MICROALGAE CULTURE FOR HATCHERIES

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Introduction

Microalgae are phototrophic microorganisms, they capture solar energy and convert it into bioenergy, thereby providing certain potential and bioactive constituents. Bacteria and microalgae are vital components in aquatic ecosystems and they exhibit mutualistic relationships. Marine microalgae are varied, specialized unique, and vast groups of single-celled microorganisms that contain chlorophyll, which traps the energy from light and uses it to convert nutrients and carbon dioxide dissolved in the seawater into organic growth.. They are the primary producers and constitute almost a quarter of the world's primary productivity (Lebeau and Robert, 2003). Unicellular marine microalgae are widely used as food in the hatchery production of commercially valuable fish and shellfish for various stages. The important components of microalgae are the diatoms, dinoflagellates, silico-flagellates (phyto-flagellates), coccolithophores, blue-green algae, and the 'hidden flora' the nanoplankton. Among these, the diatoms and phyto-flagellates are significant organisms since they form the primary link in the food chain of the sea. It is known that the success of any hatchery operation depends mainly on the availability of the basic food, the microalgae.

Microalgae serve as live food for commercially important molluscs, fish, and crustaceans. The consumers are grown directly in the presence of their food produced *in situ.* Depending on consumer requirements (Which often depend on life stages) the microalgae are consumed either directly (e.g. by herbivorous fish, bivalve molluscs, larval shrimp and prawns, zooplankton), or indirectly via the 'algae-zooplankton' food chain (e.g. most fish). Microalgae play an important part in aquaculture' as a food source, together with bacteria, they also have an important role in the oxygen and carbon dioxide balance in the cultures. They can synthesize essential PUFAs and vitamins that are unable to be synthesized by higher organisms. Fatty acids are essential nutrients for almost all living beings for their existence and health. Docosahexaenic acid (DHA), Eicosapentaenoic acid (EPA), and Arachidonic acid (AA) etc. are the major PUFAs present in marine microalgae and these would have therapeutic significance has been demonstrated by recent clinical and epidemiological studies (Patil *et* al., 2005).

Cultivated microalgae have long been integral to the hatchery production of many farmed fin fish, shellfish, and other commercially important aquaculture species. Molluscs like oysters, mussels, and clams filter them from the seawater in all stages of life. Rotifers and brine shrimps also ingest algae and are then themselves used as food for larval fish and prawns. The shrimp hatcheries use microalgae as food for the early larvae and later for water quality maintenance. In many hatchery systems algae are added to the water containing larvae to improve the 'quality' of water in green water systems. The production of live algae is very critical in successful hatchery management. In the natural environment, the larvae feed on any minute plant components which are readily available to them. But in a hatchery, the feed which is acceptable to the larvae for their growth and further development has to be identified and isolated. In the

early critical stages of the rearing larvae of fin fishes and shellfishes, the phyto-flagellates (species of *Isochrysis, Pavlova, Dicrateria, Chromulina, and Tetraselmis*) and other nanoplankters (species of *Chlorella and Synechocystis*) form the basic food. But in the post-larval stages of crustaceans and spat or juvenile stages of bivalves, the diatoms (species of *Chaetoceros, Skeletonema* and *Thalassiosira*) form the primary food. Hence the culture of microalgae is an essential pre-requisite for the rearing operations of economically important cultivable organisms in a hatchery system.

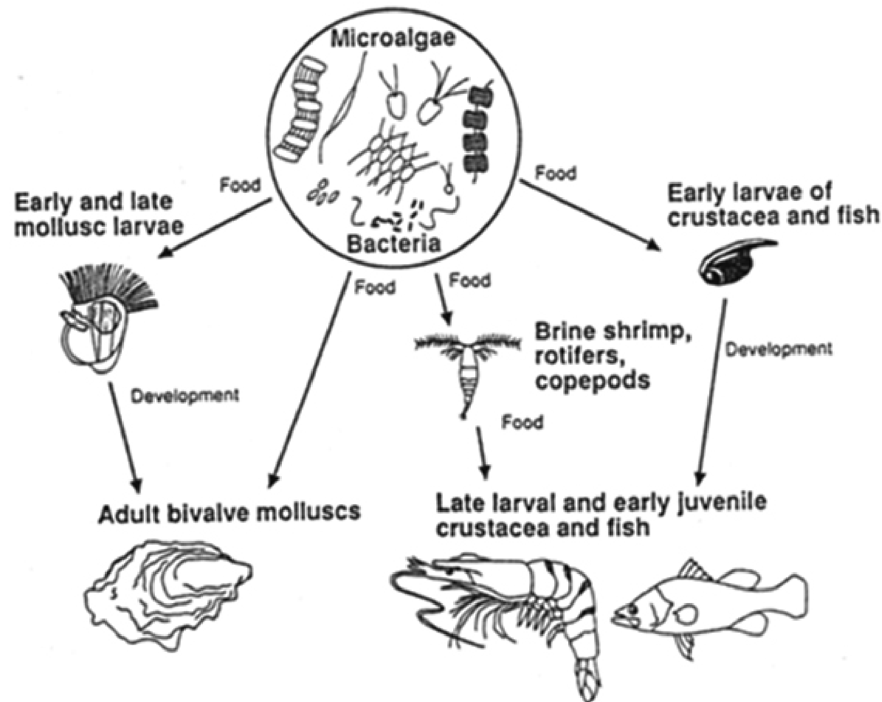


Fig. 1. The central role of micro-algae in mariculture (Brown *et al*., 1989)

