ISOLATION AND CULTURE OF MICROALGAE

Micro algae form the first link in the food chain and are used either directly as food for farmed animals (molluscs and larval stages of crustaceans) or fed to animals which themselves form prey for formed fish and prawns.

Surface water is most commonly used for the preparation of media in mass cultivation of Microalgae. Surface water must be enriched with essential growth nutrients to achieve high cell density.

Important media used for algal culture:

Walne's medium

Solution A

Constituents	Quantity
Ferric chloride ($FeCl_3$)	0.8g
Manganous chloride ($MnCl_2$. $4H_2O$)	0.4g
Boric acid H_3BO_3	33.6g
EDTA, Disodium salt	45.0g
NaH_2PO_4 $2H_2O$	20.0g
$NaNO_3$	100.0g
Make upto 1L with Freshwater	

Solution B

Constituents	Quantity
$ZnCl_2$. 6 H_2O	2.1g
Cobaltouschloride ($CoCl_2 6H_2O$)	2.0g
Ammonium molybdate $(NH_4)M)_7$ O_{24} $4H_2O$	0.9g
$CuSO_4$ $5H_2O$	2.0g
Conc. HCl	10.0ml
Make upto 100ml in Freshwater	

Solution C

Vitamin C - 0.2g (make upto 200ml with freshwater)

For culture of diatoms – add 2 ml of solution A and C and 1ml of solution B per litre of filtered water.

For culture of other algae – add 1ml of solution A, B and C per litre of filtered water.

Working stock – add 5ml of primary stock to 1 L distilled water

Isolation of pure algal strains by agar plating

1. Prepare 0.9% of agar in 1L of sea water
2. Boil the solution under the heat on a Bunsen flame
3. Add nutrients before autoclaving and cover with aluminium foil
4. Autoclave at 121^0C for 30 mins at 1 atm pressure
5. Sterilize the petridishes by autoclaving

6. Agar plates are prepared asceptically by pouring the warm autoclaved agar into the sterile petridishes near a Bunsen flame in a laminar flow chamber.
7. Streak the algal sample on to the agar surface with a sterile platinum loop.
8. Place the petridishes upside down on an illuminated glass rack
9. Cell colonies can be observed after 5-10days
10. Select the best colonies and transfer them to test tubes having culture medium and keep them on illuminated glass rack
11. A colour change is observed in the tube and then transfers the algal strains to bigger tubes.

Composition and preparation of Guillards F2 medium

Nutrients	Concentration (mg/L of seawater)
$NaNO_3$	75
NaH_2PO_4. H_2O	5
Na_2 SiO_3. $9H_2O$	30
Na_2 C_{10} H_{14} O_8 N_2 H_2O (Na_2 EDTA)	4.36
$COCl_2$ $6H_2O$	0.01
$CuSO_4$ $5H_2O$	3.15
$MnCl_2$ $4H_2O$	0.18
$FeCl_3$ $6H_2O$	3.15
Na_2M O_4 $2H_2O$	0.006
$ZnSO_4$ $7H_2O$	0.022
Thiamine HCl	0.1
Biotin	0.0005
B_{12}	0.0005

Mass culture of algae

The test tube containing stock culture is transferred to 1 L conical flask. Keep the conical flask under light of 1000 lux with uniform temperature conditions (25-26^{0}C). About 100ml of the culture grown after 8-10days in 1L conical flask is transferred to 10L capacity polythene bags. After about 10-15days the culture from the 10L polythene bags is inoculated to 20L glass carbouys. Later, once the culture reach lag phase, is transferred to 100L Perspex tank for the indoor culture and 250 L or 1000L fibreglass tanks for the outdoor culture. For the indoor culture, the container is kept in wooden racks with light and aeration facilities. Fully grown culture from the container is harvested and used as live feed in the hatcheries. For out door culture, the tanks are kept under sunlight. These containers will have the maximum concentration or the cells in the growing phase on 5th – 6th day and can be harvested.