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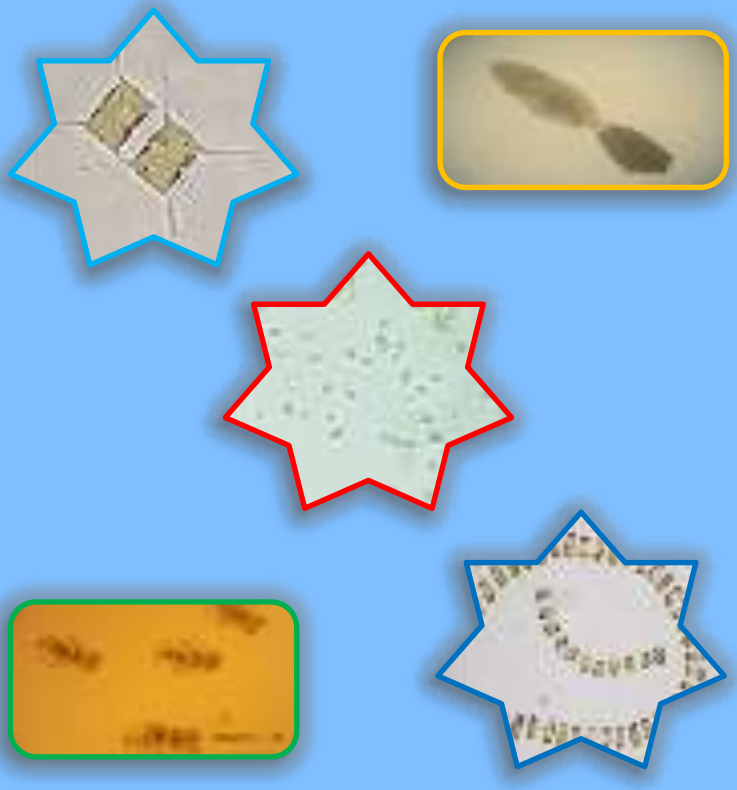
# Training Manual on and Shellfish Culture





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# Physico-chemical parameters for Micro algal culture

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## Introduction

Marine microalgae are unicellular in nature, which are the primary producers of the sea. Among microalgae, green algae, flagellate and diatom species, are the primary producers at the base of the marine food chain. It's an important source of nutrition and is used widely in the aquaculture of other aquatic organisms like finfish and shellfish, etc., either directly or as an added source of basic nutrients. They are cultured in hatcheries for larval rearing of molluscs, crustaceans and fish as a source of nutrition. In hatchery systems, micro algae is also added to the larval rearing tanks to improve 'quality' of water. The production of micro algae is very critical in successful hatchery management. Suitably treated seawater enriched with nutrients, like nitrates, phosphates, essential trace elements, vitamins and carbon dioxide is a prerequisite for any successful algal culture. High micro algal biomass with low bacteria content is important to support the growth of finfish or shellfish larvae. In this context Physio-chemical parameters and its management in algal culture systems plays a vital role in enhancing the survival, growth and production of cultivable animals.

## Parameters regulating algal growth

The most important parameters regulating algal growth are

1. Light
2. Temperature
3. Salinity
4. $p^H$ and
5. Aeration and mixing

All these factors are interdependent and a parameter that is suitable for one type of algae is not necessarily suitable for another type of algae.

**Table 1:** Optimal conditions for culture of micro algae (modified Anonymous, 1991).

| Parameters | Range | Optimum |
|---|---|---|
| Temperature (°C) | 16-27 | 18-24 |
| Salinity (g $L^{-1}$) | 12-40 | 20-24 |
| Light intensity (Lux) | 000-10000 | 2500-5000 |
| Photoperiod Hrs (light: dark) | 16:8 (min.) | 24:0 (max.) |
| $p^H$ | 7-9 | 8.0-8.5 |

**Light**

It is one of the important factors for successful microalgae culture, but the requirements vary with the species, culture depth and the density of the algal culture. For maintaining the stock cultures of all micro-algae 500 Lux (one tube light) is essential while for the mass culture containers 2000 - 10000 Lux is necessary.

