Culturing Algae

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1 Introduction

The artificial medium where the alga grows is defined as culture. Artificial culture medium is supplemented with various chemicals so that it resembles with the natural environment. The isolation of axenic culture of algae is the first and foremost step in any aquaculture process. A proper management and precautions is required in each and every step of unialgal cultures so that axenic culture can be preserved for longer period of time. It is crucial to maintain the algal reservoir free of various contaminants and bacteria. Various culture media were developed for various algae as the habitat of algae vary from one species to another. Algal culture media are divided into two broad classes viz Fresh water culture media and marine culture media. The samples which are collected are usually not pure. Therefore various purification steps should be followed so that large impurities and unwanted materials are removed. The generalized scheme of possible isolation and purification methods for microalgae are outlined below (Guillard and Morton 2003).

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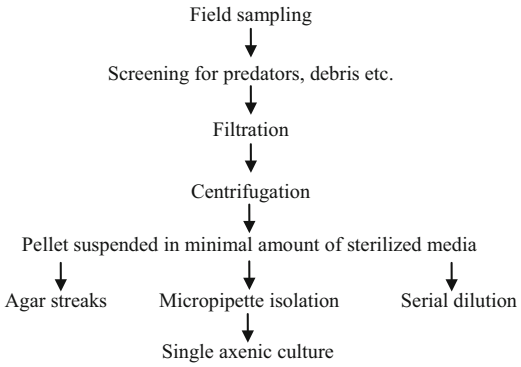

2 Sterilization and Sterile Technique

Before the start of any culture whether fresh water algae or marine algae sterilization of all the glasswares are very much essential. Sterilization is usually done to develop an aseptic culture environment by killing the microorganisms. This process is one of the most important step in any culture work so that pure culture can be maintained (Masanobu and Mary Héléne 2005). There are various types of sterilization methods such as heat sterilization, electromagnetic, chemical sterilization etc. Out of which autoclaving which is a method of heat sterilization is commonly practiced for algal cultures. All the glasswares, pipettes, plasticwares, utensils and medium (both solid and liquid) should be autoclaved before used. Autoclave is a specialized closed chamber where high steam pressure is maintained. Autoclaving is usually done at 15 lb/in. at 121 °C for 15 min (Chen and Yue 2005). Autoclaving kills all the microbes giving infection free product.

Following precautions should be taken into consideration before culturing algae.

- Laminar flow should be turned on before the start of the culture work.
- The working surface should be cleaned with 70 % ethanol.
- The Bunsen burner should be used.
- After organizing all requirements under the laminar flow, hands should be cleaned with 70 % ethanol.
- All the sterile pipette, loop or any material which are going to be used in culture should be flamed and it should be cooled before used.
- All the rim of the test tubes, petriplates should be flamed and cooled down before use.
- The loop or pipette should be cleaned after every use.

3 Preparation of Stock Solutions

Media are generally composed of three components macronutrients, trace elements and vitamins. All the three components are termed as stock solutions. Stock solutions for each and every component are made separately. The preparation of stock solutions is important because it reduces the number of repetitive chemical measurements involved in media preparation. Moreover direct weighing of media components that are required only in milligram or microgram (micronutrients or vitamins) in final formulation cannot be measured accurately. Therefore concentrated stock solutions for such components are prepared and are subsequently diluted to the final media concentration. In addition, concentrated solutions of some chemicals are more stable and can be stored for longer periods than the dilute solutions. Stock solutions are generally prepared in quantities of 100 mL to 1 liter depending on the amount of uses in final medium.

To prepare stock solutions clean flasks of various sizes are taken. Desire amount of chemicals are weighed and dissolved it in the flask containing required volume of distilled or deionised water. The flask is stirred continuously till the component is completely dissolved. If more than one component has to be added in a flask in case of trace metal solution then make sure that the first component is completely dissolved before adding the second one and so on. Some chemicals are easily dissolved while stirring but those chemicals which are not dissolved easily should be given a warm heat. Once the chemicals are fully dissolved the final volume of the stock solutions are made up to the desired volume with distilled or deionized water. The sock solutions are stored at 4 °C in a tightly sealed glass bottle or plastic bottle so that the final concentration of the solution does not change.

The sock solution for macronutrient is always prepared separately for each chemical. But for trace metal some chemicals are added together in one solution. Some of the common media used for microalgae cultures is given below.

3.1 Medium for Bold Basal (Bold 1949; Bischoff and Bold 1963)

Bold Basal medium (BBM) are used for the culture of fresh water algae. This medium can be prepared using the following components given in Table 1 in distilled water. Ten milliliters each from macronutrients, 1 mL each from EDTA, Iron, Boron and Trace Metal Solution are taken and made the volume up to 1 liter by distilled water. The pH of the culture is maintained at 6.5 for the optimal growth of the culture. Solid agar based medium are prepared by using 1.5 % agar in the liquid medium. The medium is autoclaved before use (Chen and Yue 2005).

