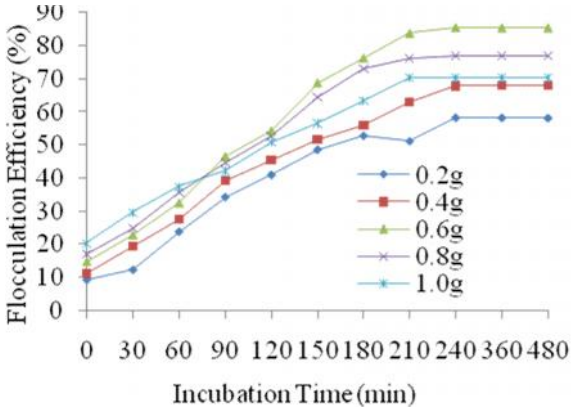
$FeCl_3$ **$Fe_2(SO_4)_3$**



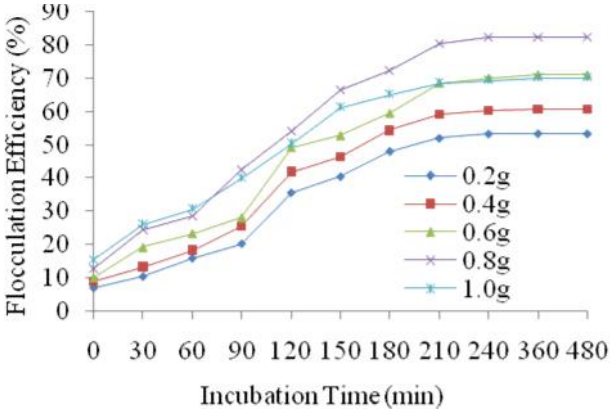
$ZnCl_2$



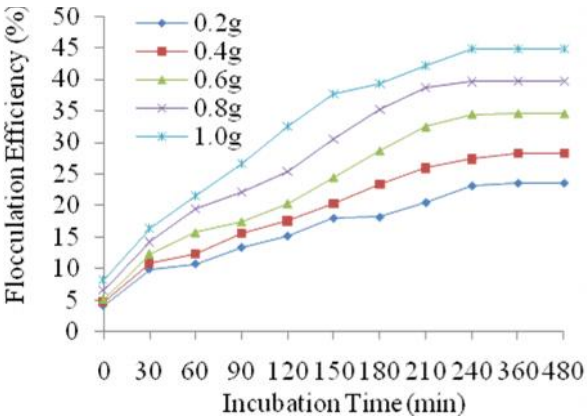
$ZnSO_4$



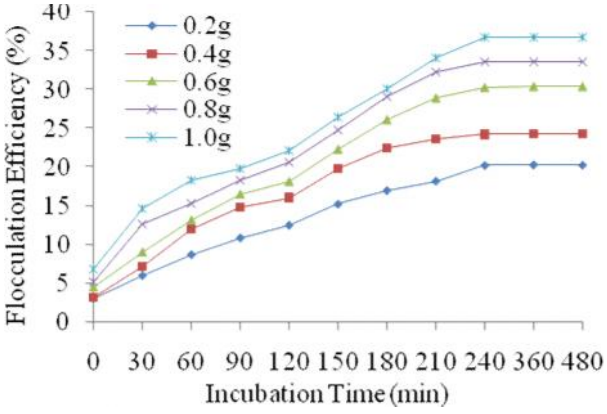
$AlCl_3$



$Al_2(SO_4)_3$



$MgCl_2$



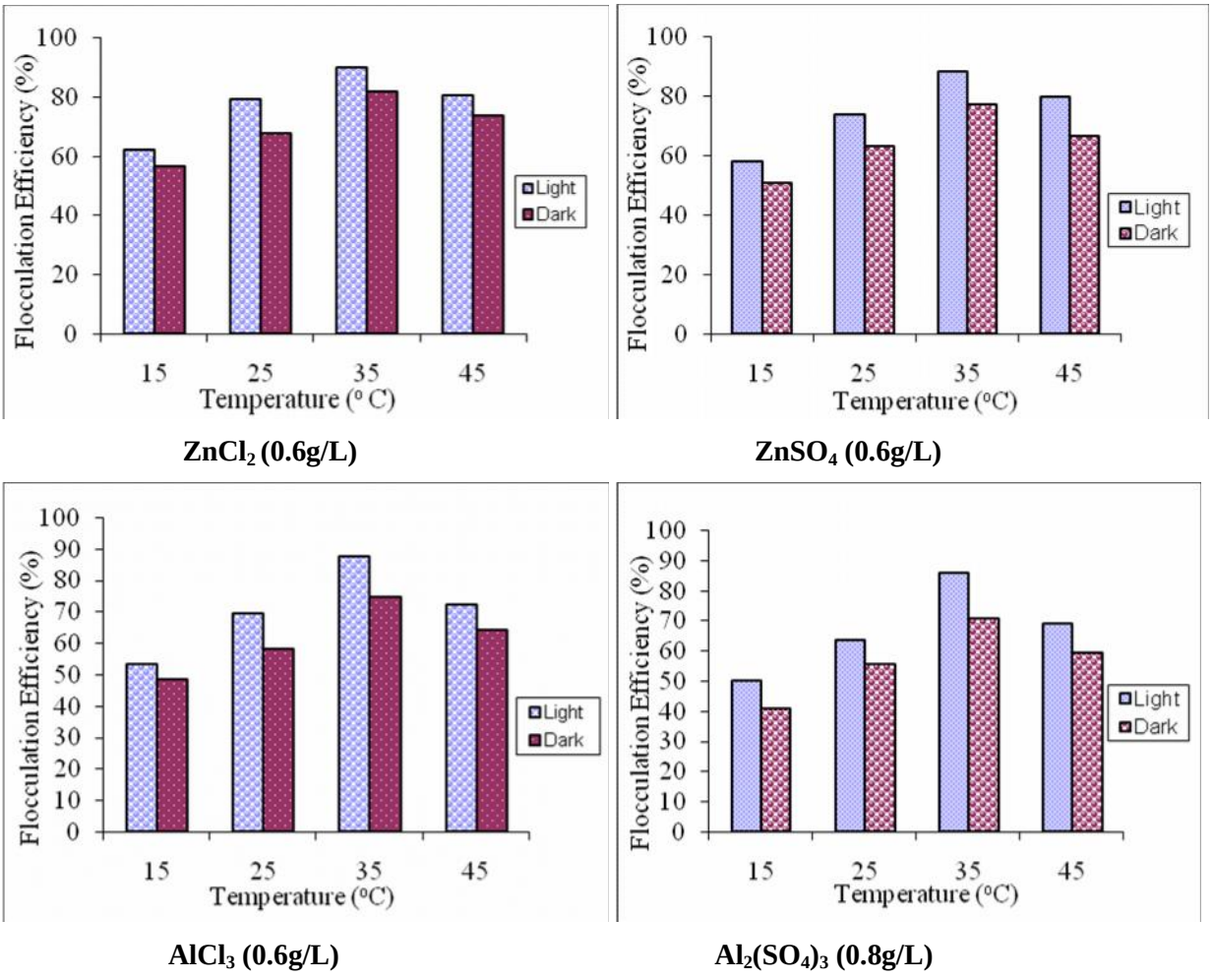
$MgSO_4$

3.2 Effect of temperature, light and darkness

Under illumination, the effective temperature was found to be 35^0C for efficient flocculants for this process (Figure 3). As the temperature reached 45^0C, flocculation efficiency declined. Temperature played a vital role in flocculation. Increase in temperature, above the normal range, up to 35^0C, increased the efficiency of flocculation. This is apparently due to the collision of cells because of the increasing mobility at higher

temperature, causing flocculant-particulate interactions and hence producing effective aggregates [10, 19]. According to Pan et al [29] influence of temperature on flocculation is clearly explained by chemical kinetics, at higher temperature the suspended particles move faster and frequency of collision is also increased, this contributes to increase in the rate of reaction. However, when temperature is too high, flocculation efficiency is lower as the formed flocs are too small and more hydrophilic. In our experiment we have found that when temperature is too low, reaction slows down, increase in temperature beyond optimum, i.e., when raised to 45^0C, decreased the agglomeration, which might be due to the susceptibility of finer cellular structures, causing cell death.

Figure 3: Effect of Temperature, light and darkness on flocculation



The flocculation was achieved more with chloride salts than that of sulfates [22]. Thus, illumination is found to be another important factor for flocculation through this study. The increase in the flocculation in the presence of light with increase in temperature is apparently due to the increase in the metabolic activity. Under darkness, reduction in inner biochemical alterations could decrease the flocculation [10]. Effects of temperature, light and darkness, along with their respective maximum flocculation efficiency values are briefly tabulated as given below (Table 1).