The role of microalgae in aquaculture hatch-eries

The role of microalgae in aquaculture hatch-eries may be summarized as follows:

- All developmental stages of bivalve molluscs are directly reliant on microalgae as a feed source. Bivalve hatcheries therefore cultivate a range of microalgal strains for broodstock conditioning; larval rearing and feeding of newly settled spat.
- Farmed gastropod molluscs (e.g., abalone) and sea urchins require a diet of benthic dia-toms when they first settle out from the plank-ton, before transferring to their juvenile diet of macroalgae.
- The planktonic larval stages of commercially important crustaceans (e.g., penaeid shrimps) are initially fed on microalgae, followed by zooplanktonic live prey.
- The small larvae of most marine finfish species and some freshwater fish species also initially receive live prey, usually in the presence of a background of microalgae. Depending on whether these microalgae are allowed to bloom within the fish larval rearing tanks, or are added from external cultures, this is referred to as the “green water” or “pseudo-green water” rearing technique.
- The zooplanktonic live prey referred to above is microscopic filter-feeders that are commonly fed on microalgae, although inert formulated feeds have been developed as a more convenient diet form for use by hatcheries.

Major classes and genera of cultured algal species

The major classes of cultured algae currently used to feed different groups of commercially important aquatic organisms include species of diatoms, flagellated and chlorococcalean green algae, and filamentous blue-green algae, ranging in size from a few micrometres to more than 100 ì. The most frequently used species in commercial mariculture operations are the diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C. calcitrans*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, *Pavlova lutheri* and the chlorococcalean *Chlorella* spp

The most frequently used species in commercial operations are the diatoms *Skeletonema costatum, Thalassiosira pseudonana, Phaeodactylum tricornutum, Chaetoceros calcitrans,* the flagellates *Isochrysis galbana, I. tahiti, Monochrysis lutheri, Tetraselmis suecica, Dunaliella* spp. and the chlorococcalean *Chlorella* spp. In freshwater aquaculture, *Chlorella, Scenedesmus* and *Chlamydomonas* are the most common, as well as *Spirulina,* a blue-green alga characteristic of alkaline waters. Cultured microalgae are mostly fed directly (Hveform) to the consumer but sometimes these are also used in a concentrated and preserved form." Drum - dried *Scenedesmus* and spray-dried *Spirulina* have been used as food for *Artemia,* sundried *Spirulina* for Tilapia, spray-dried *Spirulina* for *Brachionus,* and heat-dried *Chlorella* and freeze-dried *Isochrysis* or *Dunalilla* for larvae of *Mercenaria.* In the semi-intensive form of algae production, the blooms of natural phytoplankton are induced by artificially fertilizing with organic nutrients which stimulates primary production. This, in turn, has a positive effect on the whole food chain. This technique has been used to increase fish, prawn, shrimp, and zooplankton production in ponds (esp. the commercial species like milkfish, Tilapia, and silver carp). Although this technique has been developed and used for industrial purposes due to its economic viability (because of reduced labour and expense and also results in a greater variety of diatom species) but is not always very reliable and is now often replaced by the use of uni-algal cultures.

Nutritive value of microalgae

The main difficulty in running all the above systems is the coordination of algal growth and uptake of algae by the consumer. Therefore, a different approach is adopted where algal production is separated from the consumption step. In many cases, aquaculture and algal wastewater treatment are combined for this purpose. The production price of the algae, however, determines their potential for small or large-scale application in aquaculture. More than forty different algal species are currently used as live food for aquatic invertebrates and vertebrates and it has also been observed that one algal species is a good food and another less so or not at all. Of course, the algae must be non-toxic, be of proper size to be ingested, have a digestible cell wall, and have sufficient essential biochemical constituents. "Bivalve molluscs, e.g. cannot digest thick cell walls, whereas rotifers can do so with the aid of their mastax. For this reason, *Chlorella* is well suited for *Brachionus* cultivation, but not for oysters or clams. The amino acid concentration within the algae and the balance of amino acids play a positive role in the growth and development of consumers. Similarly, the quality of lipids is of prime importance to the nutritional value of microalgae. Different algal foods have different concentrations of certain polyunsaturated fatty acids (PUFA) which play a very important role in the health status of larvae of many fish. However because algae are a biological system, its biochemical constituents (quantitatively and qualitatively) are dependent on numerous factors that are linked and even inherent to the culture conditions. For example, algal cultures in the exponential phase contain more protein, while cultures in the stationary phase have more carbohydrates. The nutritional value of some important algae is as under:

Algae	Protein	Lipids	Carbo-hydrates	Fibre	Ash	Nucleic acid	Moisture
Spirulina	62-68	2"3	15-20	5-8	10-12	6	5-6
Chlorella	40-50	10-15	12-16	6-8	8-10	6	5-8
Scenedesmus	50-55	12-19	10-15	10-12	6-8	4-6	5-7

A generalized set of conditions for culturing microalgae (modified Anonymous, 1991)

Parameters	Range	Optima
Temperature (°C)	16-27	18-24
Salinity (g.l-1)	12-40	20-24
Light intensity (lux) (depends on the volume and density)	1,000-10,000	2,500-5,000
Photoperiod (light: dark, hours)		6:8 (min) 24:0 (max)
pH	7-9	8.2-8.7

Besides these major constituents, the algae have the proximal concentration of minerals, essential fatty acids, and amino acids. These are also rich in water-soluble vitamins and comparable with other foodstuff of high vitamin content. The main and most attractive component of micro-algal biomass is crude protein but its utilization by consumers depends upon its digestibility. The utilization of protein in an *in vivo* system is limited by the rigid algal cell wall. Proteins of algae are difficult to extract from intact cells and cell wall is to be broken to obtain maximal extractability. The cells have to be processed sometimes to make the 'material more digestible. This can be accomplished by using a drum dryer or by a short period of boiling, whereas spray drying is insufficient (especially in green algae). The quality and digestibility are assessed by values of Protein Efficiency Ratio (PER), Biological value (B~), Digestibility coefficient (DC), and Net Protein Utilization (NPU). The algal diets are well accepted by carp, and the survival rate of fish is high. It has also been shown that algae can replace soybean meal and 60% of the fish meal in the diets to give yields comparable to the control. Different types of results have been obtained for different fish. However, it is commonly observed that algae-supplemented diets proved better for the growth of fish and showed low fat content in the whole body as well as in the individual organs.

Isolation of pure algal strains

Isolation of the required species of microalgae can be done by one of the following methods.

1. **Pipette method:** Large organisms can be pipetted out using a micro-pipette under the microscope and transferred to culture tubes having suitable culture media.
2. **Centrifuge or washing method**: By repeated centrifuging of the samples in different revolutions and by inoculating the deposits, it is possible to get different organisms.
3. **By exploiting the photo-tactic movements**: By this method, most of the phyto-flagellates can be isolated. Make a dark chamber with a small hole on one side and keep the sample in a beaker nearer to the hole. Place a candle near the hole outside. Since the flagellates tend to move towards the light, it is visible after some time that these organisms crowded near the candlelight. By pipetting, these organisms can be separated and by tube culture methods, it can be raised to a pure culture.
4. **Agar plating method:** For preparing the agar medium, 1.5 gm of agar is added to 1 litre of suitable

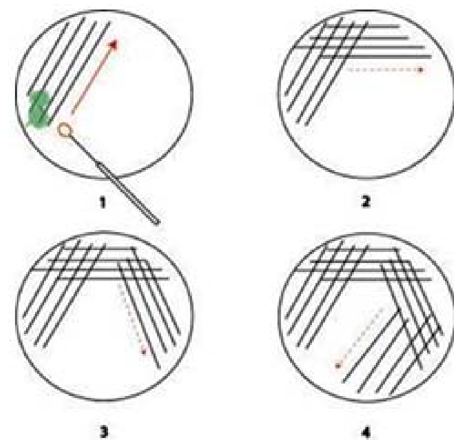
culture medium e.g. Schreiber's medium, Miquel's medium, TMRL medium, and Conway medium, or even natural seawater.

This agar solution is sterilized in an autoclave for 15 minutes under 60 kg pressure and 100°C temperature. Now this medium is poured in sterilized 15 cm diameter Petri dishes and kept for 24 hours. For isolation, the required species can be picked up by platinum needle or looped under a microscope and streaked on the surface of the agar plate.

After inoculation, these Petri dishes are placed in an incubator for 7-8 days providing light (1000 lux) and constant temperature (25°C). Within this time, the required species, if it has grown into a colony, is removed by a platinum loop under the microscope and transferred to culture tubes. Further, from culture tubes to small conical flasks and larger flasks, the algae can be grown on a mass scale.

Agar plating technique

The following agar plating technique can be used to isolate algal strains from raw seawater and for the maintenance of existing algal strains.



