MARINE MICROALGAE CULTURE TECHNIQUES FOR FINFISH AND SHELLFISH LARVAL REARING

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Aquaculture system is based on microalgae and their animal consumers. The uptake of microalgae biomass by filter-feeders is very promising from the energetic standpoint. Microalgae are the biological starting point for energy flow through most aquatic ecosystems, and are the basis of the food chain in all most all aquaculture operations. Microalgae are used for rearing larvae and juveniles of many species of commercially important molluscs, crustaceans and fish (marine and freshwater) either directly as a source of feed or indirectly through zooplankton (rotifers, copepod or *Artemia*). In addition, the microalgae are directly introduced in the larval tanks (green water techniques) during marine finfish larval rearing, where they are believed to play a role in stabilizing the water quality, provide nutrition to the larvae and enable microbial control. Thus the management of microalgae population and their culture is considered to be an integral part of hatchery operations.

Microalgal Species selection for culture

All microalgal species are not equally suitable for culture in laboratory or hatchery. A suitable species or strain of microalgae should be selected depending upon the following criteria:

i. Species of finfish or shellfish which will feed on the microalgae

ii. Mass culture potential

iii. Cell size

iv. Digestibility

v. Overall nutritional value

Common Micro algae and applications in Aquaculture

Nannochloropsis oculata

It is a small (2-4 µm) green algae belong to the family Eustigmatophyceae, rich in EPA. It is used for rotifer culture and as water conditioner in finfish hatcheries. It is also used in reef tanks for feeding corals and other filter feeders.

Isochrysis galbana

These are small (3-5µm) golden brown flagellates belonging to the family Isochrysidaceae. They are excellent feed for the production of copepods, brine shrimp, oysters, clams, mussels and scallops. Because of its richness in DHA, *Isochrysis* is used for the enrichment of zooplankton (*Artemia*) which has been raised on *Nannochloropsis* and yeast, for improving the DHA/EPA ratio in the live feed

Chaetoceros calcitrans

It is a marine planktonic diatom belonging to the family Chaetocerotaceae. The cells (*diameter* 2 - 85 mm, *length* 2 - 45 mm) are elliptical in valve view. It is the widely used species in aquaculture, as it is composed of nutritional value suitable for marine filter feeders especially molluscs. It is used in shrimp hatcheries to increase the vitamin levels.

Chlorella salina

It is a single celled green algae, spherical in shape, 2-10 mm in diameter. Used for feeding rotifers in finfish larval rearing.

Pavlova lutheri

It is a small (3-10 µm) golden brown flagellate, belong to the family Haptophyceae. It is a widely cultured species and used in the cultures of oysters, clams, mussels and scallops to increase the DHA/EPA levels in their broodstock. It is a temperate species, used for rotifer enrichment, which is mainly used in cold water fish hatcheries (cod).

Tetraselmis suecica

It is a large green (4-5µm) flagellate belonging to the family Chlorodendraceae. This microalgae forms the standard feed for oysters, clams, mussels and scallops. This particular algae is rich source of natural amino acids that stimulate feeding in marine animals and also forms excellent feed for shrimp larvae. It is used in combination with *Nannochloropsis* sp. for rotifer production and also for feeding brine shrimp.

Thalassiosira weissflogii

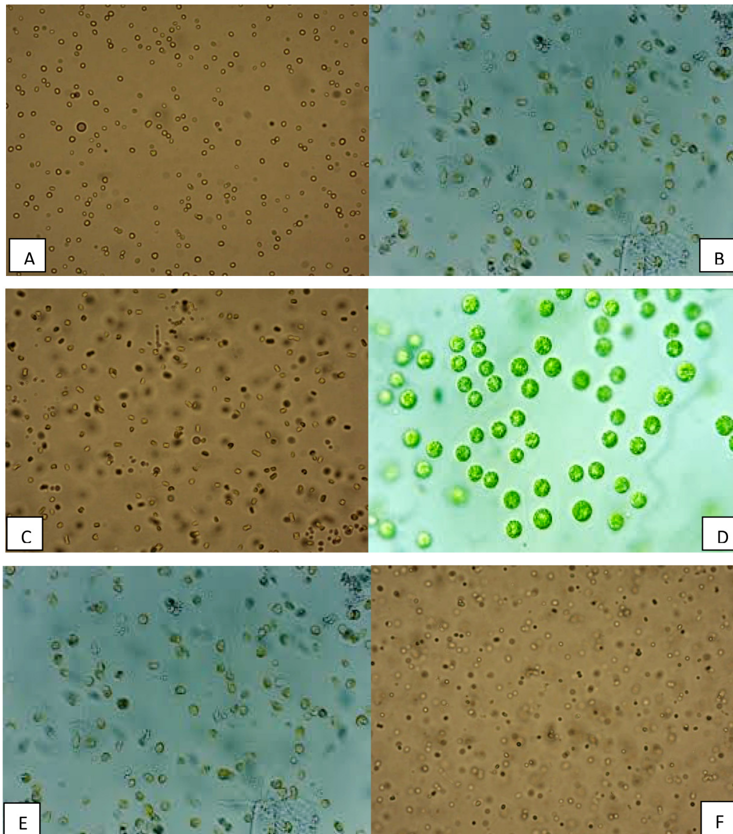
It is a large diatom (4-32 µm) cylindrical in shape belonging to family Thalassiosiraceae. It is the major species used in shrimp and shellfish larvi-culture. It is the single best algae for shrimp larvae and also good feed for copepods, brine shrimp, and broodstock conditioning of oysters (post set), clams and mussels.