Table 1 Bold basal medium composition

Component	Stock solution (gL^{-1} dH_2O)	Quantity to be used (mL)	Final concentrations (M)
Macronutrients			
$NaNO_3$	25.00	10	2.94×10^{-3}
$CaCl_2$ $2H_2O$	2.50	10	1.70×10^{-4}
$MgSO_4$	7.50	10	3.04×10^{-4}
K_2HPO_4	7.50	10	4.31×10^{-4}
KH_2PO_4	17.50	10	1.29×10^{-3}
NaCl	2.50	10	4.28×10^{-4}
EDTA solution		**1**	
EDTA	50.00		1.71×10^{-4}
KOH	31.00		5.53×10^{-4}
Iron solution		**1**	
$FeSO_4.7H_2O$	4.98		1.79×10^{-5}
H_2SO_4		**1**	
Boron solution		**1**	
H_3BO_3	11.42		1.85×10^{-4}
Trace metals solution		**1**	
$ZnSO_4.7H_2O$	8.82		3.07×10^{-5}
$MnCl_24H_2O$	1.44		7.28×10^{-6}
MoO_3	0.71		4.93×10^{-6}
Cu $SO_4.5H_2O$	1.57		6.29×10^{-6}
Co $(NO_3)_2.6H_2O$	0.49		1.68×10^{-6}

3.2 Chu 10 (Chu 1942)

Chu 10 is another medium used for the culture of various algae such as green algae, diatoms and cyanobacteria. It is an antificial medium where no chelators, vitamins and trace metals are added (Table 2). One milliliter each from component can be taken and make the volume up to 1 l by distilled water. The pH of the culture is maintained at 6.5.

3.3 Medium for Diatom (Cohn and Pickett-Heaps 1988; Cohn et al. 2003)

This is mainly for the culture of various diatom species. The medium uses soil extract. The following components are dissolved in 900 mL of dH_2O except vitamins. The final volume is brought up to 1 liter using dH_2O and sterilized. After cooling the solution vitamins are added and the pH is adjusted to 6.7. For the preparation of vitamin solution thiamine HCl is added into 950 mL of dH_2O. From all the stock solution 1 mL each is used and is brought to 1 liter. The solution is store frozen. The components of diatom medium are given in Table 3.

Table 2 Chu 10 medium compositions

Component	Stock solution ($g.L^{-1}$ dH_2O)	Quantity to be used (mL)	Final concentration (M)
$Ca(NO_3)_2$	40	1	2.44×10^{-4}
K_2HPO_4	5	1	2.87×10^{-5}
$MgSO_4.7H_2O$	25	1	1.01×10^{-4}
Na_2CO_3	20	1	1.89×10^{-4}
Na_2SiO_3	25	1	2.05×10^{-4}
$FeCl_3$	0.8	1	4.93×10^{-6}

Table 3 Compositions of diatom medium

Component	Stock solution ($g.L^{-1}$ dH_2O)	Quantity to be used (mL)	Final concentration (M)
$Ca(NO_3)_2.4H_2O$	70.85	1	3.00×10^{-4}
K_2HPO_4	54.44	1	4.00×10^{-4}
$MgSO_4.7H_2O$	24.65	1	1.00×10^{-4}
Na_2SiO_3 (27 % aq. sat. soln.)	20 mL pH 8.5	5	$\sim 3.00 \times 10^{-4}$
$FeSO_4.7H_2O$	0.278	1	1.00×10^{-6}
$MnCl_2.4H_2O$	0.02	1	1.00×10^{-7}
Soil extract	–	50	–
Vitamins solution		**1 mL**	
Thiamine HCl (Vitamin B_1)	–	1 g	2.97×10^{-6}
Biotin (Vitamin H)	–	1 g	4.09×10^{-6}
Nicotinic acid (niacin)	–	1 g	8.12×10^{-6}
Cyanocobalamin (Vitamin B_{12})	1	1 mL	7.38×10^{-10}

3.4 Medium for Volvox *(Provasoli and Pintner 1960)*

This medium is mainly for the culture of *Volvox* species. *Volvox* medium is also used for the culture of some strains of *Eudorina*, *Pandorina* and *Gonium*. In 900 mL of dH_2O calcium nitrate and glycylglycine are dissolved followed by other stock solutions. Vitamins are later added. The details components of *Volvox* medium are given below in Table 4.

3.5 Medium for BG-11 (Allen 1968; Allen and Stanier 1968; Rippka et al. 1979)

BG-11 (Blue Green Algae Medium) is mainly for the culture of Cyanobacteria. It is primarily used for freshwater, soil and marine organisms which do not require high ionic strength (Table 5). One milliliter each of the components and the trace metal was taken. The volume was made up to 1 liter by distilled water. The pH of the culture was maintained at 7.5 for the optimal growth of the culture and autoclaved.

Table 4 *Volvox* medium

Component	Stock solution ($g.L^{-1}dH_2O$)	Quantity to be used	Final concentration (M)
Glycylglycine	–	500.0 mg	2.46×10^{-3}
$Ca(NO_3)_2.4H_2O$	–	117.8 mg	5.00×10^{-4}
Na_2 ß-glycerophosphate $5H_2O$	50.0	1 mL	1.63×10^{-4}
$MgSO_4\ 7H_2O$	40.0	1 mL	1.62×10^{-4}
KCl	50.0	1 mL	6.71×10^{-4}
Biotin (Vitamin H)	0.0001	1 mL	4.09×10^{-10}
Cyanocobalamin (Vitamin B_{12})	0.0001	1 mL	7.38×10^{-11}
PIV trace metals solution		**3 mL**	
HOEDTA	–	1.398 g	1.22×10^{-5}
$FeCl_3.6H_2O$	–	0.194 g	2.15×10^{-6}
$MnCl_2.4H_2O$	–	0.036 g	5.46×10^{-7}
$ZnCl_2$	–	0.104 g	2.29×10^{-7}
$Na_2MoO_4.2H_2O$	–	0.013 g	1.56×10^{-7}
$CoCl_2.6H_2O$	4.04	1 mL	5.09×10^{-8}