Agar plates with algal streaks

- Prepare a 0.9% agar medium by weighing out 9 g of agar powder and placing it into a 2 l conical flask to which 1 l of seawater is added
- Heat the flask on a Bunsen flame and let it boil twice, i.e. heat until it boils, let it cool and let it boil a second time
- Add nutrients (see Tables 2.3 and 2.4) before autoclaving
- Cover the flask with aluminum foil
- Autoclave at 125 °C for 30 minutes at 1 atm
- Sterilise Petri dishes by incubation for 30 minutes at 150 °C
- 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, cover up the Petri dishes and leave them to cool for about 2 h
- Streak the algal sample onto the agar surface with a sterile platinum loop (previously heated to red-hot and cooled)
- Place the Petri dishes upside-down on an illuminated glass rack
- Depending on the density of the inoculum, cell colonies can be observed to grow on the surface after 5 - 21 days
- Select the best colonies and transfer them with a sterile platinum loop into a test tube filled with 5-10 ml of culture medium and shaken regularly during incubation on an illuminated glass rack when a colour change is observed in the tube, check the isolated algal strain under the microscope

5. Serial dilution culture technique

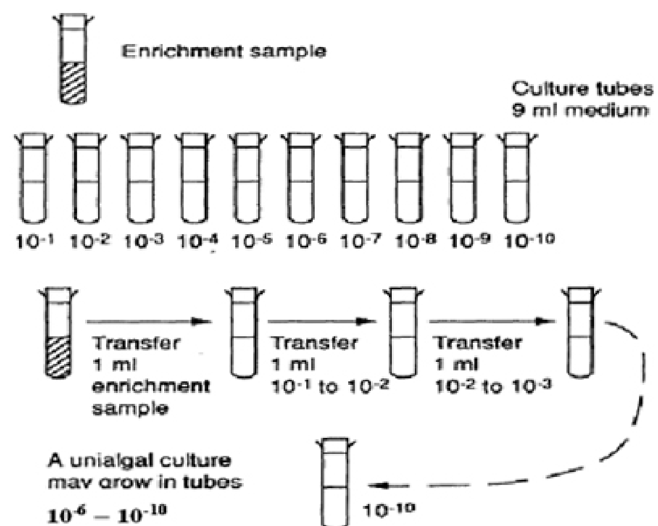
This method is used mainly for the isolation of phyto-flagellates (Sournia, 1971). In this method, mainly 5 dilution steps (the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} or 4 steps – 0.001, 0.01, 0.1, and 1 ml)

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 a 10-20 micron sieve, the filtrate has to be inoculated into five series of culture tubes in various concentrations. This has to be kept under sufficient light (1000 lux) with uniform temperature (25 °C) conditions. After 15 days, some discolouration can be seen in the culture tubes, due to the growth of microalgae. Further purification of this culture can be done by sub-culturing it in 500 ml or one-litre conical culture flasks. Once the culture is fully purified, it can be transferred into 3 or 4-litre Haufkin culture flasks and maintained as stock culture. After the isolation of the required organisms in culture tubes, they may be sub-cultured again in a few 50 ml test tubes. These test tubes are the base from which the algal food starts producing and from where the continuous supply of non-contaminated algal feed is obtained for the operation of the large-scale culture systems. Once the system is started, the test tube culture can be transferred to small culture flasks and bigger flasks by adding 3-5 ml of the stock culture. Therefore every two weeks a new set of 10 test tubes for each species should be inoculated from the previous set. The filtration of water and medium enrichment should be done no earlier than 3 days, before inoculation.

Procedure

Using an aseptic technique, dispense 9 ml of media into each of the ten test tubes with a sterile automatic dispenser or sterile 10 ml pipettes. Label tubes 10-1 to 10-10 indicating dilution factor.

- Aseptically add 1 ml of enrichment sample to the first tube (10^{-1}) and mix gently.
- Take 1 ml of this dilution and add to the next tube (10^{-2}), mix gently.
- Repeat this procedure for the remaining tubes ($1O^{-3}$ to 10^{-10}).
- Incubate test tubes under controlled temperature and light conditions:
- Temperature and photoperiod — as close to the natural environment as possible
- Light intensity — slightly lower than the natural environment
- Examine cultures microscopically after 2—4 weeks by withdrawing a small sample aseptically from each dilution tube. A unialgal culture may grow in one of the higher dilution tubes e.g. 10^{-6} to 10^{-10}. If tubes contain two or three different species then micromanipulation can be used to obtain unialgal cultures



Maintenance of stock and starter cultures

Stock cultures, otherwise known as master cultures, of the preferred species are the basic foundation of culture. They are normally supplied as monospecific (uni-algal) cultures from reputable culture collections maintained by national institutions or research laboratories. Since they are valuable, they are normally kept in specialized maintenance media, for example, Erdschreiber, or in F/2 media, or on nutrient-enriched agar plates or slopes, under closely controlled conditions of temperature and illumination. A special area or room of the algal culture room is usually allocated for this purpose.

Stock cultures are used only to provide lines of starter cultures (also known as inocula) when required. Every effort should be made to minimize the risk of contaminating the stock and starter cultures with competing microorganisms. The sterile procedures described below should be followed to ensure that contamination does not occur. Stock cultures are kept in small, transparent, autoclavable containers. For example, 500 ml borosilicate glass, flat-bottomed boiling or conical flasks fitted with a cotton wool plug at the neck, suitable for containing 250 ml of sterile, autoclaved medium, are ideal.

