

MICROALGAE CULTURE



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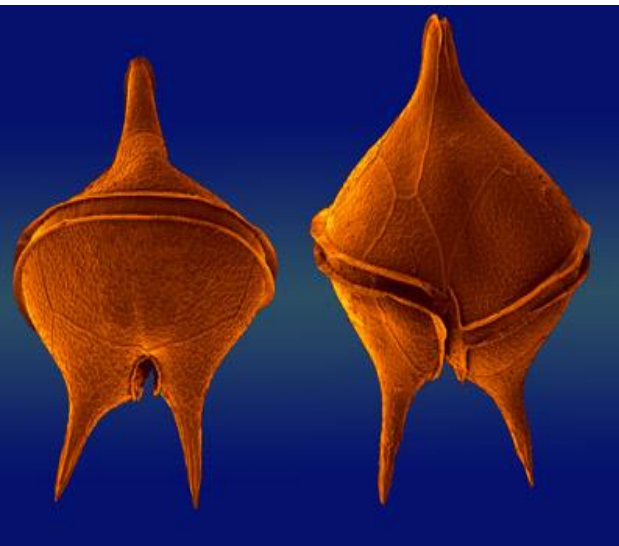
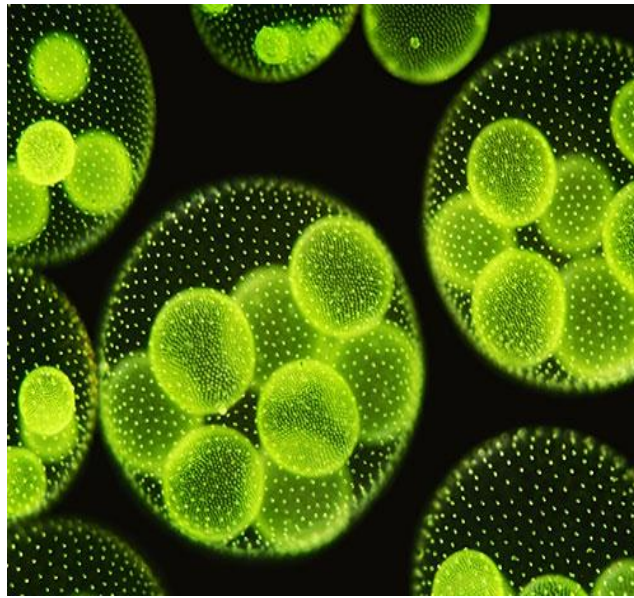
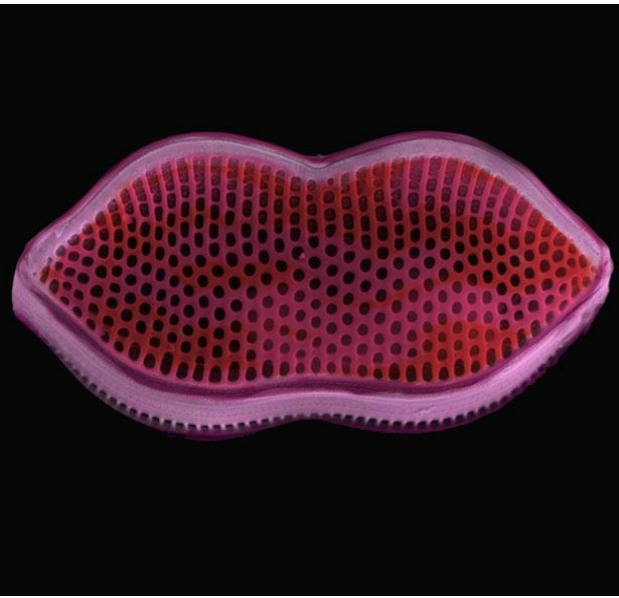
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MICROALGAE COLLECTION