HOEDTA Hydroxyethyl ethylenediamine triacetic acid

Table 5 BG-11 compositions

Component	Stock solution ($g.\ L^{-1}\ dH_2O$)	Quantity used (mL)	Final concentration (M)
Citric acid	6.0	1.0	3.12×10^{-5}
Ferric ammonium citrate	6.0	1.0	3.00×10^{-5}
$NaNO_3$		1.5 g	1.76×10^{-2}
$K_2HPO_4.3H_2O$	40.0	1.0	1.75×10^{-4}
$MgSO_4.7H_2O$	75.0	1.0	3.04×10^{-4}
$CaCl_2.2H_2O$	36.0	1.0	2.45×10^{-4}
Na_2CO_3	20.0	1.0	1.89×10^{-4}
$MgNa_2EDTA.H_2O$	1.0	1.0	2.79×10^{-6}
Trace metals solution			
H_3BO_4		2.86 g/L	4.63×10^{-5}
$MnCl_2.4H_2O$		1.81 g/L	9.15×10^{-6}
$ZnSO_4.7H_2O$		0.22 g/L	7.65×10^{-7}
$Na_2MoO_4.2H_2O$		0.39 g/L	1.61×10^{-6}
$CuSO_4.5H_2O$	79.0	1.0	3.16×10^{-7}
$Co(NO_3)_2.6H_2O$	49.4	1.0	1.70×10^{-7}

3.6 *Medium for* Spirulina *(Aiba and Ogawa 1977; Schlösser 1994)*

The main components of *Spirulina* medium are given below in Table 6. Solutions I and Solutions II are prepared separately. In case of solution II 1 mL of trace metals solution are added. The two solutions are autoclaved separately and allow them to cool. The two solutions are combined aseptically and after that 1 mL of cyanocobalamin (B_{12}) is added.

4 Isolation Methods of Algal Samples

The techniques for the isolation of microalgae first started by Beijerinck (1890). Water samples are collected in clean bottles and kept at lower temperature so that the cells remain viable. Techniques such as filtration, differential centrifugation,

Table 6 Compositions of *Spirulina* medium

Component	Stock solution (g. L^{-1} dH_2O)	Quantity used (mL)	Final concentration (M)
Solution I	**500 mL**	–	–
$NaHCO_3$	–	13.61 g	1.62×10^{-1}
Na_2CO_3	–	4.03 g	3.80×10^{-2}
K_2HPO_4	–	0.50 g	2.87×10^{-3}
Solution II	**500 mL**	–	-
$NaNO_3$	–	2.5 g	2.94×10^{-2}
K_2SO_4	–	1.0 g	5.74×10^{-3}
NaCl	–	1.0 g	1.71×10^{-2}
$MgSO_4.\ 7H_2O$	–	0.2 g	8.11×10^{-4}
$CaCl_2.2H_2O$	–	0.04 g	2.72×10^{-4}
$FeSO_4.\ 7H_2O$	–	0.01 g	3.60×10^{-5}
$Na_2EDTA.\ 2H_2O$	–	0.08 g	2.15×10^{-4}
$MgNa_2EDTA.H_2O$	1.0	1.0	2.79×10^{-6}
Trace metals solution		**1 mL**	–
$Na_2EDTA.\ 2H_2O$	–	0.8 g	2.15×10^{-6}
$FeSO_4.\ 7H_2O$	–	0.7 g	2.52×10^{-6}
$ZnSO_4.7H_2O$	1.0	1 mL	3.48×10^{-9}
$MnSO_4.\ 7H_2O$	2.0	1 mL	8.97×10^{-9}
H_3BO_3	10.0	1 mL	1.62×10^{-7}
$Co(NO_3)_2.6H_2O$	1.0	1 mL	3.44×10^{-9}
$Na_2MoO_4.2H_2O$	1.0	1 mL	4.13×10^{-9}
$CuSO_4.5H_2O$	0.005	1 mL	2.00×10^{-11}
Vitamin solution		1 mL	
Cyanocobalamin (Vitamin B_{12})	–	5 mg	3.69×10^{-9}

micropipetting, serial dilution and agar streaking are used for isolation and purification of the algal samples (Richmond 2003).

4.1 Filtration

The water samples are filtered through two layered muslin cloth or in a sieve, strainer, net of different size. In this separation method large particles and debris are discarded (Clark and Sigler 1963) to make the sample free from unwanted particles.

4.2 Differential Centrifugation

Centrifugation is the separation of larger and heavier cells from smaller cells such as algae and bacteria. But mild centrifugation for a short duration helps large dinoflagellates and diatoms to settle at the bottom forming a loose pellet. The process can be repeated several times if necessary. The centrifugation speed and time vary from species to species (Watanabe et al. 1998). This method allows the larger algae to settle at the bottom of the tube but is very difficult to obtain a unialgal culture; therefore further techniques for purifications are required.

4.3 Micropipette Isolation

This is the most commonly practiced method for the isolation of microalgae. The micropipette isolation is usually carried out with glass capillary. A few drops of water sample containing algal mixtures are placed in the cavity slide and observed under a microscope. The desired algal species is focused under the microscope and the algal cells are picked up from the side carefully into the capillary. This isolated algal cell is transferred to a drop of sterile medium. This process can be repeated several times with sterilized capillary to obtain reasonable concentration of algal cells to obtain proper growth on agar plate (Andersen and Kawachi 2005).

4.4 Serial Dilution Technique

Serial dilution technique is another method for the isolation of unialgal cells. In this technique a known volume of sample is mixed with distilled water. This technique reduces the concentration of the sample. In this process ten test tubes are taken and 9 mL of media are added in all the ten test tubes. The test tubes are labelled as 10^{-1}–10^{-10} indicating dilution factor. 1 mL of the sample is added to the first test

tube labelled as10^{-1} and mixed gently. From this test tube 1 mL of the sample is taken and added to the second test tube represented as 10^{-2} and mixed gently. The above procedure is repeated for the remaining tubes (10^{-3}–10^{-10}). The test tubes are maintained under normal temperature and light conditions. The cultures are examined periodically under the microscope.