The composition and preparation of Guillard's F/2 medium is given in Table –

Guillard's F/2 media used for culturing algae in hatcheries from Guillard (1975).

1.	Nitrate NaNO3	75.0 g per l
2.	Phosphate NaH2PO4.H2O	5.0 g per l
3.	Silicate Na2SiO3.9H2O	30.0 g per l
4.	Trace Metals	
	FeCl3.6H2O	3.5 g
	Na2EDTA	4.36 g

Dissolve in 900 ml distilled H2O
Add 1 ml of each of the following trace metal solutions

CuSO4.5H2O	0.98 g per 100 ml
ZnSO4.7H2O	2.20 g per 100 ml
CoCl2.6H2O	1.00 g per 100 ml
MnCl2.4H2O	18.00 g per 100 ml
Na2MoO4.2H2O	0.63 g per 100 ml

Make up the volume to 1 l with distilled H20 (pH ca. 2.0)
Add 1 ml per litre FSW of the above solutions (#1-4).

5.	Vitamins	
	Biotin	1.0 mg
	B-12	1.0 mg
	Thiamine HCl	20.0 mg

Dissolve in 1 l distilled H_20. Store frozen
Add 1/2 ml of vitamin solution for every 1 l of FSW.

Procedure for transferring algal cultures from flask to flask

- Wipe all inner surfaces of the inoculating booth with 85% ethanol.
- Place all flasks that will be required in the booth; i.e. all flasks to be transferred from (the transfer flask) and flasks containing sterilized media to be transferred into (new flasks).
- Close the booth and switch on the ultra-violet lamp. Leave for at least 20 minutes. (It is not safe to look directly at ultraviolet light, so a dark cover should be placed over the plexiglass (transparent acrylic plastic) viewing plate when the light is on.)
- Switch off the lamp. Ignite the small burner.
- Remove foil caps from one transfer and one new flask. Flame the neck of each flask by slowly rotating the neck through the flame.

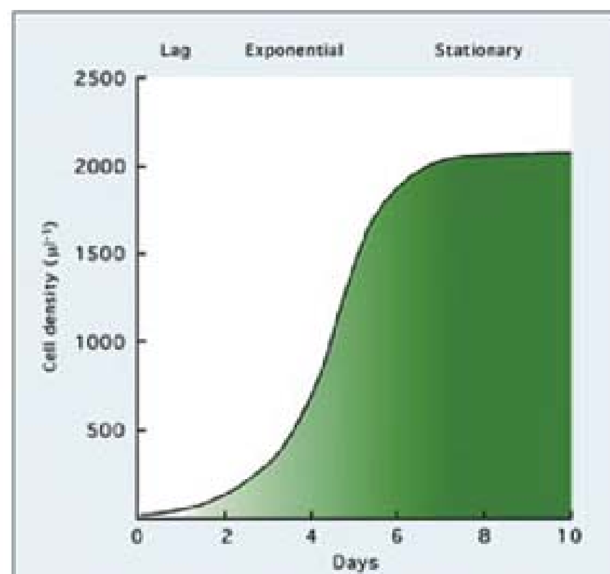
- Tilt the neck of the transfer flask toward the new flask. In one motion, remove both stoppers and pour an inoculum into the new flask. Transfer approximately 50 ml for diatom species and 100 ml for flagellates. Avoid touching the necks of the two flasks. Never touch the portion of the stopper that is inserted into the flask. Once the inoculum is added, replace the stopper in the transfer flask. Slowly flame the neck of the new flask before replacing its stopper.
- Replace the foil cap over the neck of the new flask. Using a waterproof marker pen, label the new flask with the algal species inoculated and the date of transfer.
- Repeat it for all flasks within the booth. Once completed, turn off the burner and open the booth.
- Remove all new flasks and place them in the algal incubator or a well-lit area in the algae culture facility.
- The remaining inoculum in the transfer flasks can be used to inoculate larger cultures such as 4 l flasks or carboys. (from Bourne, Hodgson, and Whyte, 1989)

Starter culture management

Procedures for the maintenance of starter cultures (inocula) are almost identical to those described above. These cultures are specifically grown to provide inocula to start larger volume cultures needed to produce food. A line of starter cultures is originally set up from the stock culture of the required species. Starter cultures, like the stocks, can be grown in 500 ml boiling flasks in 250 ml of culture medium. With larger volume starters it is advantageous to increase the level of illumination and to aerate it with an air/carbon dioxide mixture.

Growth phases of cultures

Harvesting takes place in a semi-continuous culture during the exponential phase of growth. Batch harvests are made generally at the peak of exponential growth as the cultures enter the stationary phase. An illustration of the meaning of these terms is given in Figure 19. In this case, the species cultured is the large, green flagellate, *Tetraselmis*. At inoculation from the starter culture, the starting cell density in the culture is 25 to 50 cells per ml (cells per microlitre).