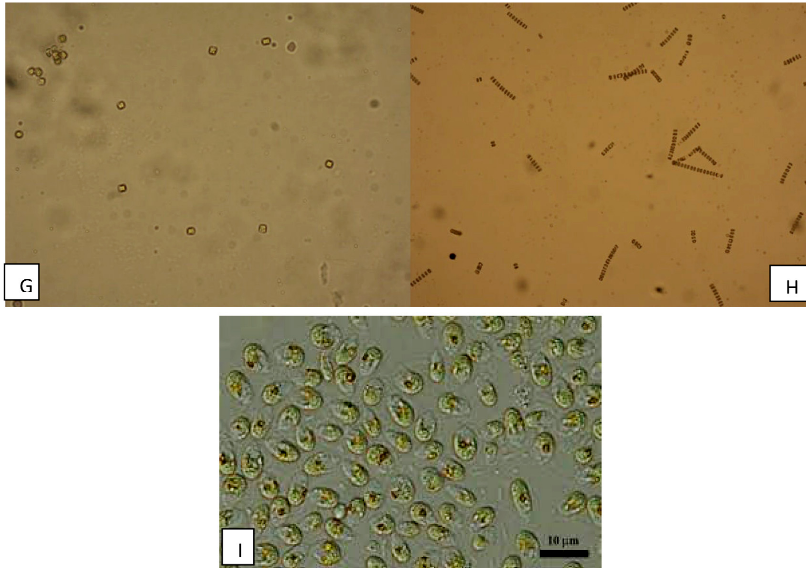
Skeletonema costatum

These are diatoms belongs to the family Skeletonemaceae. Cells are short (2-21 µm) and cylindrical appearing as long straight chains. It is widely used for extensive and intensive shrimp hatchery systems.

Dunaliella salina

It is a small green algae (9-11 µm); rod to ovoid shaped, belonging to the family Dunaliellaceae. It is mostly used in shrimp hatcheries to increase vitamin levels and also for colouration.





A. *Nannochloropsis oculata;* **B**. *Isochrysis galbana*; **C**. *Chaetoceros calcitrans*; **D**. *Chlorella salina* (source: google); **E**. *Pavlova lutheri* (source: google); **F**. *Tetraselmis suecica*; **G**. *Thalassiosera weissflogii*; **H**. *Skeletonema costatum*; **I**. *Dunaliella salina* (source: google)

Growth dynamics of microalgae

The knowledge of microalgae growth dynamics is important for aquaculturist to know when to harvest the microalgae, to estimate growth rate and population doubling time. Generally, the microalgal culture follows a characteristic pattern of growth and follows five reasonably well defined phase of algal growth in batch culture.

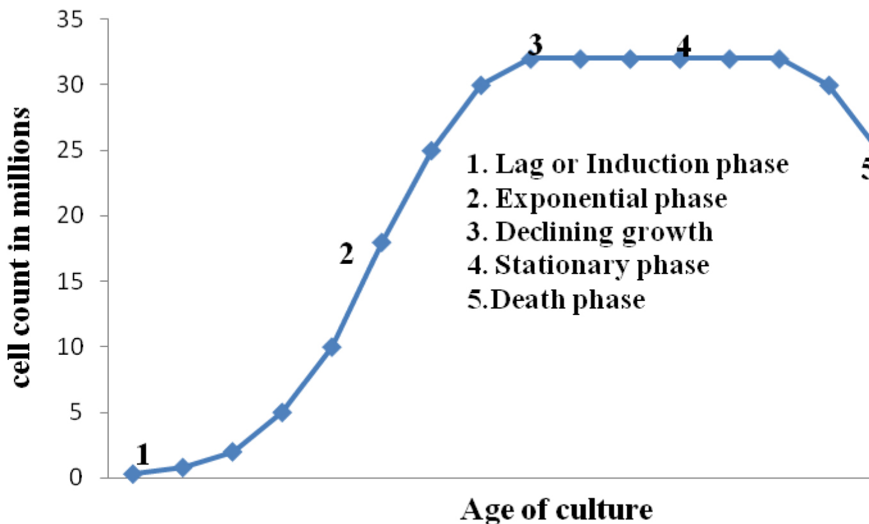
i. Lag or Induction phase

ii. Exponential phase

iii. Declining phase

iv. Stationary Phase

v. Death phase



Growth curve of micro algae (*Nannochloropsis* sp.)

