MICROALGAE CULTURE

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INTRODUCTION

Phytoplankton are the micro-algae, which form the basic food of almost all the animals in aquatic ecosystem. Most of them are unicellular They are the primary producers of organic matter in aquatic habitats. The scope of micro-algae as a possible source of protein food was recognised in the middle of the 20th century. In the past, the main attention was on Single Cell Protein (SCP) production for human consumption and later many new applications have evolved including waste-water treatment, nutrient recycling, closed life-support systems, aquaculture and the bio-conversion of solar energy.

In recent years, there has been renewed interest in producing Single Cell Protein by mass culturing the unicellular micro-algae such as diatoms (species of *Chaetoceros* and *Skeletonema*) and nannoplankters (species of *Isochrysis*, *Chlorella* and *Tetraselmis*) for feeding the larvae of crustaceans, molluscs and fishes, as the success of any hatchery operation depends mainly on providing the required species of microalgae. The larvae of prawns and fishes prefer diatoms as their basic food while the larval molluscs live on the nannoplankton flagellates, measuring less than ten microns. For rearing shrimp larvae species of *Chactoceros* and *Skeletonema* are used. Species of *Isochrysis*, *Pavlova*, *Dicrateria* and *Chromulina* are used as feed for the larvae of pearl and edible oyster, mussels, clams and sea cucumbers and mixed diatom cultures and nannoplankters are given for the juveniles and adults.

The various aspects of the micro-algae culture are: isolation of required species, identification, preparation of culture media, stock culture maintenance, indoor and outdoor mass culture and harvest and preservation of the culture.

ISOLATION

Isolation of the micro-algae can be done by any one of the following methods.

1. Pippette method:

Large organisms can be pipetted out using a micro-pipette under microscope and transferred to culture tubes which are having enriched culture media.

2. Centrifuge method:

By repeated centrifuging of the sample and by inoculating the deposits, we may get different organisms.

3. Phototactic method:

By this method, most of the phytoflagellates can be isolated. Make a dark chamber with a small hole on one side and keep a candle outside, near the hole, pour the water sample in a beaker and keep it inside the dark chamber, with its beak nearer to the hole. Since the flagellates have a tendency to move towards the light, they crowd near to the candle light. By pipetting, we can separate these organisms and a pure culture can be raised adopting culture tube method.

4. Agar plating method

For preparing the medium, 1.5 gm of agar is added to 1 litre of suitable medium or natural sea-water. This agar solution is sterilized in an autoclave for 15 minutes, poured in sterilized petri-dishes and kept for 24 hrs. For the isolation, the required species can be picked up by platinum needle under microscope and streaked on the surface of the agar plate. After inoculation, these petri-dishes are placed in an incubation chamber for 7-8 days providing 1000 lux light and constant temperature (25°C). Within this time, the required species, if it has grown into a colony, can be removed by platinum loop and transferred to culture tubes. Further, from the culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

5. Serial dilution culture technique.

In this method, 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} or 4 steps - 0.001, 0.01, 0.1 and 1 ml) are involved for the isolation of the required species. For the serial dilution technique, nearly 25 culture tubes (15 ml) are required. After filtering the seawater through 10-20 microns sieve, the filtrate is inoculated to five series of culture tubes in various concentrations. The tubes are kept under sufficient light (1000 lux) and uniform temperature (25°C). After 15 days, discolouration occurs in the culture tubes, due to the growth of micro-algae. Further purification of this culture is done by sub-culturing it in 50 ml conical flasks and then in 500 ml and one litre conical flasks. Once the culture is fully purified, it is transferred to 3 or 4 litre Hafkine culture flasks and maintained as stock culture.

After the isolation of the required species in culture tubes, it is sub-cultured in 50ml test tubes. These sub-cultures are the base from which continuous supply of non contaminated algal feed is maintained for large-scale culture operation.