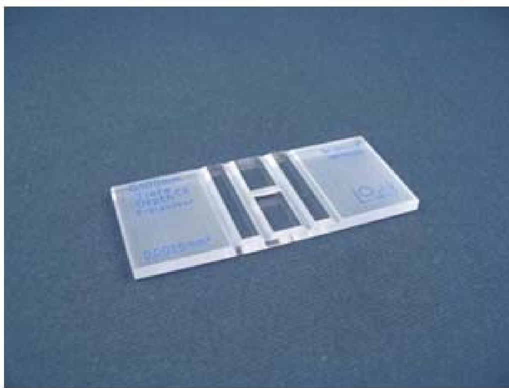


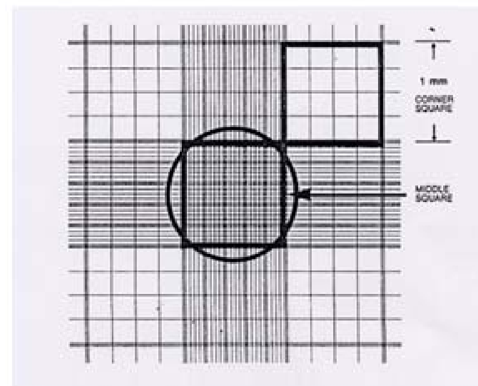
Phases in the growth of algal cultures are illustrated by a typical growth curve for a green flagellate.

After inoculation, these cells grow and divide increasingly rapidly as they acclimatize to the culture conditions. This acclimatization period, which lasts for 2 to 3 days, is called the **lag** phase. Once adapted to the conditions, the rate of cell division accelerates and an increase in the number of cells in the culture is logarithmic. This period lasts for 4 to 6 days and is called the exponential growth phase. Cell division rate then slows as light penetration through the culture and/or nutrients become limiting. The culture then enters the stationary phase, which can last for many days in the case of flagellates or only for a short time for diatoms. Cultures of flagellates remain in this phase by the recycling of nutrients from dead and decaying cells, but in the case of diatoms, which may produce self-inhibiting metabolites, that attract bacterial growth, the culture collapses.

Estimating algal density

Accurate estimates of cell density can be made using a hemocytometer. 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 0.1 mm^3. The base of each chamber is marked with a grid to aid in counting cells within the area. Before counting motile algal species, 1 or 2 drops of 4% formalin should be added to a 10 to 20 ml sample of the culture to be counted. With the coverslip in position, one or two drops of the algal sample are introduced using a Pasteur pipette to fill both chambers. Cell density is estimated as follows. The central grid of each chamber (outlined in the circle) is subdivided into 25 squares, each measuring 0.2 x 0.2 mm. The numbers of cells in 10 randomly chosen 0.2 x 0.2 mm squares are counted and the average or mean is calculated. This gives the mean number of algal cells per 0.2mm x 0.2mm x 0.1mm, or 0.004 mm^3.





Example

A. Counts of algal cells: 40 + 30 + 50 + 60 + 55 + 65 + 70 + 45 + 40 + 70 = 525

Average = 52.5 cells per 0.004 mm^3

B. Multiply the average by 250 to give the average number of cells per mm^3.

C. Since there are 1000 mm^3 in 1 ml, multiply the value calculated in B by 1 000.

In this example, the cell density would be 52.5x250 x1000 = 13.1 m (13.1 x 10^6) cells per ml.

Extensive outdoor culture

Commercial hatcheries need to produce large volumes of good quality, high-food-value algae daily to support economic-scale seed production. Outdoor tank culture makes use of natural light. Culture in rectangular or circular tanks with overhead illumination is used in shrimp hatcheries in India. This involves the fertilization of a large volume of seawater with the basic nutrients necessary for production, namely nitrogen, phosphorus, and silica in one form or another. It is possible to induce mono-specific blooms by prior fine (<2 ì particle retention) filtration of the impounded seawater and the introduction of an inoculum of the required species, as long as it is hardy and vigorous. However, it is difficult to maintain such blooms for long periods because they rapidly become contaminated with other microorganisms.

Algal culture techniques

Algae can be produced using a wide variety of methods, ranging from closely controlled laboratory methods to less predictable methods in outdoor tanks. The terminology used to describe the type of algal culture includes:

Indoor/Outdoor: Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators, and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.

Open/Closed: Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.

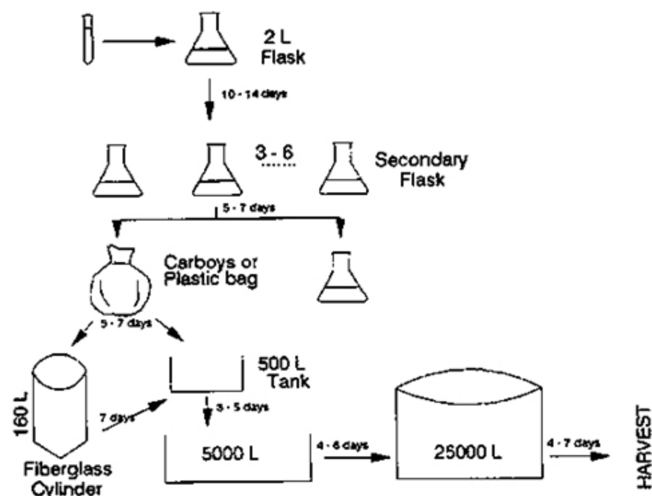
Axenic (=sterile)/Xenic: Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media, and vessels to avoid contamination. The latter makes it impractical for commercial operations.

