Microalgae Culture and Production

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Introduction

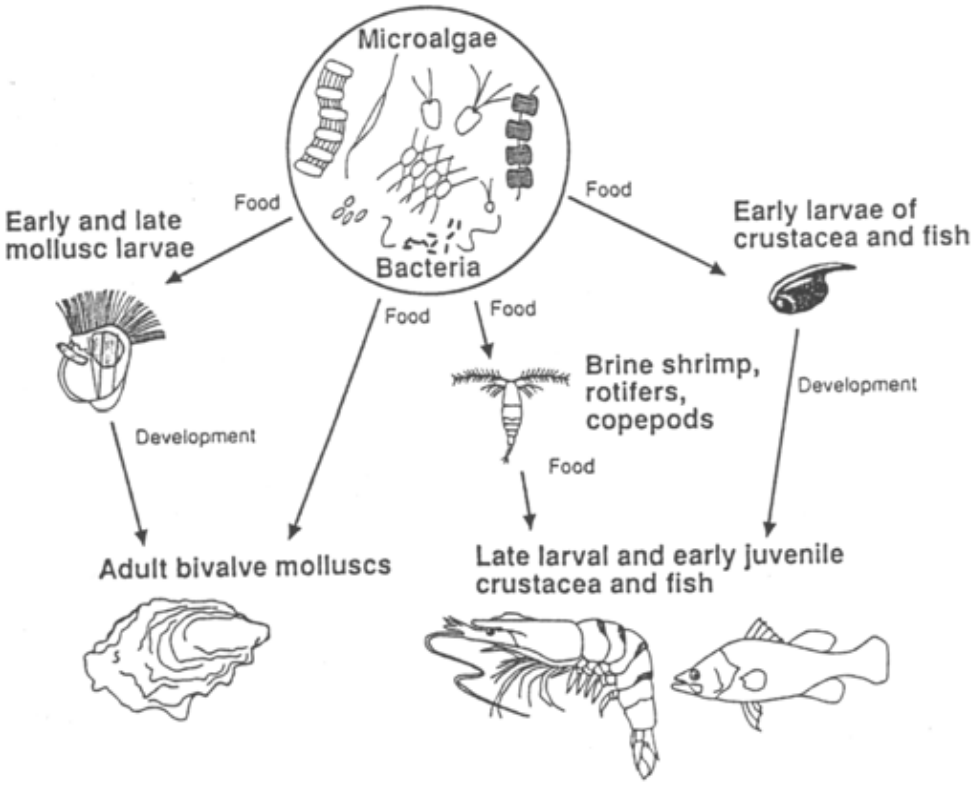
Marine micro-algae are single-celled plants and like all plants, contain chlorophyll, which traps the energy from light and uses it to convert nutrients and carbon dioxide dissolved in the sea water into organic growth. Phytoplanktons are the primary producers of the sea and they belong to different classes of algae. Unicellular marine microalgae are widely used as food in the hatchery production of commercially valuable fish and shellfish for the various stages. The important components of micro algae are the diatoms, dinoflagellates, silicoflagellates (phyto-flagellates), coccolithophores, blue-green algae and the 'hidden flora' the nanoplankters. Among these, the diatoms and phytoflagellates 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 operations depends mainly on the availability of the basic food, the micro algae.

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 not only play an important part in aquaculture' as a food source, but, together with bacteria, they also have an important role in the oxygen and carbon dioxide balance in the cultures.

Cultivated microalgae have long been integral to the hatchery production of many farmed finfish, shellfish and other commercially important aquaculture species. Molluscs like oysters, mussels and clams filter them from the sea water 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 micro algae as food for the early larvae and later for the water quality maintenance. In many hatchery systems algae are added to the water containing larvae to improve the 'quality' of water as green water systems. The production of live algae is very critical in the 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 are acceptable to the larvae for their growth and further development have 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 micro algae is an essential pre-requisite for the rearing operations of economically important cultivable organisms in a hatchery system.