i. Lag or induction phase

The Inoculation of culture into a new medium have to acclimatize with the surroundings or to the new physico- chemical conditions, so there will be no cell division for slight time thus the stage is known as lag or induction phase. The condition of the inoculum has a strong bearing on the duration of the lag phase. An inoculum taken from a healthy exponentially growing culture is unlikely to have any lag phase when transferred to fresh medium under similar growth physico-chemical conditions. In general the length of the lag phase will be proportional to the length of time the inoculum has been in phases 3 to 5. A lag phase may also occur if the inoculum is transferred from one set of growth conditions to another.

ii. Exponential phase

Once the inoculum is acclimatized to the new physico-chemical conditions, it starts multiplication and grows rapidly, thus this phase is known as exponential phase. The duration of exponential phase in cultures depends upon the volume of the inoculum, the growth rate and the capacity of the medium and culturing conditions to support algal growth. The growth rate of a microalgal population is to measure the increase in biomass over time and it is calculated from the exponential phase. Growth rate is one important way of expressing the relative ecological success of a species or strain in adapting to its natural. Cell count and

dry weight are common units of biomass determination. Once the growth phase has been plotted (time on x-axis and biomass on logarthmic y-axis) careful determination of the exponential (straight line) phase of growth is needed. Two points, N_1 and N_2, at the extremes of this linear phase are taken and substituted into the equation (Levasseur *et al* 1993).

Specific growth rate (μe)

The specific growth rate of microalgae was calculated using the equation:

$\mu = \ln (N2 / N1) / (t2-t1)$

where μ= specific growth rate (d^{-1}), N1 and N2 are the number of cells. mL^{-1} at the time t1 and t2, respectively.

Duplication time

The time for the number of cells (cells. mL^{-1}) of microalgae to double in number was calculated

using the equation

$t_g = \ln 2/ \mu$

where tg (days) is the time for cell duplication and μe is the specific growth rate, and ln 2 is the natural logarithm of 2 (approx. 0.693).

Division rate, $k = \mu / \ln 2$

The volumetric productivity, Px (cell $L^{"1}$ $h^{"1}$) $= C_x \mu$

where Cx (cell $mL^{"1}$) refers to maximum cell density achieved from the batch cultivation.

iii. Declining phase

When the death of the cells will exceed the multiplication of the cells in a culture, then the phase is known as declining phase. This phase generally occurs in culture when either a specific requirement for cell division is limiting or something else is inhibiting reproduction. The biomass is often very high and exhaustion of a nutrient salt, limiting carbon dioxide or light limitation becomes the primary cause of declining growth. At low cell densities too much CO_2 may lower the pH and depress growth. CO_2 limitation at high cell densities control any further biomass increase to be linear rather than exponential (with respect to time) and proportional to the input of

CO_2. Light limitation at high biomass occurs when the cells absorb most of the incoming irradiation and individual cells shade each other. This is known as self-shading. Microalgae are generally well adapted to surviving conditions of low incident light and may survive for extended periods under these conditions; however the growth will be linear rather than exponential.

iv. Stationary phase

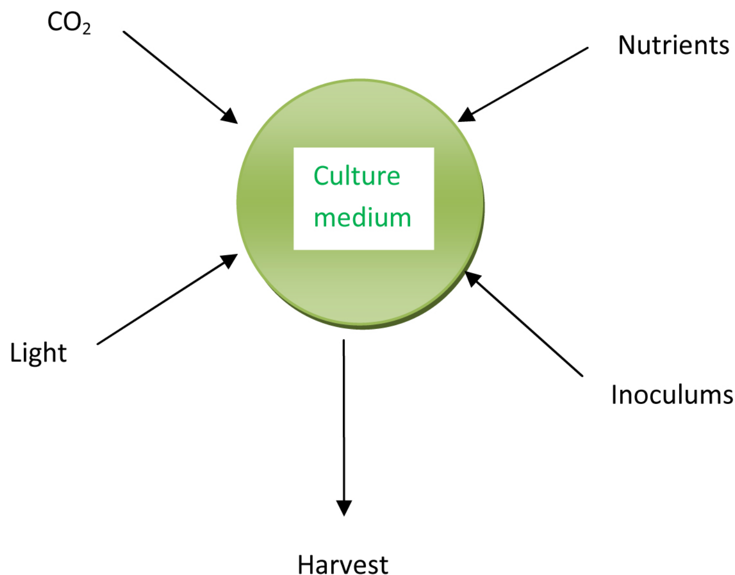
After the arrested growth, the culture will be stationary without any further cell division. The net growth of the culture becomes zero during this phase and within an hour's culture cells may undergo dramatic biochemical changes. The nature of the changes depends upon the growth limiting factor. Nitrogen limitation may result in the reduction in protein content and relative or absolute changes in lipid and carbohydrate content. Light limitation might affect the pigment content of most species and changes in fatty acid composition. Light intensities that were optimal for growth in the first 3 phases can now become stressful and lead to a condition known as photo inhibition. It is important to note that while the measured light intensity within the culture will decrease with increasing biomass, if the incident illumination is maintained relatively high then a large proportion of cells may become stressed, leading to photo inhibition and the culture can be pushed into the death phase. This is especially more if the culture is also nutrient stressed. It is preferable for many species to further reduce the incident light intensity when cultures enter stationary phase to avoid photo inhibition. The lowering of temperature combined with lower irradiance can reduce stress in many species. The longer the cells are held in this condition the longer the lag phase will be when cells are returned to good growth conditions for sub culture.