Batch, Continuous, and Semi-Continuous: These are the three basic types of phytoplankton culture which will be described in the following sections.

The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes before reaching the stationary phase, and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 l flasks, 5 and 20 l carboys, 160 l cylinders, 500 l indoor tanks, 5,000 l to 25,000 l outdoor tanks.

Batch culture systems are widely applied because of their simplicity and flexibility, allowing them to change species and remedy defects in the system rapidly. Although often considered the most reliable method, batch culture is not necessarily the most efficient method. Batch cultures are harvested just before the initiation of the stationary phase and must thus always be maintained for a substantial period past the maximum specific growth rate. Also, the quality of the harvested cells may be less predictable than that in continuous systems and for example vary with the timing of the harvest (time of the day, exact growth phase).

Another disadvantage is the need to prevent contamination during the initial inoculation and early growth period. Because the density of the desired phytoplankton is low and the concentration of nutrients is high, any contaminant with a faster growth rate is capable of outgrowing the culture. Batch cultures also require a lot of labour to harvest, clean, sterilize, refill, and inoculate the containers.



The continuous culture method: (i.e. a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out), permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

Turbidostat culture, in which the algal concentration is kept at a pre-set level by diluting the culture with a fresh medium using an automatic system.

Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined

rate. The latter adds a limiting vital nutrient (*e.g.* nitrate) at a fixed rate and in this way, the growth rate and not the cell density is kept constant.

The disadvantages of the continuous system are its relatively high cost and complexity. The requirements for constant illumination and temperature mostly restrict continuous systems to indoors and this is only feasible for relatively small production scales. However, continuous cultures have the advantage of producing algae of more predictable quality. Furthermore, they are amenable to technological control and automation, which in turn increases the reliability of the system and reduces the need for labour.

Semi-continuous culture: The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment. The culture is grown up again, partially harvested, etc. Competitors, predators, and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size.

Troubleshooting

Cultures will fail to grow, will become overly contaminated with competing micro-organisms, or will crash even in the best-run hatcheries. Below are some pointers to check to determine the source of such failures.

- Air supply: Is there adequate air entering the cultures? Are the cells sedimenting to the bottom of the culture vessel?
- Temperature: Check the min/max thermometer. Were there any increases or decreases in the temperature of the algal culture facility over the past 24 hours? Temperatures in the range 18 to 23 ^{o}C are ideal for indoor.
- PH: Check CO_2 supply; Is the CO_2 cylinder empty? Check the pH of the algal cultures using a pH probe. Is the pH too high (above 8.5)? Is the pH too low (below 7.5)? Adjust the CO_2 supply accordingly.
- Nutrients: Check records for the last time the cultures received nutrients. This is particularly important for semi-continuous cultures.
- Contamination. Are the walls of the culture container, particularly at the water/air interface, visibly foaming or fouled with what appears to be detritus?

Advantages of algae in aqua-hatcheries /aquaculture

Despite all efforts to replace live algae with nutritionally poor-inert foods; aqua culturists are still dependent on the production and use of micro-algae as live food for commercially important fish, molluscs, and crustaceans. The main advantages of using microalgae in the diets are as follows:

Natural occurrence

The microalgae are found naturally in all water bodies and can be extensively used for large-scale production. The plankton-rich sea water can be pumped into on-shore construction, or in nursery rearing of bivalve molluscs. Usually, blooms of natural phytoplankton are induced in the enclosures where the consumers are grown. This method is very economical where large quantities of micro-algae are needed.

Alternative systems

For reducing the cost of production of microalgae, it is linked with intensive wastewater treatment systems. Treatment of bio-industry wastes and effluents of food industries in particular have been expensive. In

this context, algal biomass is integrated into the effluent treatment, and the biomass obtained is used either for energy production or for feed' use. Treated effluent in aerated lagoons can serve as a medium for *Spirulina* cultivation as a dominant algal form without or with small additions of some fertilizers.

High production with minimum inputs

Algae culture can be easily grown and does not require any sophisticated apparatus or limited growth conditions when produced as a mixed population. The seawater as such can be used by continuous pumping to provide the carbon supply for intensive cultures. Nitrogen and phosphorus are added as salts. Depending on the location, availability, type of algal species, and nature of the culture system used; the cost of production and yields vary, but overall, algal systems are energy-efficient systems.