The role of microalgae in aquaculture hatcheries



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

The role of microalgae in aquaculture hatcheries 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 micro algal 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 diatoms when they first settle out from the plankton, prior to 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 are microscopic filter-feeders that are themselves 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 micrometers to more than 100 µ. 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 chlorococcal~an *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 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 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 waste-water 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 are able to 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 also the balance of amino acids playa positive role in 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 plays a very important role in the health status of larvae of many fish. But because algae are a biological system, its biochemical constituents (quantitatively & qualitatively) are dependent on numerous factors which are linked and even inherent to the culture conditions. For example, algal cultures in the exponential phase contain more protein, while cultures in 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

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Besides these major constituents, the algae have 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 is assessed by values of Protein Efficiency Ratio (PER), Biological value (B~), Digestibility co-efficient (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 be 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 growth of fish and showed low fat content in whole body as well as in the individual organs.

A generalized set of conditions for culturing micro-algae (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 volume and density)	1,000-10,000	2,500-5,000
Photoperiod (light:dark, hours)		6:8 (minimum) 24:0 (maximum)
pH	7-9	8.2-8.7

Isolation of Pure Algal Strains

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

1. *Pipette method*: Large organisms can be pipetted out using a micro-pipette under microscope and transfer 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 phytoflagellates 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 to the hole outside. Since the flagellates have a tendency to move towards the light, it is visible after sometimes that these organisms crowded near to the candle light. 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.

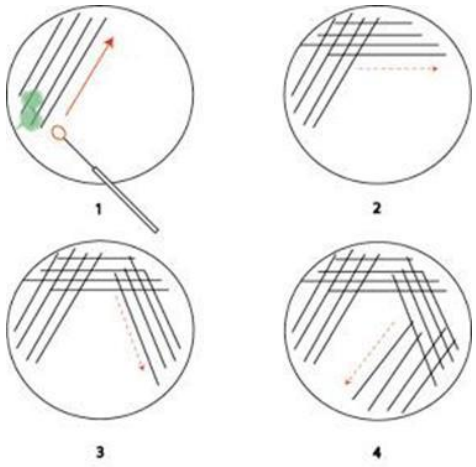
This agar solution is sterilized in an autoclave for 15 minutes under 60 kg pressure and 100°C temperature. Now this medium is poured in sterilized 15 cm diameter Petri-dishes and kept for 24 hours. For the isolation, the required species can be picked up by platinum needle or loop under microscope and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

- 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 sea water 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 & 2.4) before autoclaving cover the flask with aluminium foil;
- Autoclave at 125 °C for 30 minutes at 15 lb;
- Sterilise Petridishes by incubation for 30 minutes at 150°C;
- Agar plates are prepared aseptically by pouring the warm autoclaved agar into the sterile Petridishes near a Bunsen flame or in a laminar flow, cover up the Petridishes 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 shake it regularly during incubation on an illuminated glass rack when a colour change is observed in the tube, check the isolated algal strain under microscope.



Agar plates with algal streaks

5. ***Serial dilution culture technique:*** This method is used mainly for the isolation of phytoflagellates (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 10-20 micron sieve, the filtrate has to be inoculated to 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 micro-algae. 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, it may be sub-cultured again in few 50 ml test tubes. These test tubes are the base from which the algal food started 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 to 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 not earlier than 3 days, prior to inoculation.

