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MASS CULTURE OF PHYTOPLANKTON

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INTRODUCTION

Large-scale culture of micro-algae especially nannoplankton flagellates and selected species of diatoms are necessary for feeding the larval forms of molluscs or fishes, in a hatchery system. Since the bivalve larvae can feed on organisms measuring less than 10 microns, these forms have to be isolated from the seawater and maintained as stock culture. Utilizing the inoculum, mass culture can be carried out in the laboratory conditions as well as in the open system.

Culture containers

The containers that can be used for the laboratory mass culture of the micro-algae are 10 litre polythene bags, 20 litre glass-carbuoys as well as 100 litre persepex tanks. These containers are kept in wooden racks provided with aeration and light facilities. Fully grown culture from the stock culture is used as inoculum for the mass culture in these containers. About 200 ml of the culture is used for 10 l polythene bags, 500 ml for the 20 l glass carbuoys while 2 litre of the culture is used for the 100 litre persepex tanks. After the inoculation, on 5-6th day, the culture

will reach in the optimal growth phase and ready for supply to the hatchery. Leaving one litre of the same culture in the 20 l glass carbuoy, fresh enriched water can be added for the further mass culture in the same container.

CULTURE MEDIUM

Conway or Walne's medium (Walne, 1974), PM and TMRL medium are used for the mass culture of flagellates and other nannoplankters in a hatchery system (Gopinathan, 1982). For the mass culture of flagellates, the vitamin mixture is not necessary.

During the course of the larval rearing of oysters or any other bivalves, the flagellates forms the basic food upto the stage of spat. However, for the better growth and survival of the spat, the food has to be changed from flagellates to a mixture of diatoms and other nannoplankters. For making a culture of the mixed phytoplankton in the open area using direct sun-light, the following medium could be used in a one ton capacity fibre-glass (white colour) tank.

Potassium nitrate	..	12.0 gm
EDTA (Na)	..	6.0 "
Sodium ortho phosphate	..	6.0 "
Sodium silicate	..	6.0 "

(Sodium silicate dissolve separately in 50 ml of dist. water)

To 100 litre of fresh filtered seawater (fresh seawater can be filtered through No. 3 organdy cloth to remove zooplankton) 1/10 of this medium is added. The water is poured in 2 or 3 white-lined basins or fibre-glass tanks and kept in bright sun-light. Within 36 hours, a slight discoloration can be noted on the sides of the basin. On

examination, we can notice a mixture of planktonic diatoms and other nanoplankters. If the temperature is very high and sunlight is very bright, only the blooming of Chaetoceros spp. could be observed.

Besides the above mentioned chemicals which serve as nutrients, commercial fertilizers can be used for the mass culture of nanoplankters (eg. Chlorella) in open tanks on a economical basis. The media used for the open culture of Chlorella are:

Urea 46	...	10 mg/l
16-20-0 (NPK)	...	10 mg/l
20-0-0	...	100 mg/l

GROWTH PHASES OF ALGAL CULTURE

The usual way of the laboratory culture of the micro-algae is one in which a limited volume of medium containing the necessary inorganic and organic nutrients is inoculated with a relatively small number of cells and these exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows characteristic pattern in which the following phases of growth may usually be recognised:

1. Lag or induction phase: The cells taken from the stock culture and inoculated to a new flask have to acclimatise to the new medium. So there is no cell division for a few hours and this phase is known as lag or induction phase.
2. Exponential or growing phase: Once the cells are acclimated to multiply and grow rapidly. It is assumed that within 12-24 hours, one cell will divide into 2 and

further these cells carry the growth till the culture reaches its maximum concentration. This growing phase is known as exponential phase.

3. Declining phase: Once the cells reach maximum concentration, the growth and multiplication of the cells will be arrested and slowly the cells show the symptoms of decline. This arrested growth of the cells in the culture is known as declining phase.
4. Stationary phase: After the arrested growth for a few days the culture will be stationary without any further growth and multiplication of the cells. Actually, stationary phase is prolonged in the case of flagellates. They may develop some cover or cyst around its body for thriving the unfavourable environment. During the stationary phase, if the cells get a new environment, they may start further growth and reproduction.
5. Death phase: After a long period in the stationary phase, the cells may lose its viability and start to die and thus culture will become useless either for re-culturing or for feeding.

DETERMINATION OF ALGAL DENSITIES

Regular count of the algal cells must be made in order to schedule inoculation of the various culture flasks and mass culture containers, to monitor growth of the algal cultures and to determine the quantity of algae to be fed to the rearing larval organisms.

Since the nanoplankton organisms measure less than 10 μ , we have to use a Haemocytometer for counting. Initially, the sample has to be treated with a drop of eosin or formalin to kill the cells and after stirring well, one drop is taken with a sterilized pipette. After placing the cover-slip on

the haemocytometer, the pipette is brought to the edge of the slide and touched. The sample runs inside and thus we will get a thin film of the culture in which the cells are equally distributed. Since the haemocytometer has got 9 chambers, 4 sides having 16 divisions and 5 chambers with 25 division, we have to restrict the counting to at least 3 chambers. The average number of cells in one ml is calculated by using the equation,

$$\text{Average counts per chamber} \times 10^4 = \text{Total number of cells/ml.}$$

HARVEST AND PRESERVATION

The maintenance of the culture and constant supply to meet the requirements of the hatchery is a problem especially during adverse weather conditions. The preservation of the algae either by freezing or by sun-drying is advantageous in that during scarcity of food, the rearing operations could be successfully continued. For freezing, the culture has to be flocculated either by adding alum or lime or by adjusting the pH using NaOH. After determining the quantity of culture to be flocculated, measure the volume of NaOH solution needed to flocculate and add to the solution. Vigorous stirring should be done and the culture is left for one hour. After one hour, we can see the algal mass deposited in the bottom. The clear water is decanted and the sediment collected. The pH is brought down to its original value by adding slowly dilute HCl. Now the algae is ready for freezing or drying. Drying of the algae can be done by pouring the same in white enamel trays and keeping it in the bright sun-light. If the algae has dried up thoroughly, it can be scraped from the enamel trays and kept in glass bottles. Before freezing, the algal mass has to be treated with a few drops some protective reagents like Dimethyl sulphoxide or glycerol. The frozen algae can be kept for

3 months. Whenever adverse condition arises, the frozen feed can be used for rearing the larvae, though the protein content may be little less when compared to the live feed.

REFERENCES

- Gopinathan, C.P. 1982. Methods of culturing phytoplankton. In: Manual of research methods for fish and shellfish nutrition. CMFRI, sp. publ., 8: 113-118.
- Walne, P.R. 1974. Culture of bivalve molluscs. Fishing News (Books) Ltd. Surrey, G.B., pp. 1-173.