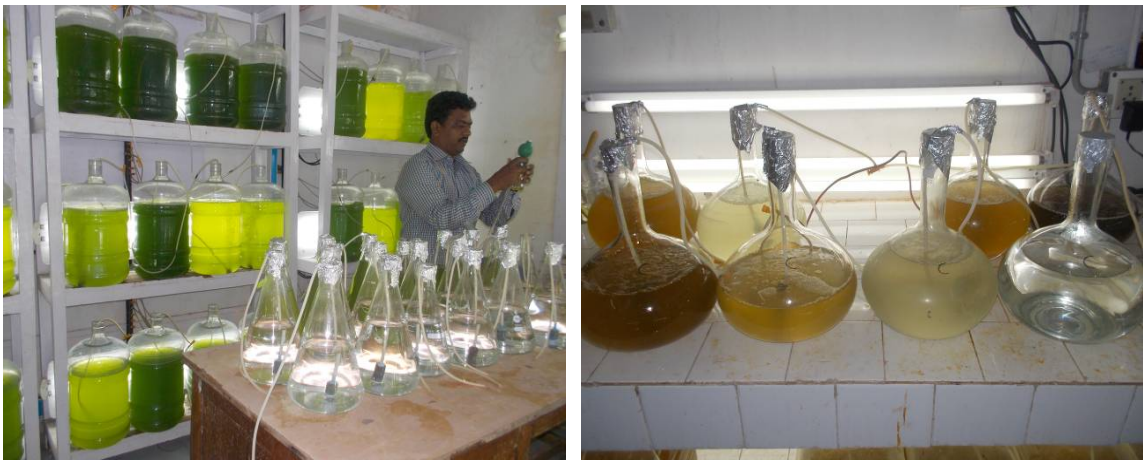

**Fig. 1. Illumination in indoor algal culture**

The light intensity must be increased and should be sufficient enough to penetrate through the culture, if cultured at higher depths, large volumes and higher cell concentrations (Example: Erlenmeyer flasks - 2000, Larger volumes - 5000-10000 Lux). Fluorescent white lights are mostly used in Indoor microalgal culture facilities, which can provide 2500 Lux, while outdoor systems and greenhouses gets ambient sunlight, if

needed fluorescent lights are used. Most of the flagellates require less light during the stationary and declining phase. Too much of light intensity will cause early declining of the culture. Fluorescent tubes should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be 16 h (min.) of light per day, although cultivated phytoplankton develops normally under constant illumination.

**Temperature**

The optimal temperature for micro-algae cultures will vary with species and is generally between 20 and 24°C. Most commonly cultured species of micro-algae from tropical/subtropical regions tolerate temperatures between 16 and 27°C. This may vary with the composition of the culture medium, the species and strain cultured. Temperatures lesser than 16°C will slow down growth, whereas those more than 35°C are lethal for a number of species. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with air - conditioning units.

**Salinity**

Marine phytoplanktons are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting seawater with freshwater. Salinities of 20-24 g $L^{-1}$ have been found to be optimal. But the salinity suitable for one algae may not be suitable for the other.

**$p^H$**

The $p^H$ range for most cultured algal species is between 7 and 9, with the optimum range being 8.0-8.5. Changes in the $p^H$ and culture condition due to precipitation of certain nutrients may lead to complete culture collapse. Reviving of the cultures affected by $p^H$ changes can be accomplished by aerating the culture. Carbon dioxide plays a dual role in microalgal culture.

**Table 2:** Temperature, light, and salinity range for culturing selected microalgae species.

| Species | Temperature (°C) | Light (Lux) | Salinity (ppt – ‰) |
|---|---|---|---|
| *Chaetoceros calcitrans* | 25 - 30 | 2000-10000 | 20 - 35 |
| *Isochrysis galbana* | 25 -30 | 2000-10000 | 10 – 30 |
| *Skeletonema costatum* | 10 - 27 | 2500-5000 | 15 – 30 |
| *Nannochloropsis oculata* | 20 - 30 | 2500-8000 | 12 - 30 |
| *Pavlova sp* | 15 -30 | 4000-8000 | 10 – 40 |
| *Tetraselmis sp* | 20 -28 | 5000-10000 | 20 - 40 |
| *Chlorella sp* | 10 -28 | 2500-5000 | 26 - 30 |
| *Thalassiosira sp* | 25 - 30 | 2000-10000 | 20 - 35 |

It provides a source of carbon to support photosynthesis, and it helps maintain $p^H$ at optimum levels. In the case of high-density algal culture, the addition of carbon dioxide allows to correct the increased $p^H$, which may reach up to $p^H$ 9 during algal growth.