Procedure

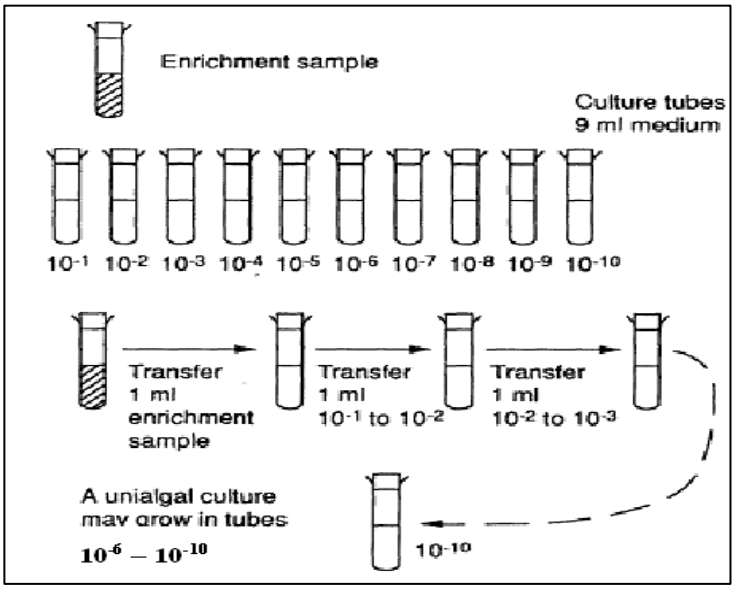
Using aseptic technique, dispense 9 ml of media into each of ten test tubes with sterile automatic dispenser or sterile 10 ml pipettes. Label tubes 10-1 to 10-10 indicating dilution factor.

- 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:

(a) Temperature and photoperiod — as close to the natural environment as possible

(b) 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 alternatively 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 off the algal culture room is usually allocated to 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 $NaNO_3$ — 75.0 g per l
2. Phosphate $NaH_2PO_4.H_2O$ — 5.0 g per l
3. Silicate $Na_2SiO_3.9H_2O$ — 30.0 g per l
4. Trace Metals

$FeCl_3.6H_2O$	3.5 g
Na_2EDTA	4.36 g

Dissolve in 900 ml distilled H_2O

Add 1 ml of each of the following trace metal solutions

$CuSO_4.5H_2O$	0.98 g per 100 ml
$ZnSO_4.7H_2O$	2.20 g per 100 ml
$CoCl_2.6H_2O$	1.00 g per 100 ml
$MnCl_2.4H_2O$	18.00 g per 100 ml
$Na_2MoO_4.2H_2O$	0.63 g per 100 ml

Make up the volume to 1 l with distilled H_2O (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

(a) Wipe all inner surfaces of inoculating booth with 85% ethanol.

(b) 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).

(c) Close booth and switch on 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 plexi-glass (transparent acrylic plastic) viewing plate when the light is on.)

(d) Switch off lamp. Ignite small burner.

(e) Remove foil caps from one transfer and one new flask. Flame the neck of each flask by slowly rotating the neck through the flame.

(f) 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.

(g) Replace 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.

(h) Repeat it for all flasks within the booth. Once completed, turn off burner and open booth.

(i) Remove all new flasks and place in the algal incubator or a well-lit area in the algae culture facility.

(j) 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)

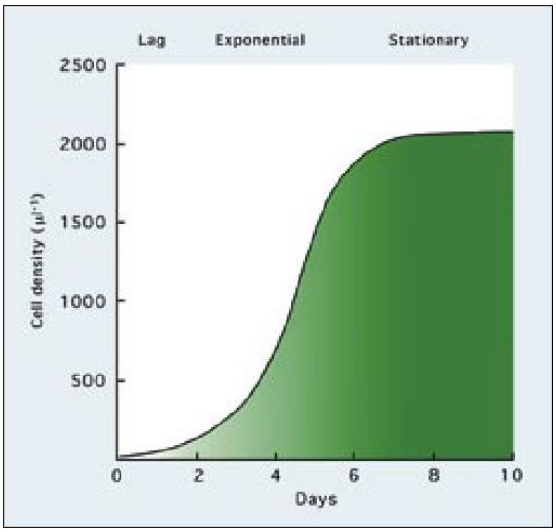
Starter culture management

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 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. 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).

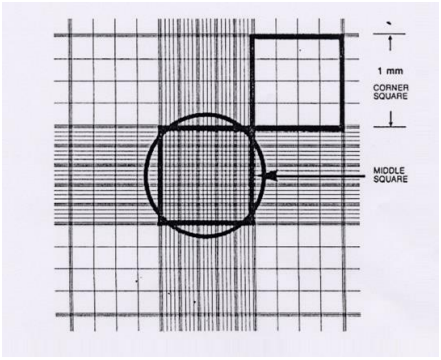


Phases in the growth of algal cultures illustrated by a typical growth curve for a green flagellate

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 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, which attract bacterial growth, the culture collapses.

