Micro-agriculture techniques for the sustainable production of live feed organisms in the laboratory

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Indian coastal waters harbour diverse groups of microalgae or phytoplankton such as diatoms, dinoflagellates, blue green algae, silicoflagellates and cocolithophores together constituting nearly 95% of primary production in the marine ecosystem. In mariculture, these microalgae form the main live feed organisms during the early developmental stages of commercially important marine finfishes, crustaceans and molluscs. Hence marine hatcheries maintain stock cultures of diatoms and dinoflagellates for producing mass cultures for feeding requirements. The marine hatchery complex at Calicut Research Centre of CMFRI maintains stock cultures of eight species of live feed organisms (Table 1).

Like agronomic practices essential for the cultivation of field crops, certain micro-agriculture manipulations determine the sustainable production of microalgae in the laboratory. Growth of microalgae in the laboratory can be influenced by external environmental conditions such as light intensity, photoperiod, temperature, dissolved nutrients, minerals *etc.* These factors not only can affect the growt and multiplication of microalgae, but also their quality and accumulation of metabolites.

Light is one of the most important environmental factors affecting the growth rate of microalgae. Liao *et al.* (1983) demonstrated that the growth rate of *Skeletonema costatum* increased with light intensity progressing through 500-10,000 lux and declined at light intensity exceeding 10,000 lux. Most types of microalgae grow well at temperatures from 20 to 25 °C (Laing, 1991). For optimum growth rate and sustainable production of microalgae, the following conditions were optimised at our laboratory.

Table 1. Species of microalgae maintained at Calicut Research centre of CMFRI

Group	Species
Chlorophyceae	*Chlorella vulgaris*
Chlorophyceae	*Dunaliella salina*
Chlorophyceae	*Nannochloropsis oculata*
Chlorophyceae	*Nannochloropsis salina*
Bacillariophyceae	*Chaetoceros calcitrans*
Haptophyceae	*Isochrysis galbana*
Haptophyceae	*Dicrateria gilva*
Prasinophycea	*Tetraselmis gracilis*

Temperature is maintained at 23° C using two split air conditioners operated alternatively. Light is provided from fluorescent lamps kept 12 inches away from the culture flask to prevent cultures getting heated up due to prolonged illumination. Photoperiod is another important factor which was set to 12 h dark and 12 h light regime using a timer-switch. Before setting the photoperiod, the stock cultures were crashing very often during the onset of exponential phase itself.

Contamination is a menace to the stock cultures which is caused due to improper handling of culture flasks, cotton plugs, seawater, media as well as vitamins and inadequate sterilisation of culture flasks. To avoid contamination of the stock cultures, the culture flasks are cleaned thoroughly with excess water, rinsed with HCl, brushed with soap solution

and finally washed under running tap water. These flasks with the chlorinated, aerated and filtered seawater are autoclaved for 30 min, cooled and then nutrients and vitamin solution are added using syringe filters (2 µ). The cotton plugs wrapped with cotton gauze are labelled to avoid cross contamination. These cotton plugs are also periodically autoclaved.

Silicate supplement is required for the culture of *Chaetoceros* and other diatoms. In addition to normal dose of nutrients and vitamins as recommended by Walne, addition of 1 ml l^{-1} of 2 µM solution of sodium metasilicate enhances the growth rate and cell multiplication. Salinity of the medium is increased to 37-38 ppt for culturing Dunaliella salina as cultures at salinity levels lower than this fail to develop after inoculation.

Immobilisation or entrapping the microalgal cells in polyurethane film or styrene balls helps in easy transport, long term storage, saves inocula and minimises the risk of contamination. Carbondioxide (CO_2) bubbling to the cultures for one minute every day dispensed through sterilised tubular vents enhances the cell density to above 50% more than those flasks which did not receive CO_2. Serial dilution of stock cultures once in every fortnight is favoured to keep them axenic and pure. Contamination due to ciliates is a serious problem that can lead to collapse of the culture. Recouping the cultures with additional supplements of nutrients and vitamins after every six days prolongs the exponential phase of the cultures and thereby the time and labour involved in cleaning the glassware, preparation of fresh medium and inoculation can be saved. Two litre cultures are thus maintained for two months in our laboratory at the Calicut Research Centre of CMFRI.

Nutrient enrichment to the live feed culture is achieved by supplementing minerals (µM solutions of Sn, Mg, Ca, Bo, Mo and Zn) or growth hormones over the normal levels. These minerals and hormones are accumulated in the cultured algal cells. Such enriched feed organisms act as vehicles carrying additional levels of minerals, hormones and amino acids to their consumers through feeding aimed at imparting certain desirable traits. Organic culture of live feed organism without using inorganic salts as source of nutrients is also possible by substituting sterilised garden soil extracts and blended seaweed extracts (Kaladharan *et al.,* 2002). While garden soil extracts offer major nutrients, seaweed extracts do offer immunostimulants besides vitamins and natural growth stimulants. By optimising the physical, chemical and environmental conditions for the cultures, the quantity and quality of live feed cultures in the laboratory can be increased considerably.