**Aeration and mixing**

Similar to light and temperature, aeration is also important for developing and maintaining healthy cultures, as well as to enhance the exponential phase of growth of micro-algae for a few days more. Air circulation is important to avert sedimentation of the algae and thermal stratification in the culture medium. It is also necessary to ensure that all cells of the algae get sufficient light and nutrients. Moreover it helps in enhancing the gas exchange activity between the culture medium and the air. The significance of aeration is that it contains carbon source in the form of carbon dioxide from atmospheric air, which is very much essential for photosynthesis of micro algae. For high density cultures, the $CO_2$ originating from the air may not be sufficient for the algal growth and pure carbon dioxide may be supplemented to the air supply. $CO_2$ addition, moreover supports the water by buffering action against $p^H$ changes by maintaining the $CO_2/HCO_3^-$

balance. Based on the scale of the culture system and type of algal species, mixing of culture media can be done by daily hand stirring (test tubes, Erlenmeyers), aeration through air blower (bags, tanks), or by utilizing motor driven paddle wheels and jet pumps (ponds). However, it should be cautioned that few of the algal species can't tolerate vigorous mixing.

Turbidity in intake water can be reduced by passing through different filtration systems. The units other than algal culture don't require $CO_2$ in seawater. Hydrogen sulfide content should be nil in water used for hatcheries. $NH_3$ and $NO_2$ level in hatchery water should be below 0.1 mg $L^{-1}$ and 0.01 mg $L^{-1}$ respectively. Dissolved oxygen content in algal culture tanks should be above 5 mg $L^{-1}$. Alkalinity and $p^H$ are interrelated while maintaining $p^H$, alkalinity will also remain under safe limits in most cases. Generally, the total alkalinity level of 80 – 120 mg $L^{-1}$ is maintained in hatcheries. Water quality parameters like salinity, temperature, $p^H$ and light intensity should be checked regularly. Generally the water intake system for any hatcheries should be devoid of pesticides and other organic and inorganic pollutants. Pathogens get entry into hatcheries through improper water quality maintenance and improper water treatment systems. So an effective water treatment system is very much essential for every hatchery.

**Water Treatment**

The micro algal culture water should be free of suspended solids, plankton (e.g., protozoans, ciliates and other algae species), bacteria, dissolved metals, pesticides and any other unacceptably high concentrations of dissolved organic compounds (DOC). Therefore, one of the most important prerequisites in successful micro algal culture is the pretreatment of water. Various standard pretreatment methods typically available are mechanical and chemical methods, sterilization or disinfection. Moreover, the choice of treatment method should be mainly based upon the type of species cultured, volume requirements, and cost.

**Mechanical Filtration**

Mechanical filtration removes suspended solids, plankton and bacteria, protozoa or another species of algae, which is a serious problem for monospecific/axenic cultures of micro-algae. The type of mechanical filtration used depends on the condition of the incoming water and the volume of water to be treated.

1. Sand filters or polyester filter bags (20 to 35µm) for large quantity.
2. Cartridge filters (10, 5, 1 µm) or diatomaceous earth (DE) filters for medium quantity.
3. To remove bacteria using 0.22 or 0.45 µm membrane cartridge filters for small quantity.

**Heat Sterilization**

Heat sterilization of filtered seawater can be either done by autoclaving (for small volumes) at 121°C (250°F) at 15 psi for 15 minutes or by a glass-lined water heater of 500 to 1000 W submersion heater (for large volumes).

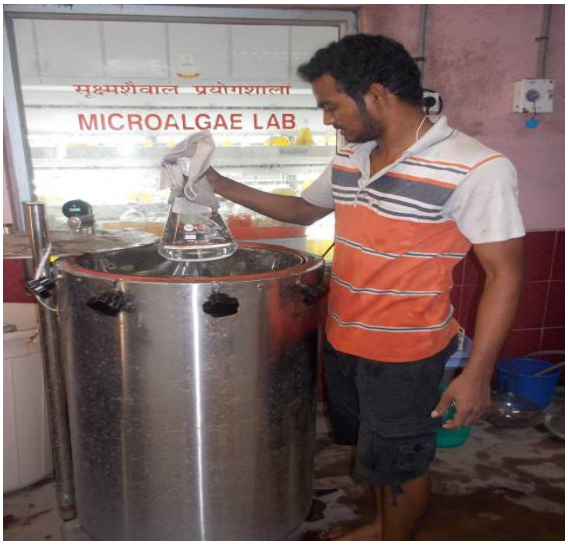


**Fig. 2 Heat sterilization by autoclaving**

Microwave sterilization is useful for small volumes of seawater. Nutrients can be added before microwaving since the temperature will not exceed 84 °C.

**Chemical Methods**

Dissolved inorganic and organic compounds (DOC), metals, pesticides, and other contaminants can prevent or retard microalgal growth, although detecting them can be complicated and costly. Activated carbon (charcoal) filtration is helpful in reducing DOC, while deionization resins are effective in removing metals and hydrocarbons. Activated carbon can be housed in a filter or a filter bag and all the water can be passed through it.

