pH induced flocculation of microalgae, *Chaetoceros calcitrans*

Biji Xavier*, Ritesh Ranjan, Sekar Megarajan, Vamsi Balla, Narasimhulu Sadhu, Chinni Bathina and Shubhadeep Ghosh
Visakhapatnam Regional Centre of ICAR-Central Marine Fisheries Research Institute, Visakhapatnam -530 003, Andhra Pradesh
*Email: bijicmfri@gmail.com

Microalgae are important in aquaculture as live feed and often the preferred food source, even if several alternatives like yeast and micro encapsulated feeds are available. Major genera of microalgae for larval

feeds include *Chaetoceros*, *Thalassiosera*, *Tetraselmis*, *Isochrysis* and *Nannochloropsis*. In "green water" feeding technique, microalgae are added as suspension and are grown simultaneously in tanks with fish larvae. The microalgae has several benefits such as positive effect on weight gain, improved resistance to disease and decreased nitrogen output into the environment. The mass culture of microalgae is a necessity for various mariculture operations in most hatcheries. However, the infrastructure requirements and the high costs involved for continuous production of the algal culture are major constraints. Recent developments reveal that fresh microalgae can be substituted with concentrated preserved algal mass for green water applications. Intensive cultivation for production of large quantities of microalgae biomass requires a proper harvesting technique. Flocculation as a method of microalgae harvest is comparatively more cost and energy efficient. Harvest by flocculation with pH adjustment for *Chaetoceros calcitrans* a small, fast-growing marine diatom used widely in aquaculture industries for culture of several marine filter feeders was successfully tested.

Axenic cultures of *Chaetoceros* was used for the flocculation study. Microalgae were cultivated in 3L Hauffkin flask with Guillard F/2 as culture medium at salinity of 30 ppt, under continuous day and night illumination by white fluorescent (2500 lux average). Cells were harvested at late logrithmatic growth phase (after 5days) and flocculation experiments performed with the adjustment of pH (8.4 – 11.9) using 5N NaOH. The pH varied from for the present study. The experiment was done in 500 ml beaker and the base was mixed to the culture at higher rate (200 rpm) by agitation using magnetic bar stirrer, to allow for steady increase and homogeneity in pH. When the required pH was reached, mixing was slowed (50 rpm) to allow for settling under gravity. Flocculation efficiency was estimated by measuring the optical density of the aliquot of the medium collected after 4 hours of flocculation. The optical density of the aliquot was measured using UV spectrophotometer (Biotech Epoch 2) at wave length of 750nm. The flocculation efficiency (%) was calculated using the formula,

$(1-B/A)*100$, where A is the optical density of the initial culture medium and B is the optical density of the sample, both at 750 nm.

Cell viability test was done using Evan's Blue stain with flocculated sample of 0.1ml diluted to 1ml and centrifuged for 5 minutes. The supernatant was discarded and 100 μl of 1% Evans blue stain was added to the pellet. The sample was incubated at normal temperature for 30 minutes after which pellets were washed and suspended in fresh filtered sea water. The cells were observed under the microscope ("Lynx" Lawrence & Mayo) and photographs taken. Broken cells appeared blue, as Evans Blue solution diffused into their protoplasm region and stained the cells blue.

Results indicated that when the pH was adjusted with 5N NaOH, the flocculation efficiency showed significant increase from an initial pH of 8.4 to the induced pH of upto 10.2 and then, it was reduced at pH of 10.3 and subsequently, became stationary upto pH of 11.9. The flocculation efficiency increased from 23% (8.4 pH) to 75% (10.2 pH). The sedimentation height also followed the same trend as that of flocculation efficiency. It varied from 0.035-0.053 mm / minute with the maximum recorded at pH of 11.9. The present study revealed that additional bases increased the precipitation and led to the formation of loose flocs. The Evans Blue staining confirmed that the cells are individually dispersed upto the induced pH of 10.2, without taking any stain, and hence the microalgal cells are intact with good viability and the culture can progress from the flocculated cells. In the cells flocculated with induced pH of 10.3 to 11.9, the cells were aggregated and the Evan's blue solution diffused into their protoplasm region and stained the cells blue in colour. It is therefore concluded that induced pH of 10.2 is optimum with better flocculation efficiency and sedimentation height for the harvesting of *Chaetoceros calcitrans* and that the microalgal culture is able to progress from the flocculated cells.