- **Horizontal hauling**
- **Plankton mesh: 48 μm .**
- **Collection time: Early morning**
- **Transported to Laboratory for identification followed by isolation**



MICROALGAE IDENTIFICATION



MICROALGAE IDENTIFICATION

Kingdom:	Plantae
Phylum:	Chlorophyta
Class:	Trebouxiophyceae
Order:	Chlorellales
Family:	Chlorellaceae
Genus:	<i>Chlorella</i>
Species:	<i>C. marina</i>



Unicellular, ellipsoidal to spherical in shape

MICROALGAE IDENTIFICATION

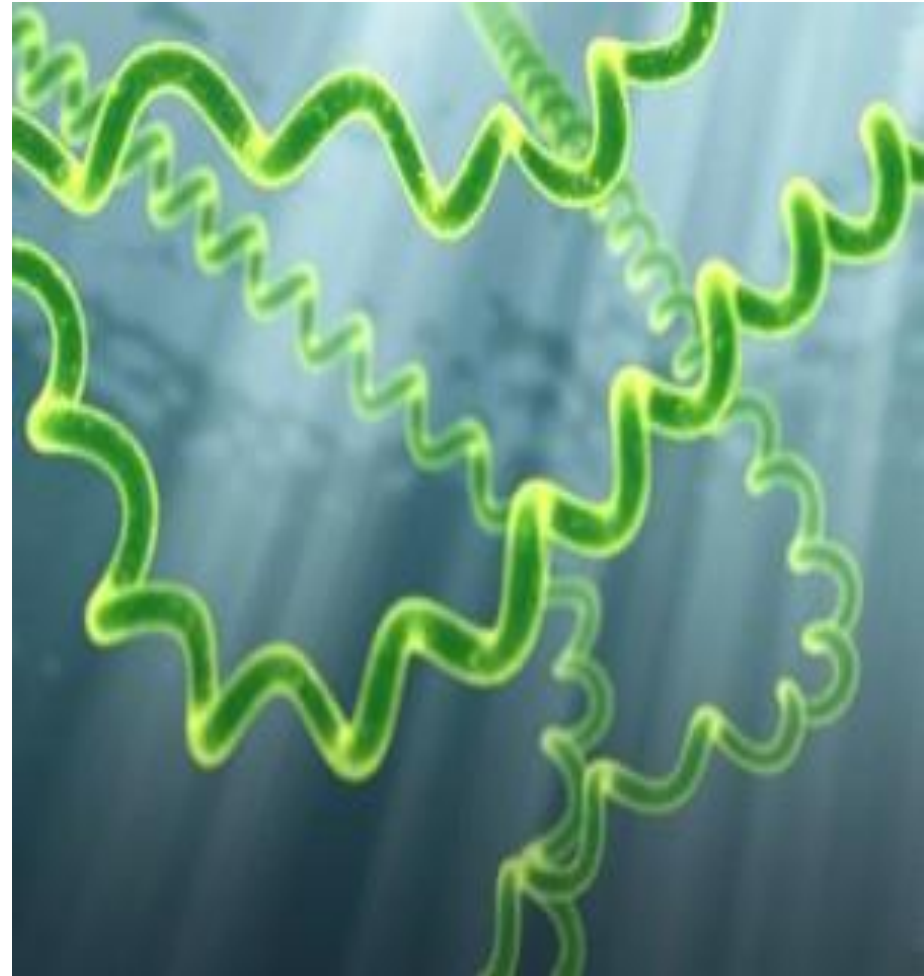
Kingdom:	Chromista
Phylum:	Bacillariophyta
Class:	Mediophyceae
Order:	Thalassiosirales
Family:	Skeletonemaceae
Genus:	<i>Skeletonema</i>
Species:	<i>S. costatum</i>



Cells are cylindrical with rounded ends. Cells form long straight chains with help of spines