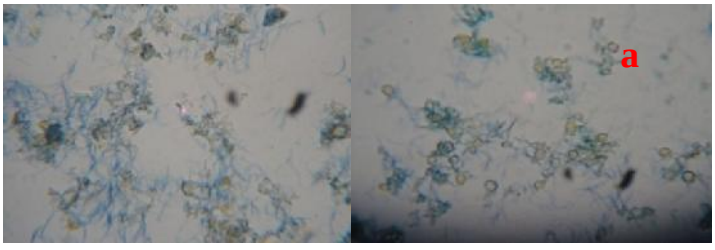
Table 1. Flocculation efficiency (%) of flocculants on *Nannochloropsis oculata* at different temperatures in light and under darkness

Temperature (^{0}C)	Flocculation Efficiency (%)							
	$ZnCl_2$ (0.6g L^{-1})		$ZnSO_4$ (0.6g L^{-1})		$AlCl_3$ (0.6 g L^{-1})		$Al_2(SO_4)_3$(0.8 g L^{-1})	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
15	62.31	57.58	58.32	50.82	53.41	48.11	49.23	40.25
25	79.66	68.26	74.11	62.43	69.22	55.23	61.74	50.88
35	92.34	83.14	90.49	80.14	88.49	74.77	85.91	68.79
45	81.81	73.96	79.98	66.97	74.23	64.09	69.32	57.60

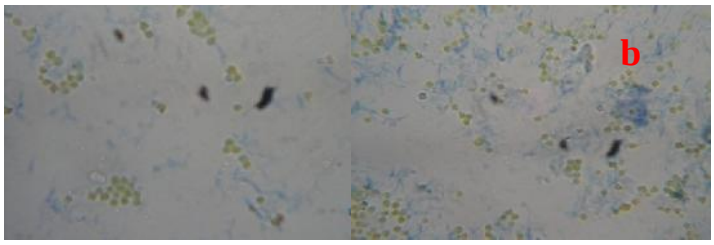
3.3 Cell viability check

Structural stability was investigated by Evans blue staining for the microalgal culture involved in flocculation (Figure 4). The culture when treated with Zinc salts, cells were found to be highly intact, irrespective of the concentration of the flocculants (Figure 4b). The cells were partially distorted when exposed to 0.6 gL^{-1} of Aluminium Chloride, whereas the cells exhibited a complete cell lysis on exposure of aluminium sulfate at a concentration of 0.4gL^{-1} (Figure 4c). The cells were completely destructed even at a lower concentration of Ferrous salts, $FeCl_3$ (0.4gL^{-1}) and $Fe_2(SO_4)_3$ (0.6gL^{-1}), thus proving them to be unsuitable for flocculation (Figure 4a).

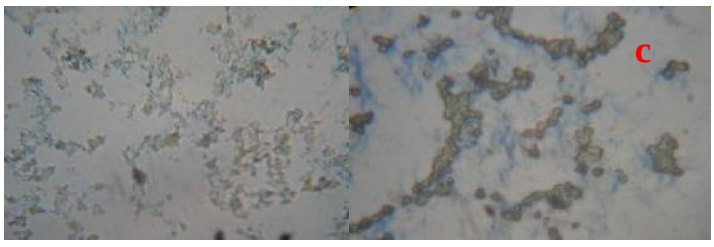
Figure 4: Viability of microalgal cells upon exposure to various chemical flocculants



$FeCl_3$0.4g L^{-1} Fe_2 $(SO_4)_3$0.6g L^{-1}



$ZnCl_2$ 0.6 g L^{-1} $ZnSO_4$0.6g L^{-1}



$AlCl_3$ 0.6 g L-1 Al_2 $(SO_4)_3$0.8g L^{-1}

4. Conclusion

The present investigation indicated that Zinc Chloride would be the best flocculant, though it required a longer time of 240 min and a higher concentration of 0.6g/L, to flocculate the microalgal cells. $AlCl_3$ salts were found to destabilise the structure of cells leaving them unsuitable for flocculation. Higher the temperature than optimal range, 35^0C, lower was the flocculation efficiency. The study also revealed that illumination was also one of the most essential parameters for effective flocculation while darkness declined the process.Thus for biodiesel production, to harvest microalga, $ZnCl_2$ would the efficient chemical flocculant.

References

1 Costa, J.A.V. and de Morais, M. G., The role of biochemical engineering in the production of biofuel from microalgae, Bioresource Technology, 2011, 102: 2-9.

2 Keera, S.T., El Sabagh, S.M. and Taman, A.R., Transesterification of vegetable oil to biodiesel fuel using alkaline catalyst, Fuel, 2011, 90: 42-47.

3 Chisti, Y., Biodiesel from microalgae, Biotechnology Advances, 2007, 25: 294-306.

4 Patil, V., Tran, K.Q. and Giselrod, H. R., Towards Sustainable Production of Biofuels from Microalgae: A Review, Int. J. Mol. Sci., 2008, 9: 1188-1195.