The most common and simplest method of chemical sterilization is chlorination. Mostly this type of sterilization is preferred for large volumes of atleast 4 L. An active chlorine level of 10-20 ppm in the water for 12 to 24 hours is sufficient to kill most pathogens. Filtered seawater can be sterilized (20 ppm active chlorine) with sodium hypochlorite solution in 200 ml of liquid chlorine (10% sodium hypochlorite) per 1000 liter of seawater. Sterilization occurs in a short period of time, usually 10 to 30 minutes, but a longer time without aeration (12 hours or overnight) is given for a margin of safety. Before use, neutralize the residual chlorine by adding a sodium thiosulphate solution at the rate of 1 ppm ($1g/m^3$) for every 1 ppm of chlorine left in the solution along with vigorous aeration for 2-3 hrs.

**Disinfection**

After the removal of suspended particulates through the mechanical filtration, disinfection of culture water can be done either through UV or ozone or by using both is found to be more effective. Ultraviolet radiation (germicidal energy) is an efficient, simple and reliable way to kill microorganisms in culture water. However, the killing power of UV is affected by turbidity/coloration of the incoming water, distance from the source, exposure time (flow rate) and species. Dosage of UV is measured as $mW\text{-}sec/m^2$. The dosage range varies from 2-230 $mW\text{-}sec/m^2$ at 254 nm. Minimum dosages vary widely for different microorganisms: 15 $mW\text{-}sec/m^2$ for most bacteria, 22 $mW\text{-}sec/m^2$ for

water-borne algae, 35 m W-sec/$m^2$ for bacteria/viruses, 100-330 m W-sec/$m^2$ for protozoans, fungi and moulds.

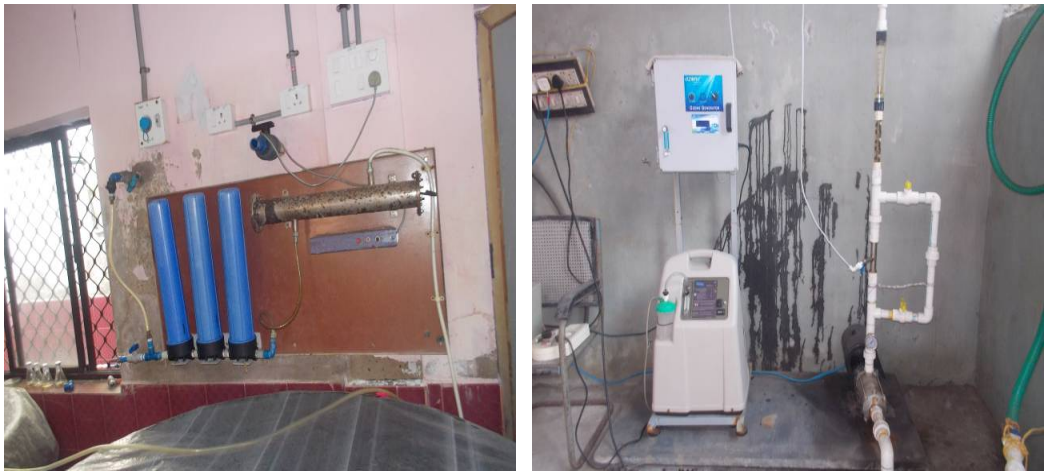

**Fig. 3. Disinfection by Ultraviolet radiation and Ozone treatment**

The ozone as a strong oxidizing agent is more effective in removing dissolved organics, pesticides, colour and nitrates. Due to unstable and highly corrosive nature of ozone, it should be handled with special materials because it gets quickly reverts back to $O_2$. Ozone oxidation can kill microorganisms, but for a given period of contact time; disinfection of water requires a certain dissolved ozone concentration. A residual ozone concentration of 0.1-2.0 mg $L^{-1}$ for a period of 1-30 minutes is required to be maintained for complete disinfection. Moreover, disinfection also depends upon the target microorganism. Care should be taken since a residual level of even 0.01 mg $L^{-1}$ can kill fish and shrimp larvae.

Mostly different hatcheries follow different types of mechanical filtration followed by Heat sterilization for indoor algal culture. For outdoor algal culture, filtration followed by Chemical methods or by disinfection with either UV or Ozonization is followed. For a complete treatment of seawater, filtration should be accompanied by either physical treatments like autoclaving, UV and ozonization or by employing chemical methods for greater degree of sterilization.

## References

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Training Programme on

# "Live Feed for Marine Finfish and Shell fish Culture"

17th - 22nd March 2016

**Visakhapatnam Regional centre of CMFRI**

Ocean View Layout

Pandurangapuram,Visakhapatnam