MICROALGAE IDENTIFICATION

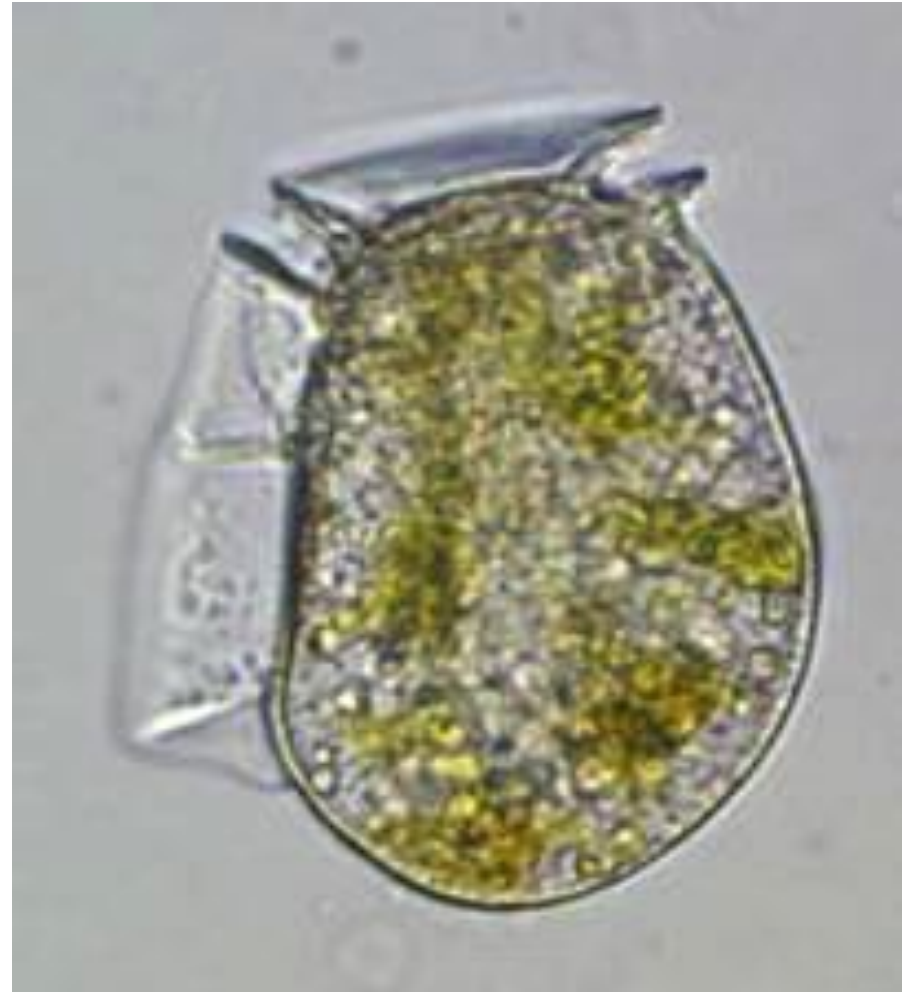
Kingdom:	Eubacteria
Phylum:	Cyanobacteria
Class:	Cyanophyceae
Order:	Spirulinales
Family:	Spirulinaceae
Genus:	<i>Spirulina</i>
Species:	<i>S. platensis</i>



Unicellular, tiny green filaments coiled in spirals of varying tightness and number