v. Death phase

This is last phase of growth dynamics, when the cells may lose its viability and start to die. The death phase of culture is generally very rapid, thus the term 'culture crash" is often used for this phase. The cultures of some species will loose their pigmentation and appear washed out or cloudy, whereas cells of other species may lyse (no recognizable cells) but the culture colour will be maintained. The latter is an important consideration and one reason why colour should not be relied upon to gauge culture health. Bacteria which may have been kept in check during exponential and early stationary phase may "explode" as cell membrane

integrity becomes progressively compromised or leaky and a rich carbon source for bacterial growth is released. Free pigment and bacterial growth are further reasons why measures of turbidity or fluorescence should not be used beyond early stationary phase as surrogate biomass indicators, or especially as indicators of culture health. Occassionally cell growth of some species can reoccur after a culture has apparently died. In this instance most vegetative cells will have died, and possibly most of the bacteria, releasing nutrients back into the media. Then either the very few remaining vegetative cells or more likely germination of cysts or temporary cysts will be able to fund this secondary growth.

Microalgae culture



Process of micro algal culture with various inputs

Microalgae culture is a form of aquaculture involving farming of different species of microalgae in a confined environment. The culture has three components namely, culture medium contained in a suitable vessel; the algal cells growing in the medium and air, to allow exchange of carbon dioxide between medium and atmosphere. An autotrophic alga need light, CO_2, water, nutrients and trace elements for their growth. Some auxotrophic algae, which cannot prepare biochemical compounds like vitamins through photosynthesis, need additional biochemical compounds to be added in the culture medium.

Algal culture techniques

The following are the different algal culture techniques used for microalgae culture.

1) **Batch culture:** In this culture the resources present in the culture medium are abundant; the micro algae grow according to sigmoid curve. Once the resources are utilized by the cells, sub culturing is followed by transferring a small volume of the existing culture to the large volume of fresh culturing medium.

2) **Continuous culture:** In this culture, the resources are potentially infinite. Culture is maintained on chosen point on the growth curve by the regulated addition of fresh medium.

3) **Indoor/outdoor:** Indoor culture allows the control over illumination, temperature, nutrient level, contamination with predator and other competing algae; however, outdoor culture will not have any control on the above said parameters.

4) **Open/closed:** Open cultures in uncovered tanks and ponds are more contaminated than closed cultures in test tube, conical flask etc.

5) **Axenic (sterile) / xenic:** Axenic cultures are pure cultures free from all foreign contaminants and this type of culture requires strict sterilization of glass wares, culture media and vessels to avoid contamination, where as xenic culture might be a contaminated one.

Microalgae culture media

Algal nutrient solutions or culture medium are made up of a mixture of chemical salts and water. The culture medium provides the nutrient needed for the growth of algae. These nutrients solutions are formulated specifically for its use in aquatic environments and their consistency is more precise for laboratory culture. The culture medium is constituted with the addition of macronutrients, micro nutrients and vitamins. Macro nutrients include nitrate, phosphate and silicate. Micro nutrients contain various trace metals. Vitamins like thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin (B_7) are commonly required for the growth of most of the micro algae.

i. Macronutrients

Nitrogen and phosphorus are the important macronutrients, for the growth and metabolism of algal cells, which are added to the culture medium as Nitrate ($NaNO_3$ /KNO_3) and phosphate (NaHPO4.H2O). Nitrogen is a key element for the formation of protein and nucleic acids accounts to 7-20% of micro algal cell dry weight. Phosphorous plays a major role in the formation of energy carrier molecule (ATP) which forms 1% dry weight of the algae. Algae require inorganic carbon source in the form of CO_2, carbonate or bicarbonate for its photosynthesis. Silicate is necessary for the cell wall development of diatoms and is added in sodium silicate form (Na_2SiO_3 $9H_2O$) to the culture medium.

ii. Micronutrients

Micro nutrients are trace metals which are present in algal cells in extremely small quantities (<4 ppm), which are essential for the physiological growth of algae. Iron (Fe,) Manganese (Mn), Cobalt (Co), Zinc (Zn), Copper (Cu) and Nickel (Ni) are the most important trace metals required by algae for the various metabolic functions. Deficiencies in trace metals may lead to slow algal growth and excess concentration may inhibit growth, impair photosynthesis and finally damage the cell membrane of the algae. Typical trace metal stock solutions may consist of chloride or sulphate salts of zinc, cobalt, manganese, selenium, and nickel, and they are kept in a solution containing the chelator EDTA. Iron is an important trace metal required for the algae for its normal growth, photosynthesis and respiration. Iron is usually kept as a separate solution, may be added as ferric chloride or ferrous sulphate. EDTA is used as chelator and is available as disodium salt ($Na_2EDTA.2H_2O$) that is readily soluble in water.

iii. Vitamins

Vitamins are organic micronutrients, which are essential for photosynthetic microalgae. Algal species require different combination of vitamins, mostly Vitamin B_{12} (Cyanocobalamin), Vitamin B_1(Thiamine) and Vitamin B_7 (Biotin). The general order of vitamin requirements for algae is vitamin B_{12} > thiamine > biotin. Vitamins are normally added aseptically (through a 0.22-mm filter) after the medium has been autoclaved.