4.5 Streaking Cells Across Agar Plates

A loop is loaded with a small amount of sample and then the sample is spread with the loop across the medium supplemented with 1.5 % agar (Heaney and Jaworski 1977). The origin of the streak typically has a mixture of algae that are not separated, but as the distance from the origin increases, single cell begins to separate. When the colonies started appearing, the cells are again taken from the agar plates with a loop and then restreaked on a new agar plate (Richmond 2003). This process can be repeated several times until a pure culture is obtained.

4.6 Raising of Mass Culture

Once the pure cultures are obtained, then the cultures are transferred to both into the liquid and solid media to develop small scale culture. After inoculation the culture experience an adaptation phase called the lag phase where the algae trys to stabilize the culture condition. At this stage the cells could not able to divide. When the cultures are allowed to grow continuously, a slight colour change can be noted. The cultures are light green indicating that the algal cells enter another stage where the algae started dividing. This stage is called the exponential stage (Fig. 1a–d). once the culture attained full growth, it is necessary to subculture so that algae maintain in its healthy state. Aliquot of algal cells can be aseptically transferred into a flask of freshly prepared medium and is cultivated under the same culture condition (Devi 2008).

5 Antibiotic Treatment

In order to obtain pure unialgal cells antibiotic treatment is sometimes necessary. Some of the common antibiotics used in the microalgal culture are penicillin, streptomycin and gentamycin. It diminishes the bacterial growth without affecting the algal growth. The concentration of antibiotics used in the medium differ from one species to another as the capability to tolerate the level of antibiotic concentration vary. A range of 50–500 mg/l concentrations is generally used. Krauss (1962) also presented data showing that streptomycin is inhibitory to a wide range of algae

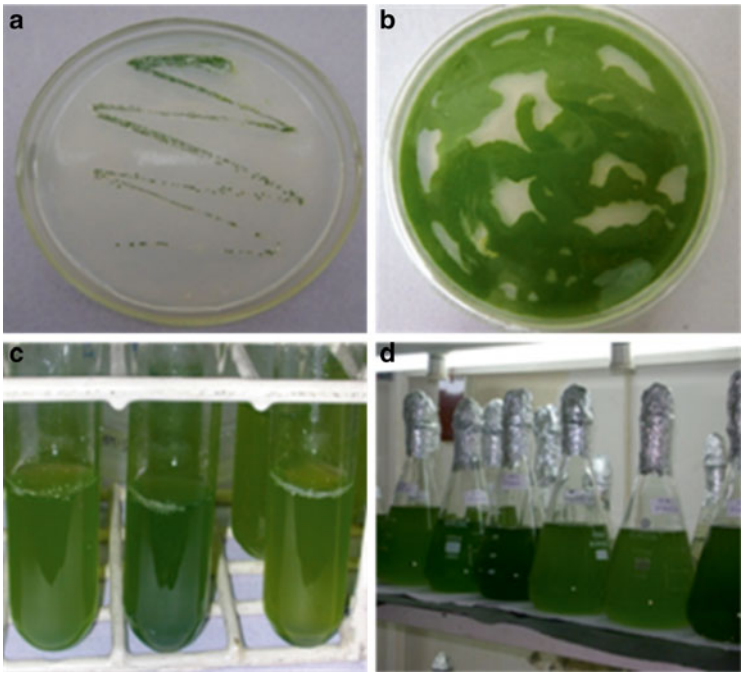

Fig. 1 (**a**–**d**) Various steps of Microalgal culture. (**a**) Streaking (**b**) Unialgal culture in solid medium (**c**) Unialgal culture in liquid medium (**d**) Raising of mass culture in the laboratory

between 1 and 40/μg/mL. Various combinations of broad-spectrum antibiotics such as chloramphenicol, tetracycline, streptomycin and neomycin are used for short exposure periods at various low concentrations, together with benzyl penicillin which is effective only against Gram-positive bacteria (Jones et al. 1973). The common antibiotics used in the algal culture are chloramphenicol, tetracycline, and bacitracin. The antibiotics prepared for the used in the algal culture are preserved in frozen condition until use.

6 Modern Microalgal Isolation Method

Some automated isolation method for microalgae has been developed as the earlier method needs skill and time consuming. Not only this it is very difficult to isolate manually a very small algal cells (Li 2002). Flow cytometry is another automatic isolation method for cell sorting (Fig. 2). It is a laser based technology mainly developed for cell counting, single cell isolation by suspending cells in a stream of fluid. The main principle involved in Flow cytometry is light scattering, excitation and emission of fluorochrome molecules to generate specific multi-parameter data from particles and cells in the size range of 0.5–40 μm in diameter.