MICROALGAE IDENTIFICATION

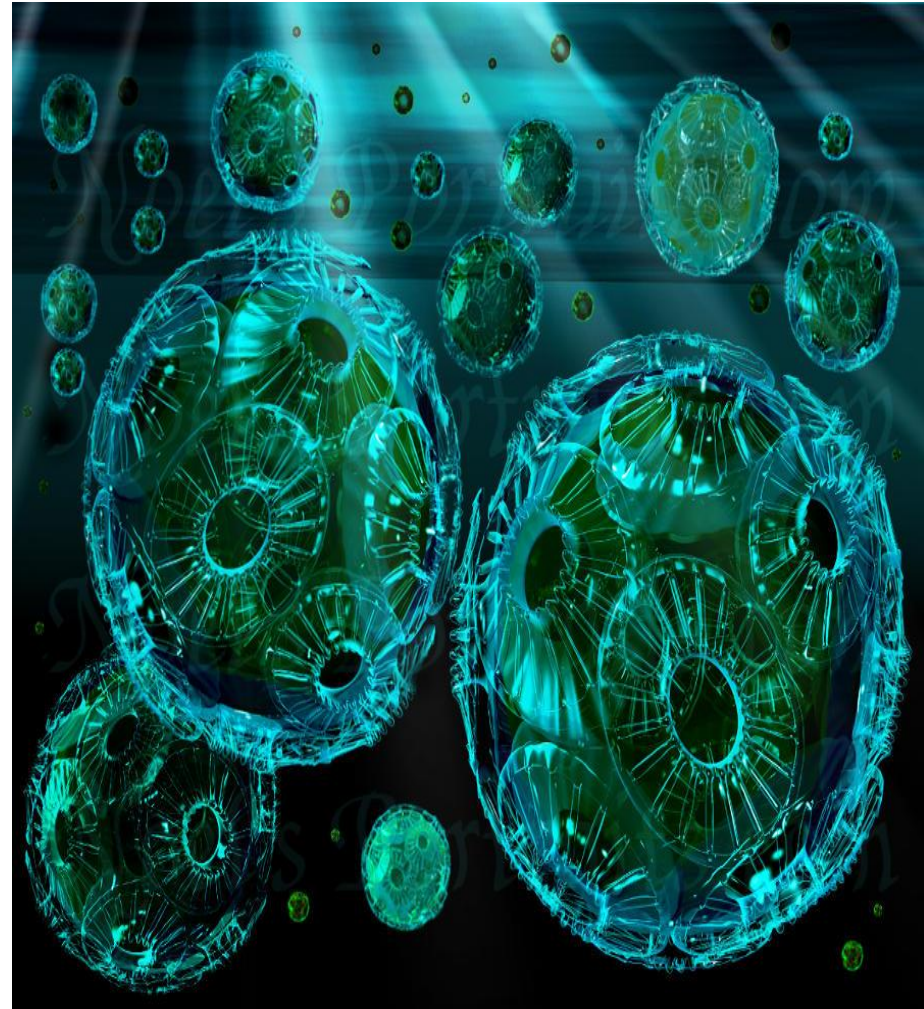
Kingdom:	Chromista
Phylum:	Miozoa
Class:	Dinophyceae
Order:	Dinophysiales
Family:	Dinophysaceae
Genus:	<i>Dinophysis</i>
Species:	<i>D. acuminata</i>



Cells are oval or elliptical in shape. Armoured planktonic forms

MICROALGAE IDENTIFICATION

Kingdom:	Chromista
Phylum:	Haptophyta
Class:	Coccolithophyceae
Order:	Isochrysidales
Family:	Noelaerhabdaceae
Genus:	<i>Emiliana</i>
Species:	<i>E. huxleyi</i>