Stock Solutions preparation

In order to simplify routine medium preparation, stock solutions are prepared with accurate weighing of the chemicals in the specific culture media, dissolved in the specific volume of distilled water. There are two terms used in stock solution preparation, working stock and primary stocks. Working stocks are the small quantity (aliquot) of solution which is directly used for the preparation of final medium so that adding a small volume of liquid stock solution is easier and quicker than weighing of individual dry chemicals. Primary stocks are formed from several single substance solutions and finally combined to form the working stock. Stock solutions made from some chemicals (EDTA) may need heat treatment to dissolve completely in the water, otherwise it may lead to unnecessary precipitation of the nutrients in the medium. But, vitamin stocks should be prepared with normal distilled water and should not be exposed to any heat treatments and are advised to be kept in dark bottles.

Culture media

The selection of culture media mainly depends on the type algae species cultured. Diatoms like *Chaetoceros, Skeletonema*, *Thalassiosera*, *Tetraselmis* etc., need silicates for the formation of silicious cell wall in addition to nitrate, phosphate, trace metals and vitamins. Most of the culture media for micro algal culture are composed of chemicals, trace metals and vitamins. Most commonly used culture media used for stock culture and mass culture of micro algae in the laboratory is 'Conway' or 'Walne's medium (Walne,1974) and Guillard's F/2 media. This media is mainly used for indoor culture of *Nannochloro*psis, *Chlorella,* and diatoms like *Chaetoceros, Skeletonema, Thalassiosera* and *Tetraselmis.*

Composition of Conway / Walne's medium used for micro algal culture

Solution (A)

1.	Potassium Nitrate (KNO_3)	100gm
2.	Sodium di-hydrogen orthophosphate ($NaH_2PO_4.2H_2O$)	20gm
3.	EDTA di-sodium salt (Na_2EDTA)	45gm
4.	Boric Acid (H_3BO_3)	33.4gm
5.	Ferric Chloride ($FeCl_3$)	1.3gm
6.	Manganous Chloride ($MnCl_2.2H_2O$)	0.36gm
7.	Distilled Water	1lit

Solution (B)

1.	Zinc Chloride ($ZnCl_2$)	4.2gm
2.	Cobalt Chloride ($CoCl_2.6H_2O$)	4.0gm
3.	Copper Sulphate ($CuSO_4.5H_2O$)	4.0gm
4.	Ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$)	1.8gm
5.	Distilled Water	1lit
6.	Concentrated HCl	

Solution (C)

1.	Vitamin B_1 (Thiamine)	2gm
2.	Vitamin B_{12} (Cyanocobalamin)	100mg
3.	Distilled Water	1lit

Solution (D)

1.	Sodium silicate ($Na_2SiO_3.5H_2O$)	40ml
2.	Distilled water	1lit

Stock culture: Only autoclaved seawater should be used.

Working Solution for mass culture: Add 1ml of Solution A, 0.5ml of B, 0.1 ml of C and 1ml of D into 1lit sea water. D: Only for Diatoms (*Chaetoceros* sp., *Skeletonema* sp., *Thalassiosera* sp.)

Composition of Guillard's F/2 media used for micro algal culture

Solution (A)

1.	Sodium nitrate ($NaNO_3$)	75g	1 L distilled water
2.	Sodium phosphate ($NaH_2PO_4.H_2O$)	5g	

P.S.Solution*

1.	Copper Sulphate ($CuSO_4 .5H_2O$)	10g	1 L distilled water
2.	Zinc Sulphate ($ZnSO_4.7H_2O$)	22g	1 L distilled water
3.	Cobalt Chloride($CoCl_2.6H_2O$)	10g	1 L distilled water
4.	Manganous Chloride ($MnCl_2.4H_2O$)	180g	1 L distilled water
5.	Sodium Molybdate ($Na_2MoO_4.2H_2O$)	6g	1 L distilled water

Prepare each solution separately in 1lit bottles

Solution (B)**

1.	EDTA di-sodium salt (Na_2EDTA)	4.36g	1 L distilled water
2.	Ferric Chloride ($FeCl_3.6H_2O$)	3.15g	

Add 1ml of each solution (P.S solution1-5) each to 1 lit of EDTA & $FeCl_3$ mixed solution

Solution (C)***

1.	Thiamin HCl	20g	1 L distilled water
2.	Biotin	100mg	1 L distilled water
3.	Cyanocobalamine (B_{12})	100mg	1 L distilled water

Add 5ml of each solution into 1lit of sea water

Solution (D)****

1.	Sodium Silicate ($Na_2SiO_3.9H_2O$)	35g	1 L distilled water

Stock culture: Only autoclaved seawater should be used.