CULTURE MEDIA

For the successful culture of micro-algae, various chemical culture media have been used depending on the type of organisms cultured and their growth phases. Since the micro-algae require nutrients such as nitrates and phosphates, roughly in a ratio of 10: 1 (N:P) for normal growth and reproduction, the culture media used in the laboratory should have sufficient quantities of these elements besides other growth promoting substances including trace metals and vitamins. Since the diatoms require

silica for building up the cell walls, the culture media should have a silicate source besides nitrates, phosphates, chlorides and trace metals. Similarly, for the nannoplankton flagellates such as species of *Isochrysis, Dicrateria, Pavlova*, trace metals and vitamins are required for their growth and multiplication. Usually for culturing the flagellates, *Chromulina* and *Tetraselmis* 'Conway' or Walne's medium is used in the laboratory for the maintenance of stock culture as well as mass culture. The composition of the Walne's culture medium, is given below:

'Conway' or Walne's medium

A. Potassium nitrate 100 gm; Sodium ortho-phosphate 20 gm; EDTA (Na) 45 gm; Boric acid 33.4 gm; Ferric chloride 1.3 gm; Manganese chloride 0.36 gm; and Dist. Water 1 litre.

B. Zinc chloride 4.2 gm; Cobalt chloride 4.0 gm; Copper sulphate 4.0 gm; Ammonium molybdate 1.8 gm; and Dist. Water 1 litre

C. Vitamin B_1 (Thiamine) 200 mg in 100 ml dist. water; Vitamin B_{12} (Cyanocobalamine) 10 mg in 100 ml dist. water

Prepare A, B and C (each) in different reagent bottles. Add 1 ml of A, 0.5 ml of B and 0.1 ml of C to 1 litre of filtered and sterilized seawater.

For the preparation of various phytoplankton mixture in the outdoor tanks, using direct sunlight, the following medium could be used:

Potassium nitrate 13.2 gm; Sodium orthophosphate 6.6 gm; EDTA (Na) 6.6 gm; Sodium silicate 6.6 gm

Dissolve the first 3 chemicals in 25 ml of dist. water and sodium silicate separately in 25 ml of dist. Add this to 1 ton of fresh seawater filtered through 0.33 mm mesh organdy net, to remove zooplankton, providing sufficient aeration. Within 24 hrs, a slight yellow discolouration develops. On examination under microscope, we can notice the growth of diatoms and nannoplankters. When the temperature is very high and sunlight is very bright, *Chaetoceros* spp. blooms could be observed. Besides the above mentioned laboratory prepared chemicals, which act as nutrients, commercial fertilizers can be used for the mass culture of micro-algae. In open tanks, for economy purposes, the following fertilizing medium can be used.

Urea 46	...	**10 mg/l**
16-20-0	...	**100 mg/l**
20-0-0	...	**100 mg/l**

GROWTH PHASES OF THE ALGAL CULTURE

The usual way of the laboratory culture of 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 then exposed to suitable conditions of light, temperature and aeration. Increase in cell numbers in such a culture follows a 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 room and inoculated to a new flask have to acclimatise to the new medium. So there will be no cell division for a few hours and this stage is known as lag or induction phase.

2. Exponential phase

Once the cells are acclimatised to the medium, multiplication starts and the culture reaches its maximum concentration in 12-18 hrs. This growing phase is known as exponential phase.

3. Declining Phase

Once the cells reach the maximum concentration, the growth and multiplication of the cells will be arrested and slowly show the symptom of decline. This stunted growth phase is known as declining phase.

4. Stationary Phase

After growth stops the culture will be stationary for a few days without any further cell division. Actually, stationary phase is prolonged in the case of flagellates. For this, they may develop some cover or matrix around body for withstanding the unfavourable conditions. In 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 viability and start to die and thus the culture will become useless, either for reculturing or for feeding.

DETERMINATION OF ALGAL CELL DENSITIES

Regular counts of the algal cells must be made in order to schedule inoculation of the mass culture from the containers, monitor growth of the algae and determine the quantity of algae to be fed to the larval organisms.

Since most of the nannoplankton organisms measure less than 10 microns, a Haemocytometer is used for counting. For this, a sample is prepared and 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 should be brought to the edge of the haemocytometer and touch it. The sample will run inside and thus we will get a thin film of the culture in which the cells should be equally distributed. Since the haemocytometer has got 9 chambers, we have to take the counts of 4 side chambers having 16 divisions to determine the number of cells in one ml using the following equation.