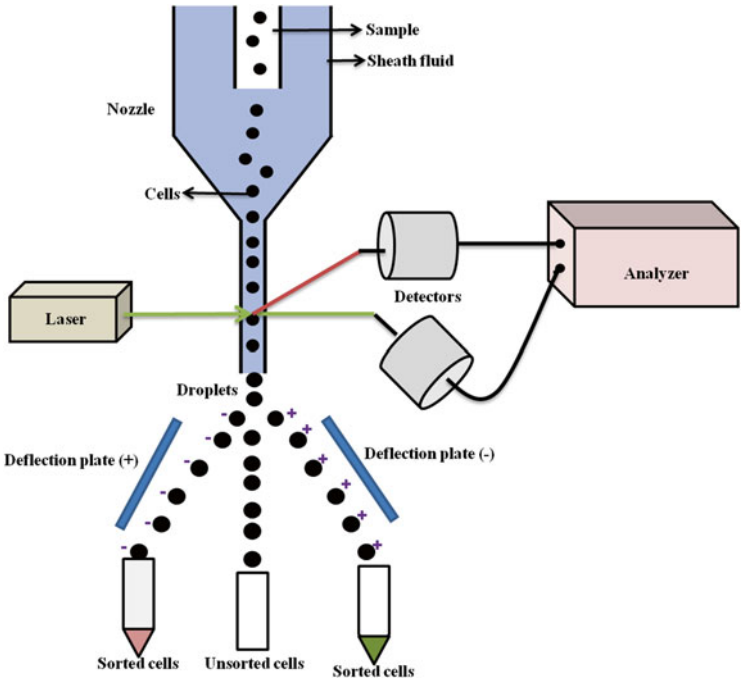

Fig. 2 Schematic diagram of flow cytometry

The light source used in flow cytometry are mainly lasers as lasers produce a high intensity of monochromatic light. Lasers have a very small size hole to focus light so that large single cells are excited thereby reducing the chance of more than one cells.

When the cells intercept a light source they scatter light and fluorochromes are excited to a higher energy state. The light which are scattered and emitted from cells are converted to electrical pulses with the help of optical detectors. Confocal lenses picked up all the collimated light and are passed to different detectors. Photomultiplier tube (PMT) is the most common type of detector used in flow cytometry. Logarithmic amplification is most often used to measure fluorescence in cells which help in expansion of weak signals and compression of strong signals. The data of flow cytometry are stored in computer files. Haugen et al. (1987) tested four fragile algae (*Chroomonas*, *Micromonas*, *Tetraselmis*, and *Gyrodinium*) for possible damaging effects by flow cytometry and neither flow cytometric fluidics nor laser exposure caused cell loss or inability to grow. Several authors have reported successful isolation of microalgae by flow cytometric sorting. Sensen et al. (1993) started unialgal cultures of *Cyanophora*, *Haematococcus*, *Monomastix*, *Scherffelia*, and *Spermatozopsis* (Andersen 2005).

7 Outdoor Cultivation of Microalgae

For the mass cultivation of microalgae, they can be grown either in the open or closed systems. In early and late 1970s, the production of algae was initiated in East Europe, Israel and Japan and the algae were mainly cultured in the open ponds for food. Open ponds are of two types namely natural lakes, lagoons or ponds and artificial system. Of these the open ponds which include raceway tanks or circular ponds etc. are very common because they are easier to handle. The major benefit of open system is that they are easy to handle than the other systems. But the raceway pond faces some disadvantages such as requirement of large land areas, evaporation looses contamination etc. At the same time mixing of the culture is very difficult in open systems thereby producing less biomass.

7.1 Raceway Ponds

Raceways are oval shaped, closed loop which are filled with water up to certain level so that the culture can be mixed and circulated properly. In raceway ponds, paddlewheels are used to maintain constant mixing of the algae. This wheel works continuously to avoid any settlement below the pond. They are built in concrete and covered with white plastic. Raceway ponds for mass culture of microalgae have been used since the 1950s. The largest raceway-based biomass production facility occupies an area of 440,000 m^2 (Spolaore et al. 2006) (Fig. 3).

Fig. 3 Microalgae cultivation in the field (image courtesy © D.Sahoo)

7.1.1 Advantages of Open Culture

Compare to closed system, open system has the following advantages such as

- Open culture system is cheaper.
- The open system does not require existing agricultural land, since they can be established in wasteland.
- Energy requirement is low.
- Regular maintenance and cleaning is much easier in open culture system.

7.2 Closed Culture System

The closed culture system for microalgae is usually carried out by using photobioreactors. Photobioreactors are of various types such as tubular, flat panel or column reactors (Janssen et al. 2002; Carvalho et al. 2006). They used external light supplies for their growth.

7.2.1 Tubular Photobioreactor

Tubular photobioreactors used long, transparent tubes which can be horizontal (Carlozzi et al. 2006), vertical (Converti et al. 2006; Perner-Nochta et al. 2007), inclined (Vunjak-Novakovic et al. 2005), or as a helix (Hall et al. 2003). Mechanical pumps or airlifts create the pumping force which allow CO_2 and O_2 to be exchanged between the liquid medium and the aeration gas (Hall et al. 2003; Converti et al. 2006), while almost no gas-exchange takes place in the tubes (Fig. 4).

Fig. 4 A tubular photobioreactor used for growing microalgae in a power plant (image courtesy © D. Sahoo)

7.2.2 Flat Panel Photobioreactors

In case of flat panel photobioreactors the algal culture is mixed across the flat panel system (Richmond et al. 2003). The light is absorbed by the algae which are above the culture. They are good for immobilization of algae. It has been reported that with flat-panel photobioreactors, high photosynthetic efficiencies can be achieved.

7.2.3 Column Photobioreactors

Column photobioreactors are reactors which can be in vertical position or can be bubbled from below. The light are passed from the transparent walls through the culture. This source of light can be given internally (Suh and Lee 2001). This type of photobioreactors give a very good mixing of the culture, the rate of gas transfer are very high.

8 Seaweed (Macro algae) Culture in the Laboratory

Seaweeds are macroscopic, multicellular benthic marine algae mainly found growing in the sea and brackish water. They comprise of green, brown and red algae. Seaweeds collected from the field are brought to the laboratory. They are cleaned manually by removing attached epiphytes and epifauna. To maintain a culture of seaweeds vegetative tissue or the spores are used. The cultures are kept in such chambers where the temperature, light can be manipulated. Direct use of natural sea water for macroalgal culture is seldom acceptable as the growth of the macroalgae is very slow. But for large scale cultivation the macroalgae are usually cultured in natural seawater (Fig. 5). For laboratory culture it is necessary to supply nutrients and other trace metal in the media for the growth. Thus for the culture of seaweeds in the laboratory artificial sea water is used along with various other nutrients required for the proper growth of the desired species (Fig. 6). To observe the development of the thallus it is very important to culture the algae of interest in the artificial conditions. But it is always difficult to culture the desire macroalgae in natural seawater. By improving the culture media composition algal culture of macroalgae became widely studied in 1960s (Bold 1942; Tatewaki 1966).