Estimating algal density

Accurate estimates of cell density can be made using a haemocytometer. 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. Prior to 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 cover slip in position, one or two drops of the algal sample are introduced by means of a Pasteur pipette to fill both chambers. Cell density is estimated as follows.



The central grid of each chamber (outlined in the circle) is sub-divided 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 µ 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.

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.

1. Air supply: Is there adequate air entering the cultures? Are the cells sedimenting to the bottom of the culture vessel?
2. Temperature: Check 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 23ºC are ideal for indoor.
3. PH: Check CO_2 supply; Is the CO_2 cylinder empty? Check 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 CO2 supply accordingly.
4. Nutrients: Check records for the last time the cultures received nutrients. This is particularly important for semi-continuous cultures.
5. 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

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

Natural occurrence

The microalgae are found naturally in water bodies (fresh as well as marine) 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 in the effluent treatment and biomass obtained is used either for energy production or for feed' use. Treated effluent in aerated lagoons can serve as a media for *Spirulina* cultivation as a dominant algal form without addition of any nutrients 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 sea water 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 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 nutritive potential of the algae bio-mass. As system of a biological nature, it is influenced by various environmental factors which 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, fibre, 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 combinational with other conventional foods. Mixture of waste grown algae *(Spirulina, Chlorella, Scenedesmus)* in combination with soybean meal have been used resulting in increased yields. Supplementations are more. Useful in areas where fresh algae cannot be produced on mass scale and preserved and concentrated powder is available. It enriches the diet with many essential aminoacids, fatty acids and water soluble vitamins.

Disadvantages of algae in 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 but 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 to grow selected species with known food value in large volumes. Where only small quantities of algae are needed for a restricted period of time, there are a number of culture methods and devices by which uni-algal cultures can be produced under well-defined conditions. But up-scaling environmental conditions, rapidly leads to collapse of the cultures or take over 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 out-door, 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 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. Sometimes, the algal cultures may get contaminated with toxic substances like heavy metals etc. which may lead to

mortality or oxygen depletion associated with algal blooms and fish kill, and these may be correlated to toxicity. Certain algae, on the other hand, may be of larger size and cannot be ingested by particular aquatic community or may have thick cell wall which cannot be digested. The biochemical composition of particular species of algae may show high values of essential constituents like proteins, lipids, fatty acids etc., however, it will be rated nutritionally poor, if these have low digestibility, ultimately leading to poor growth of aquatic animals.

Variation in nutritional value

Although algae have been described as nutritionally rich but the nutritional value of an alga and its biochemical composition varies and is dependent on a number of environmental factors, growth conditions and state of growth. In outdoor cultures, where growth conditions vary and there is direct influence of environmental conditions, one can never predict the exact chemical composition at particular time. Unwanted changes in nutritive value sometimes occur when one attempts to counteract or prevent environmentally induced unbalanced growth conditions.

High production cost

Depending on the technology used, microalgae production may cost from very less to very high. The cost is meagre when sewage grown algae are harvested and used, as these are bypr6ducts of waste water treatment process. Although high production costs should always be considered in relation to the commercial value of the end product, it is clear those pure cultures of algae for nursery rearing and grow-out of bivalve mollusc and for farming herbivorous fish is costly. Major production costs are made up by labour, pumping, nutrients and mixing.

Conculsion

Thus it has been demonstrated that algae can be successfully incorporated into common fish diets. But the overall prospects for aquaculture will be governed primarily by economic considerations.

Where artificial and expensive feeds are used, including algae that were grown on artificial medium, the trend is to produce and market luxury foods such as shrimp, oysters and clams in order to demonstrate an economic gain. In contrast, the possibilities for recycling waste materials markedly improve the economics of aquaculture, particularly if credits are taken for savings in the cost of waste water treatment. However, public health standards must be met.

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