***P.S. Solution:** Each solution (1L) should be prepared separately in 5 different bottles

****Solution B:** 1L of EDTA & $FeCl_3$ mixed solution with P.S. solution (1ml of each)

***** Solution C:** 1L of sea water with Thiamin, Biotin and Cyanacobalamin solution (5ml each)

****** Solution D:** Only for Diatoms (*Chaetoceros*)

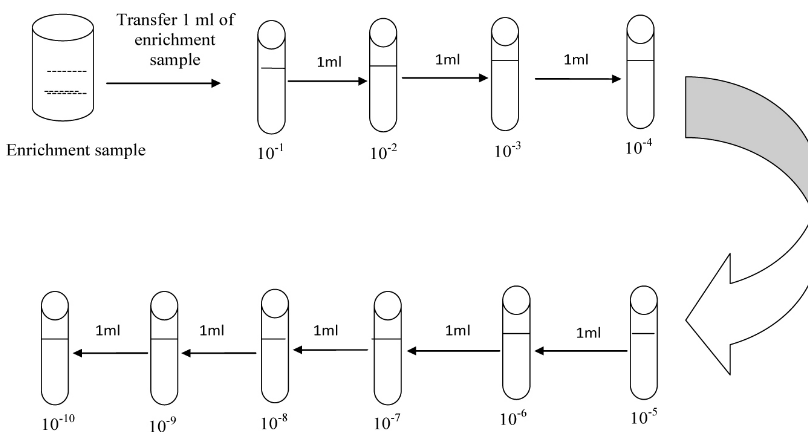
Working Solution for mass culture: Add 1ml of each Solution A, B, C and D into 1 L sea water. D: Only for Diatoms (*Chaetoceros* sp., *Skeletonema* sp., *Thalassiosera* sp.)

Isolation of microalgae

Once the decision of which microalgae needs to be cultured is taken, the first step is to isolate the pure culture. Even though the cultures are available from specialized culture collection centers, isolation of endemic strain is advisable because the strain can grow better in local environmental conditions. Collection of micro algal sample is the crucial step which can be done using plankton nets of mesh size 10, 30 and 120 µm. The collected micro algae need to be kept in sterile nutrient solution to keep them alive and allow them to grow. Some amount of the sample can be concentrated and preserved in 10% buffered formalin for the identification in the laboratory. While sample collection both biotic (light, water temperature, dissolved oxygen, dissolved carbon dioxide, nutrient concentration, pH, salinity) and abiotic factors (pathogen) at the sampling site need to be considered. Technique used for the isolation of micro algae includes technique of dilution, single cell isolation by micropipette and agar streaking.

i. Dilution technique

This isolation technique is suitable for organisms which are abundant in samples and ineffective for rare organisms. This technique enables isolation of a single cell by repeated attempts. In dilution technique, inoculates large number of test tubes with culture media (dilution ranging from 10^{-1} - 10^{-10}). After inoculating, incubate the test tubes under controlled temperature and light. Examine the culture after 2-4 weeks by taking a small sample aseptically from each dilution tube. Uni-algal culture may grow in any of the higher dilution tubes. The success of the present

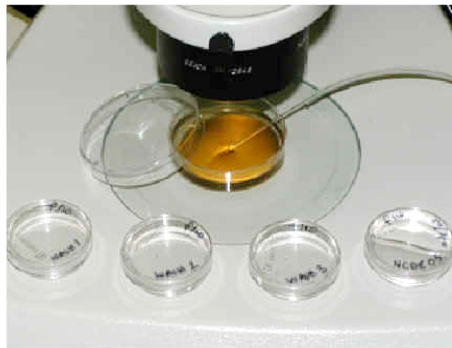


Serial dilution technique for isolation of micro algae

technique is highly dependent on the accuracy of a measured amount of cell culture during transfer from one medium to another. Axenic isolates are not possible with dilution technique because bacteria are more abundant than algae.

ii. Single cell isolation by micropipette

This is the most common isolation technique, which is performed with a pasture pipette or glass capillary under microscopic observation. These single cells are transferred to sterile droplets of water or suitable media. The technique requires expertise and precision. The cells can get damaged due to shear stress caused by micropipette or capillary tips. Caution is necessary for successful implementation of the present technique. Ultrapure droplets are required, especially for marine samples, to distinguish between microalgal cells and other particulate matters.