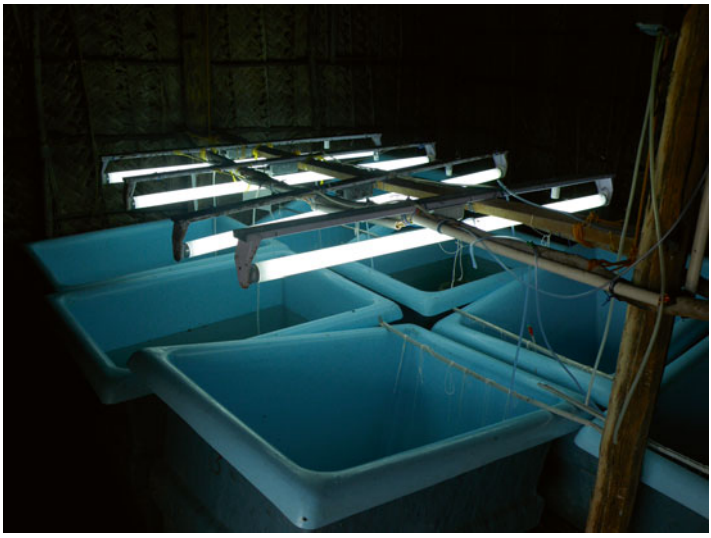

Fig. 5 Tank culture of seaweed for pilot cultivation

Fig. 6 Laboratory culture of seaweed

9 Isolation and Culture

Macroalgae have high potential for regeneration. Therefore the unialgal culture of macroalgae is generally practiced by cutting off the vegetative cells. Algal cultures can also be developed from zoospores and planogametes, zygotes, carpospores, tetraspores, or aplanospores (Kawai et al. 2005). Macroalgae are cultured in a sterilized glass or petridishes filled with enriched medium which are covered with parafilm. After every 2–4 weeks or depending on the material and temperature culture media are changed. If the culture was still contaminated the isolation processes are repeated several times.

But before the start of any culture work, the thalli are allowed to acclimatize for 4–5 days in sterilized seawater with desired salinity. The thalli are weighed properly and are cut into small pieces of 2–3 cm. They are transferred into 500 mL of cleaned conical flask containing 200 mL of sterilized liquid media. The most commonly used culture media for macroalgae is F/2 medium and PES medium.

Direct use of natural sea water is not acceptable for the culture of macroalgae as natural seawater contains various elements and lots of organic compounds which are not always favored for the growth of macroalgae. So it is very much necessary to add extra nutrients and trace metals to make better growth of the algae. Therefore artificial sea water is enriched by adding macronutrients, trace elements and vitamins for the laboratory culture of macroalgae. Compositions of some of the common marine media are given below.

9.1 Aquil or Artificial Seawater Media (Morel et al. 1979; Price et al. 1989)

Aquil is an artificial seawater medium that has been widely used with various modifications and supports the growth of many marine taxa. The details compositions of Aquil modified medium is given in Table 7. To prepare the media in 600 mL of distilled water each of the anhydrous salts is dissolved individually. In another 300 mL of distilled water each of the hydrous salts are dissolved individually. The two solutions are combined and 1 mL of trace metal solution and 1 mL of vitamins solution are added. The final salinity is maintained at 35 psu.

9.2 Medium for Von Stosch (Grund) (Guiry and Cunningham 1984)

Von Stosch (1963) modified enriched seawater medium from Grund medium. This medium is used by Guiry and Cunningham (1984) in the culture of *Gigartina* and various other red seaweeds. Nine hundred fifty milliliters of filtered natural seawater is taken and 10 mL of each of the following stock solutions given in Table 8 are added aseptically.

Table 7 Composition of aquil medium

Component	Stock solution (gL^{-1} dH_2O)	Quantity to be used	Final concentration (M)
Anhydrous salts			
NaCl	—	24.540 g	4.20×10^{-1}
Na_2SO_4	—	4.090 g	2.88×10^{-2}
KCl	—	0.700 g	9.39×10^{-3}
$NaHCO_3$	—	0.200 g	2.38×10^{-3}
KBr	—	0.100 g	8.40×10^{-4}
H_3BO_3	—	0.003 g	4.85×10^{-5}
NaF	—	0.003 g	7.15×10^{-5}
Hydrous salts			
$MgCl_2.\ 6H_2O$	—	11.100 g	5.46×10^{-2}
$CaCl_2.\ 2H_2O$	—	1.540 g	1.05×10^{-2}
$SrCl_2.\ 6H_2O$	—	0.017 g	6.38×10^{-5}
Major nutrients (In 900 mL of distilled water 1 mL each stock solutions are added and final volume is brought to 1 liter)			
$NaH_2PO_4.\ H_2O$	1.38	1 mL	1.00×10^{-5}
$NaNO_3$	85.00	1 mL	1.00×10^{-4}
$Na_2SiO_3.\ 9H_2O$	28.40	1 mL	1.00×10^{-4}
Trace metal stock solution (In 900 mL of distilled water, EDTA is dissolved and 1 mL each stock solutions are added and final volume is brought to 1 liter)			
EDTA (anhydrous)	—	29.200 g	1.00×10^{-5}
$FeCl_3.\ 6H_2O$	—	0.270 g	1.00×10^{-6}
$ZnSO_4.\ 7H_2O$	—	0.230 g	7.97×10^{-8}
$MnCl_2.\ 4H_2O$	—	0.0240 g	1.21×10^{-7}
$CoCl_2.\ 6H_2O$	—	0.0120 g	5.03×10^{-8}
$Na_2MoO_4.\ 2H_2O$	—	0.0242 g	1.00×10^{-7}
$CuSO_4.\ 5H_2O$	4.9	1 mL	1.96×10^{-8}
Na_2SeO_3	1.9	1 mL	1.00×10^{-8}
Vitamin stock solution (In 900 mL of distilled water, thiamine HCl is dissolved and 1 mL each stock solutions are added and final volume is brought to 1 liter)			
Thiamine. HCl (vitamin B1)	–	100 mg	2.97×10^{-7}
Biotin (vitamin H)	5.0	1 mL	2.25×10^{-9}
Cyanocobalamin (vitamin B12)	5.5	1 mL	3.70×10^{-10}