5 Bajhaiya, A.K., Mandotra, S.K., Suseela, M.R., Toppa, K. and Ranade, S., ALGAL BIODIESEL: the next generation biofuel for India: Review Article, Asian J. Exp. Biol. Sci., 2010, 1 (4): 728-739.

6 Xiao, M., Intan, R. and Obbard, J. P., Biodiesel production from Microalgae oil-lipid feedstock via immobilized whole-cell Biocatalysis, Proceedings Venice 2010, Third International Symposium on Energy from Biomass and Waste Venice, Italy; 8-11 November 2010.

7 Demirbas, A., Biodiesel from oilgae, biofixation of carbon dioxide by microalgae: A solution to pollution problems, Applied Energy, 2011, 88: 3541-3547.

8 Chisti Y., Biodiesel from microalgae: A Review, Biotechnology Advances, 2007, 25: 294-306.

9 Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A. and Bux, F., Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production, Bioresource Technology, 2011, 102: 57-70.

10 Harith, Z.T., Yusoff, F. M., Mohamed, M. S., Mohamed Din, M. S. and Ariff, A. B., Effect of different flocculants on the flocculation performance of microalgae, *Chaetoceros calcitrans*, cells, African Journal of Biotechnology, 2009, 8 (21): 5971-5978.

11 McKendry, P., Energy production from biomass (part 2): conversion technologies, Bioresource Technology, 2002, 83: 47-54.

12 Cheng, Y.S., Zheng, Y., Labavitch, J.M. and VanderGheynst, J.S., The impact of cell wall carbohydrate composition on the chitosan flocculation of *Chlorella*, Process Biochemistry, 2011, 46: 1927–1933.

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

14 Zhang, X., Hu, Q., Sommerfeld, M. Puruhito, E. and Chen, Y., Harvesting algal biomass for biofuels using ultrafiltration membranes, Bioresource Technology, 2010, 101: 5297–5304.

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

16 Benemann, J R., CO_2 Mitigation with Microalgae System, Energy Conver. Manage., 1997, 38 (Suppl.): S475.

17 Poelman, E., De Pauw, N. and Jeurissen, B., Potential of electrolytic flocculation for recovery of micro-algae, Resources, Conservation and Recycling, 19: 1-10.

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

19 Uduman, N., Qi, Y., Danquah, M.K. and Hoadley, A.F.A., Marine microalgae flocculation and focused beam reflectance measurement, Chemical Engineering Journal, 2010, 162: 935-940.

20 Xu, L., Guo, C., Wang, F., Zheng, S. and Liu, C.Z., A simple and rapid harvesting method for microalgae by in situ magnetic separation. Bioresource Technology, 2011, 102: 10047–10051.

21 Knuckey, R.M., Brown, M.R., Robert, R. and Frampton, D.M.F., Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds, Aquacultural Engineering, 2006, 35: 300-313.

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

23 Borges, L., Moron-Villarreyes, J.A., Montes D'Oca, M.G. and Abreu, P.C., Effects of flocculants on lipid extraction and fatty acid composition of the microalgae *Nannochloropsis oculata* and *Thalassiosira weissflogii*, Biomass and Bioenergy, 2011, 35: 4449-4454.
24 Pittman, J. K., Dean, A.P. and Osundeko, O., The potential of sustainable algal biofuel production using waste water resources, Bioresource Technology, 2011, 102: 17-25.
25 Chen, C.Y., Yeh, K. L., Aisyah, R., Lee, D.J. and Chang, J.S., Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review, Bioresource Technology, 2011, 102: 71–81.
26 Yang, Z., Shang, Y., Lu, Y., Chen, Y., Huang, X., Chen, A., Jiang, Y, Gu, W., Qian, X., Yang, H. and Cheng, R., Flocculation properties of biodegradable amphoteric chitosan-based flocculants, Chemical Engineering Journal, 2011, 172: 287– 295.
27 Thapa, K. B. and Qi, Y., Hoadley, F.A., Interaction of polyelectrolyte with digested sewage sludge and lignite in sludge dewatering, Colloids Surfaces A, Physiochemical and Engineering Aspects, 2009, 334: 66-73.
28 Oh, H.M., Lee, S.J., Park, M.H., Kim, H.S., Kim, H.C., Yoon, J.H., Kwon, G.S. and Yoon, B.D., Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus sp.* AM49, Biotechnology Letters, 2001, 23: 1229-1234.
29 Pan, Y., Shi, B. and Zhang, Y., Research on Flocculation Property of Bioflocculant PG . a21 Ca. Modern Applied Science, 2009, 3 (6): 106-112.

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