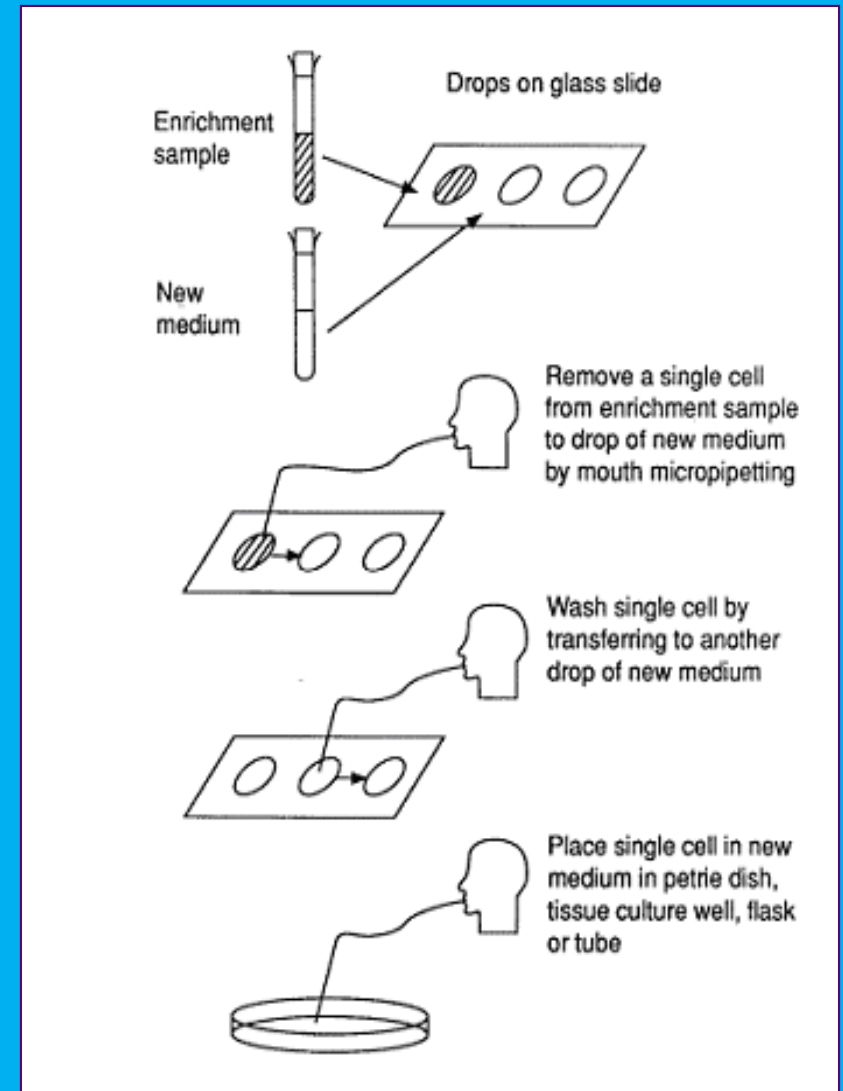
Peculiar armoured, appearance, possess calcium carbonate platelets

ISOLATION OF MICROALGAE

PIPETTE METHOD

Large microalgae will be pipetted out using a micro-pipette under microscope

Isolated algae will be transferred to culture tubes having suitable culture media



AGAR PLATING METHOD

1.5 gm of agar will be added to 1 litre of suitable culture medium



Agar solution will be sterilized in an autoclave for 15 min. under 60 kg pressure & 100°C temp.



Medium will be poured in sterilized 15 cm dia Petri-dishes & kept for 24 hours



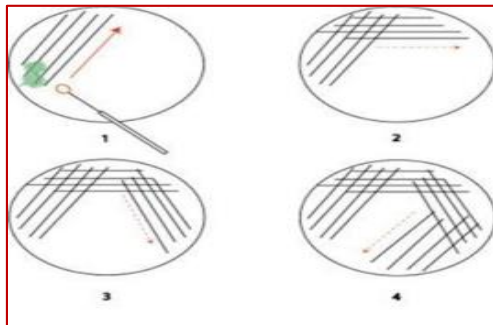
If required species grown into a colony will be removed by platinum loop under microscope & transferred to culture tubes



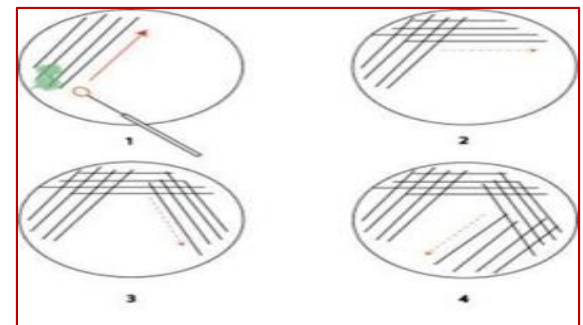
Petri-dishes will be placed in an incubator for 7-8 days providing 1000 lux light & constant temperature of 25°C