Table 8 Components of Von Stosch (grund) medium

Component	Stock solution (gL^{-1} dH_2O)	Quantity used	Concentration in final medium (M)
Na_2 ß-glycerophosphate	5.36	10 mL	2.48×10^{-4}
$NaNO_3$	42.52	10 mL	5.00×10^{-3}
$FeSO_4.\ 7H_2O$	0.28	10 mL	1.00×10^{-5}
$MnCl_2.\ 4H_2O$	1.96	10 mL	1.00×10^{-4}
$Na_2EDTA.\ 2H_2O$	3.72	10 mL	1.00×10^{-4}
Vitamin stock solution (In 950 mL of distilled water, thiamine HCl is dissolved and 1 mL each stock solutions are added and final volume is brought to 1 liter)			
Thiamine. HCl (vitamin B1)	–	200 mg	5.93×10^{-6}
Biotin (vitamin H)	0.1	1 mL	4.09×10^{-9}
Cyanocobalamin (vitamin B12)	0.2	1 mL	1.48×10^{-9}

Table 9 Composition of f/2 medium

Component	Stock solution (g. L^{-1} dH_2O)	Quantity used (mL)	Final concentration (M)
$NaNO_3$	75	1	8.82×10^{-4}
NaH_2PO_4. H_2O	5	1	3.62×10^{-5}
Na_2SiO_3. $9H_2O$	30	1	1.06×10^{-4}
Trace metal solution		1	
Vitamin solution		0.5	
f/2 trace metal solution			
950 mL of dH_2O is taken and EDTA and other components are added making the final volume to 1 liter with distilled water			
$FeCl_3$. $6H_2O$		3.15 g	1.17×10^{-5}
Na_2EDTA. $2H_2O$		4.36 g	1.17×10^{-5}
$CuSO_4$. $5H_2O$	9.8	1	3.93×10^{-8}
Na_2MoO_4 $2H_2O$	6.3	1	2.60×10^{-8}
$ZnSO_4$. $7H_2O$	22.0	1	7.65×10^{-8}
$CoCl_2$. $6H_2O$	10.0	1	4.20×10^{-8}
$MnCl_2$. $4H_2O$	180.0	1	9.10×10^{-7}
f/2 vitamin solution			
950 mL of dH_2O is taken and dissolved thiamine. HCl add 1 mL each of the stocks and bring final volume to 1 liter with dH_2O. The final solutions are stored in the freezer			
Thiamine HCl (vitamin B1)		200 mg	2.96×10^{-7}
Biotin (vit. H)	1.0	1	2.05×10^{-9}
Cyanocobalamin (vitamin B12)	1.0	1	3.69×10^{-10}

9.3 F/2 Medium (Guillard and Ryther 1962; Guillard 1975)

f/2 medium is widely used enriched seawater medium for the culture of marine algae (Table 9).

9.4 Provasoli Enriched Seawater (PES) Medium (Provasoli 1968)

To prepare the Provasoli Enriched Seawater (PES) medium 900 mL of distilled water is taken and to this the compositions are added. The final volume is made up to 1 liter with distilled water (Table 10).

Table 10 PES stock solution

Component	Stock solution (gL^{-1} dH_2O)	Quantity used	Concentration in final medium (M)
Tris base	—	5.0 g	8.26×10^{-4}
$NaNO_3$	—	3.5 g	8.24×10^{-4}
Na_2 b-glycerophosphate·H_2O	—	0.5 g	4.63×10^{-5}
Iron–EDTA solution	(See following recipe)	250 mL	—
Trace metals solution	(See following recipe)	25 mL	—
Thiamine · HCl (vitamin B1)	—	0.500 mg	2.96×10^{-8}
Biotin (vitamin H)	0.005	1 mL	4.09×10^{-10}
Cyanocobalamin (vitamin B12)	0.010	1 mL	1.48×10^{-10}
Iron–EDTA solution			
Into 900 mL of dH_2O, EDTA is dissolved followed by iron sulphate and the final volume is brought to 1 liter			
$Na_2EDTA·2H_2O$	—	0.841 g	1.13×10^{-5}
$Fe(NH_4)_2(SO_4)_2·6H_2O$	—	0.702 g	8.95×10^{-6}
Trace metals solution			
Into 900 mL of dH_2O, EDTA is dissolved and then individually dissolved the following components. The final volume is brought to 1 liter			
$Na_2EDTA·2H_2O$	—	12.74 g	1.71×10^{-4}
FeCl3·6H2O	—	0.484 g	8.95×10^{-6}
H_3BO_3	—	11.439 g	9.25×10^{-5}
$MnSO_4·4H_2O$	—	1.624 g	3.64×10^{-5}
$ZnSO_4·7H_2O$	—	0.220 g	3.82×10^{-6}
$CoSO_4·7H_2O$	—	0.048 g	8.48×10^{-7}

10 Treatment of Thalli in Disinfectant Series

Before the start of culture, the thalli are treated with various disinfectant series so that the culture is free from contamination. For disinfectant series the thalli are treated with 1 % IKI solution (Markham and Hasmeier 1982) for 1 min to eliminate surface microbes. Sterilization is done by using broad spectrum antibiotic mixture (Polne-Fuller and Gibor 1984) to prevent the growth of bacteria. The sterilized healthy thalli are cultured in 300 mL of autoclaved seawater in a flask. To this 1 % of GeO_2 (Germinium dioxide) is added (Markham and Hasmeier 1982) to prevent the growth of diatoms.