Rich nutritional value

The chemical composition of various algae used as fish feed is a good reflection of the nutritive potential of the algae biomass. As a system of a biological nature, it is influenced by various environmental factors that alter the proportions of the individual cell constituents. However, this proportion can also be modified according to needs by specific cultivation measures. The composition of the algal dry matter is made up of all essential components like proteins, lipids, carbohydrates, fiber, ash CQ, etc., and is comparable with the soya seeds. The most attractive component of microalgal biomass is crude protein. Besides these, it also contains a good amount of vitamins and minerals. The aquatic species show increased growth when fed on algal diets.

Supplemental value to conventional food

As the algal mass is a good source of proteins, minerals, etc. thus can be utilized in combination with other conventional foods. A mixture of waste-grown algae *(Spirulina, Chlorella, Scenedesmus)* in combination with soybean meal has been used resulting in increased yields. Supplementations are more. Useful in areas where fresh algae cannot be produced on a mass scale and preserved and concentrated powder is available. It enriches the diet with many essential amino acids, fatty acids, and water-soluble vitamins.

Disadvantages of algae in aqua hatcheries /aquaculture

Predation-contamination

The major difficulty with large-scale algal cultures, like other monocultures, is susceptibility to infection by viruses, bacteria, fungi, and exposure to predators such as protozoans, rotifers, crustaceans, and even micro-planktonic larvae of benthic organisms. Although contamination of larger organisms can be reduced or controlled bacterial and viral infections are still a problem. Large-scale treatment with chemicals, however, is not usually advisable, especially from the toxicological and economical point of view

Upscaling and collapsing of pure cultures

A major handicap in the cultivation of algae is the difficulty of growing selected species with known food value in large volumes. Where only small quantities of algae are needed for a restricted period, there are several culture methods and devices by which uni-algal cultures can be produced under well-defined conditions. But up-scaling environmental conditions rapidly leads to the collapse of the cultures or takeover by other species better adapted to the prevailing outdoor conditions.

Species control in induced blooms of natural phytoplankton

As mentioned earlier, up-scaling of pure algal cultures is difficult to achieve, one may go for induction of blooms in natural phytoplankton. As the cultures are grown outdoors, there is another difficulty. In

obtaining control over the species composition of the bloom induced. This is, however, an absolute necessity, if the nutritional requirements of the consumers are to be met. But by manipulating the growth parameters such as nutrient supply, retention time, pH, temperature, mixing, etc. one can obtain certain control over the composition of the algal population under large-scale cultivation.

Toxicity and digestibility

Evidence of nutritional quality is not only one of the basic requirements for utilizing the algal biomass in aqua-feeds. Equally important is proof of the toxicological safety and digestibility of the material. There have been reports that algae may contain certain toxins, however, no toxins have been found in samples of *Spirulina* and *Scenedesmus* and there are no confirmed indications of the occurrence of such toxins in other algae. Certain algae, on the other hand, maybe of larger size and cannot be ingested by particular aquatic communities or may have thick cell walls which cannot be digested.

Variation in nutritional value

Although algae have been described as nutritionally rich the nutritional value of an alga and its biochemical composition varies and is dependent on several environmental factors, growth conditions, and state of growth. In outdoor cultures, where growth conditions vary and there is a direct influence of environmental conditions, one can never predict the exact chemical composition at a particular time

High production cost

Depending on the technology used, microalgae production may cost from very little to very high. The cost is meagre when sewage-grown algae are harvested and used, as these are by-products of the wastewater treatment process. Although high production costs should always be considered concerning the commercial value of the end product, it is clear those pure cultures of algae for nursery rearing and growth of bivalve molluscs and for farming herbivorous fish are costly. Major production costs are made up of labour, pumping, nutrients, and mixing.

Harvesting and preserving micro-algae

In most cases, it is unnecessary to separate microalgae from the culture fluid. Excess and off-season production may, however, be concentrated and preserved. The various techniques employed to harvest microalgae have been reviewed by Fox (1983) and Barnabé (1990). High-density algal cultures can be concentrated by either flocculation or centrifugation. Products such as aluminium sulphate and ferric chloride cause cells to coagulate and precipitate to the bottom or float to the surface. Recovery of the algal biomass is then accomplished by, respectively, siphoning off the supernatant or skimming cells off the surface. Due to the increased particle size, coagulated algae are no longer suitable as food for filter feeders. Centrifugation of large volumes of algal culture is usually performed using a cream separator; the flow rate is adjusted according to the algal species and the centrifugation rate of the separator. Cells are deposited on the walls of the centrifuge head as a thick algal paste, which is then resuspended in a limited volume of water. The resulting slurry may be stored for 1-2 weeks in the refrigerator or frozen. In the latter case, cryoprotective agents (glucose, dimethylsulfoxide) are added to maintain cell integrity during freezing. However, cell disruption and limited shelf-life remain the major disadvantages of long-term preserved algal biomass. Concentrated cultures of *Tetraselmis suecica* kept in darkness at 4°C maintain their viability, whereas the latter is completely lost upon freezing. Furthermore, cultures stored in hermetically sealed vials lose their viability more rapidly than those kept in cotton-plugged vials.