Isolation of single cell with the help of microscope

iii. Streaking cells along agar plates

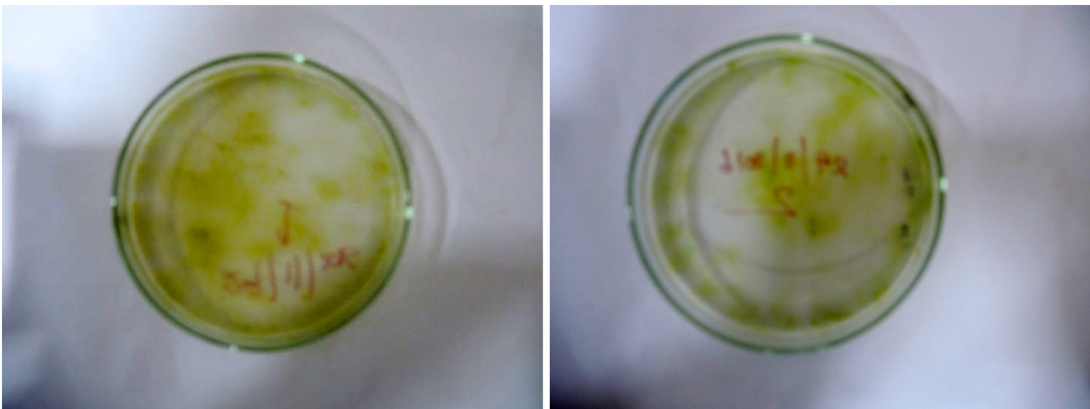
Isolation of cells on agar plate is an old and common method. For successful isolation onto agar the desired algae should be able to grow well on agar plates. For preparing the agar medium, 0.8 to 2 gm of agar is added to a litre of natural filtered seawater in a conical flask. The flask containing agar is usually heated on a flame and boiled twice till the agar is dissolved in water. The culture media 'or' nutrients (solution A and B of Conway media or F2 media) are added to the agar solution. The flask mouth is usually covered with Aluminium foil and then the flask is autoclaved for 15 minutes under 15 lbs pressure and 121°C temperature. The petri dishes are usually sterilized by keeping for 30 minutes at 150 °C in hot air oven. The solution C of culture media is added aseptically to the autoclaved agar flask once the temperature has been brought down to 40 °C. Then, agar plates are

prepared aseptically by pouring the warm autoclaved agar into the sterile petri dishes near a Bunsen flame or in a laminar flow, followed by covering up the petri dishes and leaving them to cool for about 2 h.

Agar plates for streaking of micro algal culture

Streaking of micro algal culture on Agar plates

Isolation is accomplished by streaking the sample across the agar surface (0.8-2%). The streaking can be done with the help of 'loop' or spreader. After streaking, the agar plate is incubated until colonies of the cells appear. The isolated colonies are removed from the agar plate and can be further subcultured onto other agar plates. Axenic cultures without contamination can be produced with this method.



Micro algae (*Nannochloropsis* sp.) colonies on agar plates isolated on agar plates

Maintanance of algae culture

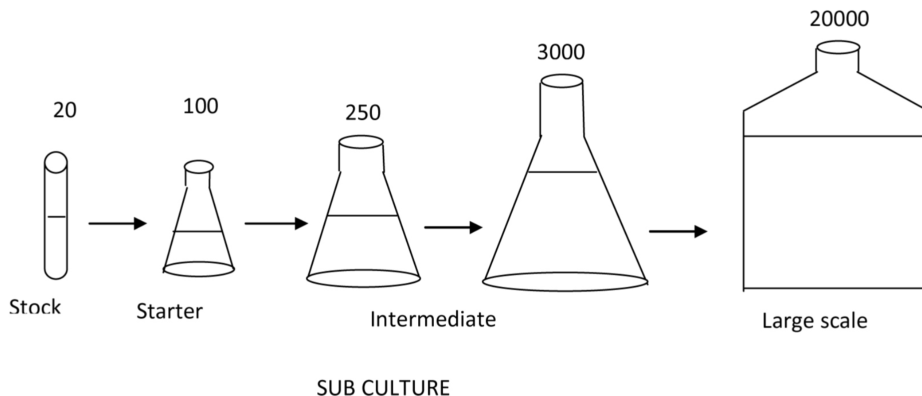
Algal multiplication is normally dependant of various steps like culture water sterilization, nutrient enrichment, inoculation of new culture with the pure algal strain, microscopic observations for growth of the algal cells and finally mass culture of algae in larger containers.

Stock culture

It provides reservoir of algae cells from which large cultures can be initiated. Stock cultures are kept in small volume containers.

Subculture

Subculture involves inoculating some cells from old stock culture to fresh culture medium, so that the cells can continue to grow, devide and healthy. If subculturing is not done, cells in the stock culture will die eventually. Precaution maust be taken while transfering the stock culture, so that contaminants from air should not enter into the stock culture.