10.1 Preparation of 1 % GeO_2 (Markham and Hasmeier 1982)

Four grams of sodium hydroxide (NaOH) is boiled and 250 mg of GeO_2 is added all at one time. The mixture is stirred properly with glass rod so that the compounds are dissolved properly and the solution is kept for cooling. pH is adjusted to 7.8–8.0 by 1 N HCl. The final volume is brought up to 250 mL with distilled water. From this, 1 mL is used in 100 mL of culture medium.

10.2 Preparation of 1 % IKI Solution (Markham and Hasmeier 1982)

In 300 mL of distilled water 2 g of potassium iodide (KI) and 1 g of iodine is dissolved. 1 mL of IKI solution is taken and added in 250 mL of sterilized seawater.

11 Preparation of Broad Spectrum Antibiotics (Polne-Fuller and Gibor 1984)

The following components are dissolved in 100 mL of distilled water and filter sterilized. Dissolve Nystatin in DMSO separately and add after filter sterilization.

Component	Quantity used
Penicillin G	1 g
Streptomycin sulphated	2 g
Kannamycin	1 g
Nystatin	25 mg
Neomycin	200 mg

There are different microalgal culture collection centres located all over the world from where the pure microalgal culture can be obtained. Some of the important microalgal culture collection centres are given below in Table 11.

Table 11 List of microalgal culture collections around the world

Collection centres	Name	Country
Academy of Sciences of the Czech Republic, Institute of Botany (CCALA)	Czech Collection of Algae and Cyanobacteria (http://ccala.butbn.cas.cz/)	Praque
Centre for Ecology and Hydrology (CCAP)	Culture Collection of Algae and Protozoa (http://www.ccap.ac.uk/)	Scotland
Chinese Academy of Sciences, Culture Collection Committee (CTCCCAS)	Freshwater Algae Culture Collection (http://www.ctcccas.ac.cn/typecc/danshui/en.html)	China
CSIRO, Marine research (CS)	CSIRO Collection of Living Microalgae (http://www.marine.csiro.au/algaedb/search.htm)	Hobart, Tasmania
Charles University Prague, Department of Botany (CAUP)	Collection of Algae of Charles University Prague (http://botany.natur.cuni.cz/algo/caup-list.html)	Praque
Duke University, Chlamydomonas Genetics Center (CGC)	Chlamydomonas Genetics Center Collection (http://www.chlamy.org/strains.html)	USA
Institut Pasteur, Paris (PCC)	Pasteur Culture Collection of Cyanobacteria (http://www.pasteur.fr/ip/easysite/pasteur/en/research/collections/crbip/general-informations-concerning-the-collections/iv-the-open-collections/iv-iii-pasteur-culture-collection-of-cyanobacteria)	Paris
Loras College, Iowa, USA	The Loras College Freshwater Diatom Culture Collection (http://www2.bgsu.edu/departments/biology/facilities/algae/html/DiatomCulture.html)	Lowa, USA
Thailand Network on Culture Collections (TNCC)	BIOTEC Culture Collection (http://www.1a.biotec.or.th/TNCC/dbstore/BCC_search.asp)	Thailand
Philipps-University Marburg, Department of Cell Biology and Applied Botany (CCAM)	Culture Collection of Algae Marburg (marburg.de/~cellbio/welcomeframe.html)	Germany
Provasoli-Guillard National Center for Culture of Marine Phytoplankton, ME (CCMP)	Provasoli-Guillard National Center for Culture of Marine Phytoplankton (https://ncma.bigelow.org/)	Maine
Station Biologique Roscoff, Phytoplancton (RCC)	Roscoff Culture Collection of Marine Phytoplancton (http://www.sb-roscoff.fr/Phyto/RCC/index.php)	France
University of Caen Basse, Laboratoire de Biologie et Biotechnologies Marines (ALGOBANK)	Algobank (http://www.unicaen.fr/algobank/accueil/)	

(continued)

Table 11 (continued)

Collection centres	Name	Country
University of Coimbra, Department of Botany (ACOI)	Coimbra Collection of Algae (http://www.uc.pt/botanica/ACOI.htm)	Portugal
University of Cologne, Institute of Botany (CCAC)	Culture Collection of Algae at the University of Cologne (http://www.ccac.uni-koeln.de/recherche.shtml)	Germany
University of Göttingen, Sammlung von Algenkulturen (SAG)	Culture Collection of Algae at the University of Göttingen (http://www.uni-goettingen.de/en/184982.htmlcgi-bin/epsag/website/cgi/show_page.cgi?kuerzel=start)	Germany
University of Texas at Austin, School of Biological Sciences (UTEX)	Culture Collection of Algae at the University of Texas at Austin (http://web.biosci.utexas.edu/utex/)	Austin, USA
University of Toronto, Department of Botany (UTCC)	University of Toronto Culture Collection of Algae and Cyanobacteria (http://www.botany.utoronto.ca/utcc/List%20of%20Cultures.html)	Toronto

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