Required species will be picked up by platinum needle under microscope and streaked on the surface of agar plate



Further, microalgae will be sub-cultured in to small conical flasks and larger flasks and finally grown on a mass scale



PHOTOTACTIC MOVEMENTS

Dark chamber with a small hole on one side will be made



Sample in a beaker will be kept nearer to the hole



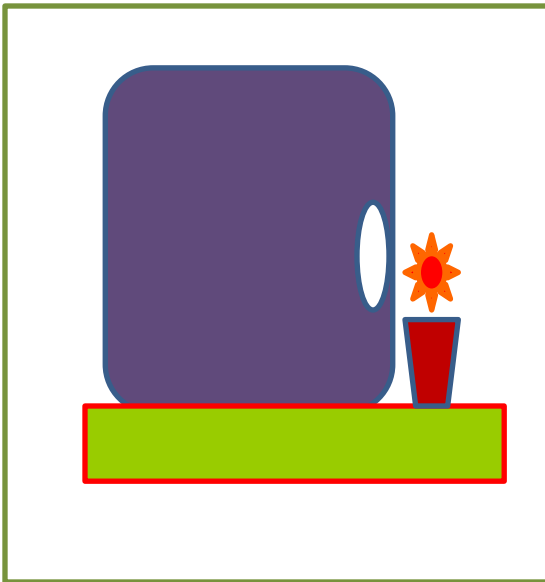
A light will be provided near to the hole outside



Since the flagellates have a tendency to move towards the light, it will crowded near to the candle light



Dinoflagellates will be separated using pipette and sub-cultured using tube as a pure culture



SERIAL DILUTION TECHNIQUE

9 ml of media will be dispensed into each of ten test tubes with sterile 10 ml pipettes in aseptic condition

Tubes will be labeled as 10^{-1} to 10^{-10} indicating dilution factor

1 ml of enrichment sample will be added to the first tube (10^{-1}) and mixed gently

Test-tubes will be incubated under controlled temperature and light conditions

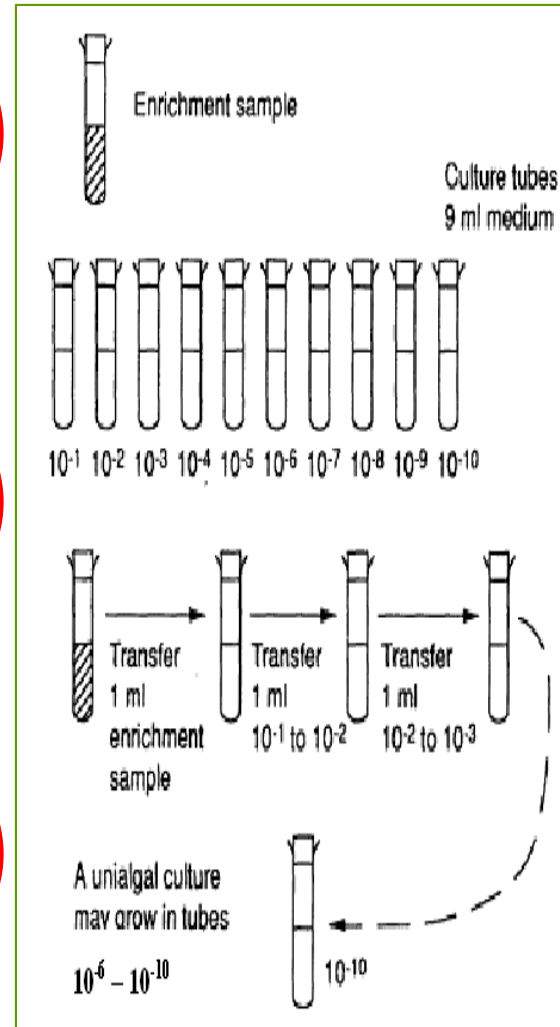
Repeat this procedure for the remaining tubes (10^{-3} to 10^{-10})