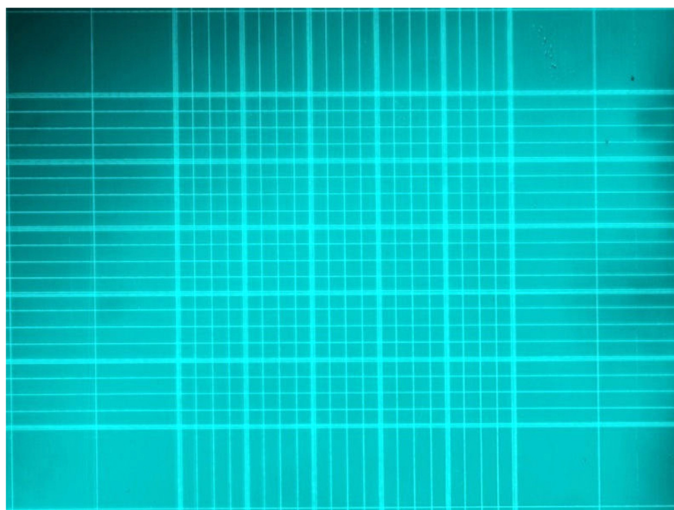
SUB CULTURE

Determination of Algal cell densities

Regular algal cell counting is essential to schedule the inoculation of mass culture from the containers, to know the growth of the algae and to determine the quantity of algae to be fed to the larval organisms. Most of the nannoplankton organisms are less than 10 µm, counting is normally done in Sedgwick Rafter Counting Chamber or Haemocytometers. Haemocytometers are thick glass slides with two chambers on the upper surface, each measuring 1.0 x 1.0 mm. A special cover slip is placed over these two chambers giving a depth of 0.1 mm making the total volume of

each chamber $0.1mm^3$. Sample is prepared and treated with eosin or formalin to kill the cells. After placing the cover slip on the haemocytometer, pipette with sample should be brought to the edge of the haemocytometer and touch it to release the sample. The sample will run inside and form a thin film of the culture in which the cells should be uniformly distributed. Haemocytometer has got 9 chambers (4 sides having 16 divisions and 5 chambers of multiple divisions). The counting of algal cells can be carried out in the middle chamber, which is consisting of 5 x 5 cells. Again the each cell has 16 divisions (4 x 4). In this division, the presence of microalgal cells are counted in 4 corner and center cells and then the following formula is applied:

Total number of cells/ ml = (Av. Number of cells from 5 square) x 25 x 10^4

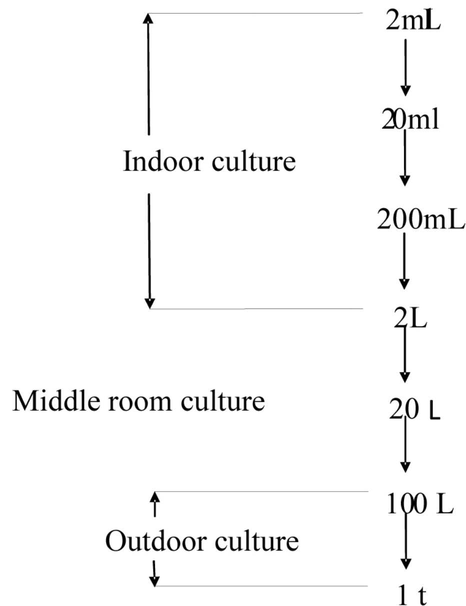


An image of Haemocytometer

Mass culture of microalgae

Since large quantity of micro algae is needed for zooplankton as well as fish larval rearing system, the cultures need to be multiplied within the limited period of time. So mass culture needs to be done with suitable culture medium to meet sufficient requirement. The mass culture can be of batch culture, semi-continuous or continuous culture. A small inoculum equal to 0.5 % of the volume of the new culture will normally generate new healthy cultures for healthy cells of a robust species. However, if the species is delicate or the culture is less healthy, then a

larger inoculum of 10% - 20% may be needed to support a new culture. (The stock cultures at VRC of CMFRI are transferred with 0.5 ml inoculum into 10 ml fresh medium representing a 5% inoculums).The general pattern for the mass production of microalgae should be followed according to the following flow chart.



Flow chart for algae culture

For the mass culture of micro algae media named TMRL and PM is reported to be effective. However, agricultural chemicals are used generally for mass culture of microalgae.

Composition of TMRL medium 100 ml (Tung Kang Marine Res. Lab)

Potassium nitrate	10gm
Sodium orthophosphate	1gm
Ferric Chloride	0.3gm
Sodium Silicate	0.1gm
Commercial fertilizer medium/1t	
Ammonium sulphate	100
Urea	10
Tripple super phosphate	10

The maximum density of algal cells in outdoor culture depends mainly on factors like atmospheric light and temperature, salinity of sea water without any contaminants suitable to the cultured species, and a good density of indoor inoculums. In outdoor culture with 100 l tanks around 8-10 million cells per ml can be achieved within 2 days of inoculation and around 2-3 million cells per ml can be achieved in 1 t outdoor tanks with suitable environmental conditions.

Outdoor mass culture of micro algae in 100 l carboys

Outdoor mass culture of micro algae in 1t FRP tanks

Harvest of the culture

The culture should be harvested during the exponential phase of the micro-algae after determining the cell concentration. If the culture has entered the declining or stationary phase, the metabolites will be very high and the cells may not be in healthy condition. Rearing larval organisms or zooplankton may not show the expected growth if fed with this feed. The trouble which comes frequently like air pressure, salinity, pH, culture contamination, nutrient media for culturing the algae needs immediate attention. Frequent observation to avoid contamination is essential as the algal feed is very much important in terms of nutrients for the other organisms like rotifers and copepods to feed on and also pure culture should not be contaminated while adopting the green water technology for the larval rearing system for marine fishes or shell fishes.

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