Harvesting of *Nannochloropsis oculata* by chemical flocculation

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Microalgae culture forms an inevitable component in aquaculture and mass culture on a commercial scale is essential to satisfy requirement in the hatchery for use as functional foods and nutraceuticals. However, the current microalgal production technologies are not cost-effective and face several bottlenecks, among which is the harvesting of microalgal biomass. Typical strategies currently applied for harvesting microalgae include centrifugation, filtration, various forms of flocculation (e.g., chemical inorganic and organic agents, alkaline flocculation, bio-flocculation using microorganisms, and electro-coagulation), sedimentation, and flotation. Among these harvesting methods, flocculation combined with sedimentation of microalgal flocs is considered best with reported cell recovery of > 90% and with low cost. However, the biomass thus recovered with chemical flocculant may cause harm to the final product but it is still regarded as a promising technique. In the present study, the flocculation efficiency of $ZnCl_2$ and $ZnSO_4$ were tested as chemical flocculants for the harvest of *Nannochloropsis oculata*.

Marine microalgae, *Nannochloropsis oculata* culture was used as the culture inoculum. The algae was cultured in photobioreactor (Celeritus Engineering, Ahmedabad) with Conway medium at temperature of 18-21^{0}C, pH of 7.8-8.4, salinity of 23-25 ppt and light intensity of 2000 lux. Flocculation experiments were carried out in stationary phase cultures (28 million / ml). 900 ml of the culture was taken in 1000 ml glass beaker and different concentrations of flocculants were added. Based on our previous experiment on electro flocculation study, chemical compound with Zinc was considered. Two different flocculants were used; $ZnSO_4$, and $ZnCl_2$ at varying concentration ranging from 0.2 g/L to 1 g/L. The flocculation efficiency was measured after 4 hours from all the experiments which were done in triplicates. The initial microalgal biomass concentration in the beaker was estimated from the optical density of 750 nm. After 240 minutes, the optical density of the supernatant was measured at half the height of the clarified culture. Culture broth containing no flocculant was used as control. Flocculation efficiency was calculated using the formula:

Flocculation efficiency (%) = (1–A/B) x 100; Where, A= OD value of sample at 750 nm and B = OD value of control at 750 nm

$ZnSO_4$ with 0.8g/L performed maximum flocculation efficiency of 92.54 % followed by 0.2g/L (71.79 %). Least efficiency was recorded in 1.0 g/L which was similar ($p>0.05$) to 0.4g/L. Whereas, with various concentration of $ZnCl_2$, the harvesting efficiency was varied from 70.55 %–77.54 %. The highest efficiency was registered with 0.4 g/L of $ZnCl_2$ followed by 0.2 and 0.6 g/L. Not much difference ($p>0.05$) was observed between 0.2 and 0.6 g/L. Lowest harvesting efficiency (70.55%) was recorded in $ZnCl_2$ with concentration of 1.0g/L.

Evan's Blue stain was used for testing the cell viability of the flocculated *Nannochloropis* cells. For staining, a 20 mL sample of flocculated *Nannochloropis* was treated with 1 ml of 1% (w/v) stock solution of Evan's Blue. The samples were allowed to stand at room temperature for a minimum of thirty minutes before microscopic examination. A subsample of each stained suspension was then inspected at 40 X magnification using an

Improved Neubauer Haemocytometer (Superior Co., Berlin, Germany). ZnSO4 (0.8%) performed better with superior viability compared to other concentrations (0.2, 0.4, 0.6 &1.0g/L). During the Evan's blue staining, most of the *Nannochloropsis* cells were greenish in colour and hence, were not stained (80% viability). *Nannochloropsis* cells flocculated with various $ZnCl_2$ concentrations had white precipitations. However, after staining with Evan's blue, the cells were greenish and individually dispersed (>40% viable cells).

Pre-treated sea water passing through slow sand filter, UV filter and further treated with ozone was used for the inoculation of flocculated microalgae. The flocculated microalgal cells to be used as inoculum were diluted and mixed properly with the help of magnetic stirrer to ensure the uniform distribution of individual cells. The initial cell count was maintained at $5x10^5$ cells/ml. These were cultured at temperature of 18-21^{0}C, pH of 7.8-8.4, salinity of 23-25 ppt and light intensity of 2000 lux with Conway medium. Cell count was estimated after 7 days of inoculation. *Nannochloropsis* flocculated with 0.8g/L $ZnSO_4$ could produce maximum cell count of $9x10^6$ cells/ml. The rest of the concentrates, with other concentrations of $ZnSO_4$, also could perform, albeit with less number of cell counts. Whereas, *Nannochloropsis* cells flocculated with $ZnCl_2$ took more time to increase the cell count, and among various concentrations of $ZnCl_2$, least concentration performed better (0.2g/L). It is concluded that, chemical flocculation of *Nannochloropsis oculata* with 0.8 g/L $ZnSO_4$ may be an effective method for harvesting of large volume of microalgae culture with good harvesting efficiency. Further studies are required for its commercial applications in various industries and fish hatcheries.