From 1st test tube 1 ml of medium will be added to the next tube (10^{-2}) & mix gently

Cultures examined microscopically after 2-4 weeks by withdrawing a small sample aseptically from each dilution tube

A unialgal culture will grow in one of the higher dilution tubes (10^{-6} to 10^{-10})

If tubes contain two or three different species then micromanipulation will be used to obtain unialgal cultures



AFTER ISOLATION

After isolation of the required species, it will be sub-cultured in 50 ml test tubes.



The test tube culture will be transferred to small culture flasks and to bigger flasks by adding 3-5 ml of the stock culture



Every two weeks a new set of 10 test tube for each species will be inoculated from the previous set



The filtration of water and medium enrichment should be done not earlier than 3 days, prior to inoculation



INDOOR ALGAL CULTURE

Indoor algal stock culture will be maintained in special air conditioning room



Stock cultures will be kept in 1 and 2 litres culture flasks, 5 and 15 litres plastic containers



Seawater filtered using 1 μ filter bag and sterilized by using autoclave. After cooling water will be transferred to the culture flask plugged with cotton or aluminum foil



INDOOR ALGAL CULTURE

All vessels used for algal culture will be sterilized properly and dried in an oven before use

About 10 ml of the inoculum in the growing phase will be transferred to the culture flasks and the culture will be placed in front of 2 tube lights (5000 lux)

The culture will be provided with 12:12 hrs. light and dark cycle



INDOOR ALGAL CULTURE

After 8-10 days, the maximum exponential phase will be obtained

The temperature and salinity will be maintained between 23 and 25°C & 28 and 30 ppt. respectively

The continuous aeration will be provided for culture

After 8-10 days, when the maximum exponential phase has reached, light will be reduced to 1 tube light for further growth



ALGAL CULTURE MEDIUMS

- **Erd-Schreiber's & Miquel's medium.**
- **Conway's or Walne's medium.**
- **TMRL. Medium (Tung Kang Marine Res. Lab).**
- **Mixed culture medium.**
- **Guillard's Medium or F2 medium**

ALGAL CULTURE MEDIUMS



COMPOSITION OF MEDIUM

Solution-A

Solution-B

Solution-C

SOLUTION-A

- Potassium nitrate - 100gm
- Sodium orthophosphate - 20gm
- EDTA(Na) - 45gm
- Boric acid - 33.4gm
- Ferric chloride - 1.3gm
- Manganese chloride - 0.36gm
- Distilled water - 1 litre

SOLUTION-B

- Zinc chloride - 4.2gm
- Cobalt chloride - 4.0gm
- Copper sulphate - 4.0gm
- Ammonium molybdate - 1.8gm
- Distilled water - 1 litre

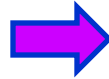
SOLUTION-C

- Vitamin B1 (Thiamin) - 20mg
- Vitamin B12 (Cyanocobalamine) - 10mg
- Distilled water - 100ml

Solution A, B and C (each) in different reagent bottles are prepared. 1ml of A, 0.5 ml of B and 0.1ml of C to 1 litre of filtered and sterilized seawater will be added

OUTDOOR MASS CULTURE

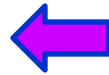
Large scale production of microalgae will be done in 100/1000 liters FRP tanks



Mass culture will be done using ammonium sulphate, super phosphate & urea in the ratio of 10:1:1



Continuous and vigorous aeration will be provided to the culture



For 100 litres of seawater 2 litres of inoculum will be added to the culture tank