Average counts per chamber x 10^4 = Total number of cells/ml

MASS CULTURE

Containers used for mass culture of micro-algae are 10 litre capacity polythene bags, 20 litre glass carbuoys and 100 litre perspex cylindrical tanks for the indoor culture and 250l, 500l, 1 ton FRP tanks and 5 ton concrete tanks for the outdoor culture. Fully grown stock culture is used for mass culture, providing sufficient light and aeration. In normal conditions, the algae will bloom and the culture may have 1-2 million cells/ml. Leaving 1/10th of the same culture, fresh enriched medium can be added for further growth in the same container.

HARVEST AND PRESERVATION OF THE CULTURE

Harvest should be done during the exponential phase of growth of the micro-algae after determining the cell concentration. If the culture has entered the declining or stationary phase, the metabolites will be more and the cells may not be in healthy condition. The rearing larval organisms may not show the expected growth if fed with this algae.

The maintenance of the culture and constant supply of the same whenever required is a problem in the hatchery, especially during adverse weather conditions. Preservation of the algal either by freezing or by drying could be done so that during adverse condition, the hatchery operations may be successfully conducted. For freezing or drying, the culture has to be flocculated either by adding lime or adjusting the pH using sodium hydroxide. After knowing the quantity of the culture to be flocculated, measure the volume of sodium hydroxide solution needed to flocculate to get one degree rise in pH. Suppose the pH of the culture is 8.4, raise to 9.4 by adding sufficient quantity of sodium hydroxide solution. After vigorous stirring, leave the culture for one hour. After one hour, the algal mass deposited at the bottom of the tank has to be collected in a bucket by decanting the clear water. Then bring the pH of culture to the original level by adding sufficient quantity of dilute hydrochloric acid. Now the algae are ready for freezing or sundrying. Drying of the algae can be done by pouring the mass in white enamel trays and keeping the same in bright sun-light. If the algae have dried up thoroughly, scrap the powder from the tray and keep it in glass bottles. Before freezing the algal mass in polythene bags few drops of preservatives like glycerol or dimethyl sulphoxide are to be added. The frozen algae may not have the same protein content as in the live condition. Whenever adverse conditions arise, the frozen algae can be used for rearing the larval organisms.

ECONOMICS

For the production of a small scale unit of micro algae culture, the initial investment and the operational cost per month are given below:

A. Initial investment

		Rs.
1.	A.C. Room (8' x 12' size) for stock culture maintenance	75,000.00
2.	Transparent fibre-glass cum asbestos roofed room (18' x 12' size) for mass culture	85,000.00
3.	Office-cum-lab room (12' x 8' size)	50,000.00
4.	Perspex tanks (100l - 4 nos)	6,000.00
5.	FRP tanks - 250 l (4 Nos) and 1 ton tanks (4 Nos)	30,000.00
6.	Chemicals, glasswares etc.	5,000.00
7.	Lighting arrangements & electrical fittings	5,000.00
8.	Wooden racks (4 Nos)	6,000.00
9.	Air compressor	15,000.00
10.	Pump and motor 1 HP (2 Nos)	12,000.00
11.	Microscope - 1 No.	6,000.00
12.	Miscellaneous items	5,000.00
	Total	**300,000.00**

B. Operational cost per month

1.	Wages for 2 labourers	3,000.00
2.	Monthly salary for 2 Technicians	4,000.00
3.	Expenditure for electricity	2,000.00
4.	Cost of chemicals for the culture medium	3,000.00
5.	Other expenses	1,000.00
6.	Interest on the investment (15%)	4,500.00
	Total	**17,500.00**

As seen from above the operational cost per month for producing 250 litres per day of the pure culture of micro algae (about 1 million cells / ml) in the laboratory conditions and 1 tonne of the mixed culture (about 0.8 million cells / ml - dominated by the diatoms) developed in outdoor conditions per day will cost about Rs.17,500/-. The initial investment for the microalgae culture unit is about Rs. 3 lakhs.