

Water quality parameters for Microalgae culture

Ritesh Ranjan, Joe K Kizhakudan, Chinnibabu Bathina, P Damodara Rao
and R P Venkatesh

ICAR-CMFRI, Visakhapatnam Regional Centre, Pandurangapuram,
Andhra University Post, Visakhapatnam - 530003

Water quality for aquaculturists refers to the quality of water that enables successful propagation of the desired organisms. Treated seawater enriched with nutrients, like nitrates, phosphates, essential trace elements, vitamins and carbon dioxide is a prerequisite for any successful microalgae and other live feed culture. High microalgae biomass with less contamination is important to support the growth of finfish and shellfish larvae. In this context water quality requirements and its management in different live feed culture systems plays a vital role in enhancing the survival, growth and production of cultivable animals.

Water Treatment

The water used for live feed culture should be free of suspended solids, plankton (e.g., protozoans, ciliates and other algae species), bacteria, pesticides and unacceptably high concentrations of dissolved or-organic compounds (DOC). Therefore, one of the most important prerequisites in successful live feed culture is the pretreatment of water. But the water pumping and treatment facilities will be common for the whole hatchery facility. So various standard pretreatment methods such as mechanical and chemical methods, sterilization or disinfection are followed by different hatcheries. Moreover, the choice of treatment method should be mainly based upon the type of species cultured, volume requirement, and cost. The whole process should be aseptic and at the same time should be economical for commercial hatcheries. Generally the following three processes are used for water treatment in hatcheries.

I. 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.

- i. Sand filters or polyester filter bags (20 to 35 μm) for large quantity

- ii. Cartridge filters (10, 5, 1 μm) or diatoma-ceous earth (DE) filters for medium quantity
- iii. To remove bacteria using 0.22 or 0.45 μm membrane cartridge filters for small quantity

II. 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 1,000 W submersion heaters for large volumes of 20 liter and above. Microwave sterilization is useful for small volumes of seawater to prepare micro nutrient and trace mineral stock since they are volatile. Nutrients can be added before microwaving since the temperature will not exceed 84 °C. So depending upon the volume and usage, the method of heat sterilization can be decided.



Heat sterilization by autoclaving

III. Chemical Methods

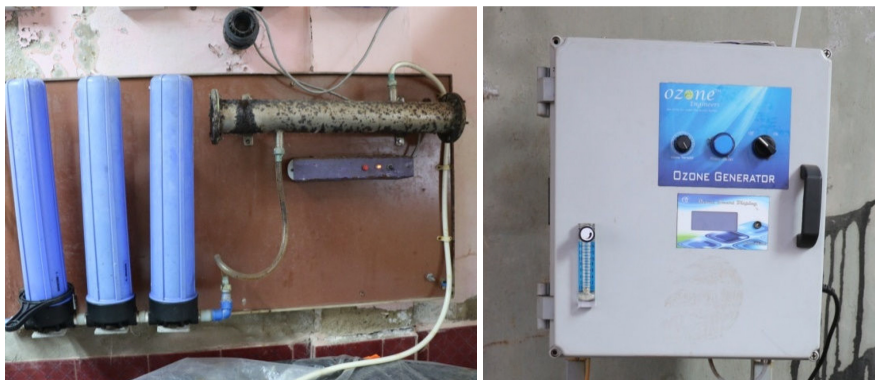
Dissolved inorganic and organic compounds (DOC), metals, pesticides, and other contaminants can prevent or retard micro algal growth, although detecting them can be complicated and costly. Activated carbon (charcoal) filtration is helpful in

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 (outdoor mass culture). An active chlorine level of 10-20 ppm in the water for 12-24 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 ($1\text{g}/\text{m}^3$) for every 1 ppm of chlorine left in the solution along with vigorous aeration for 2-3 hrs in sunlight.

Disinfection

After the removal of suspended particulates through 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 UV source, exposure time (flow rate) and species of organisms present. Dosage of UV is measured as $\text{mW}\cdot\text{sec}/\text{m}^2$. Minimum dosages vary widely for different microorganisms: $15\text{ mW}\cdot\text{sec}/\text{m}^2$ for most bacteria, $22\text{ mW}\cdot\text{sec}/\text{m}^2$ for water-borne algae, $35\text{ mW}\cdot\text{sec}/\text{m}^2$ for bacteria/viruses, and $100 - 330\text{ mW}\cdot\text{sec}/\text{m}^2$ for protozoans, fungi and moulds. So a common standard dosage can be fixed within this range from $2-230\text{ mW}\cdot\text{sec}/\text{m}^2$ at 254 nm for better disinfection of water.



Disinfection by Ultraviolet radiation and Ozone treatment

Ozone as a strong oxidizing agent is more effective in removing dissolved organics, pesticides, colour and nitrates. Due to unstable nature it gets quickly reverted back to O_2 , however, being highly corrosive and hazardous to health, it should be handled with precautions. 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 live feed culture, if needed UV or ozone can be used. 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 (small volume), UV and ozonization or by employing chemical methods (large volume) for greater degree of sterilization.

Microalgae culture environment

The most important parameters regulating microalgae growth are

- i. Light
- ii. Temperature
- iii. Salinity
- iv. pH
- v. 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.

Optimal conditions for culture of micro algae (modified Anonymous, 1991).

Parameters	Range	Optimum
Temperature (°C)	16-30	18-24
Salinity (g L ⁻¹)	12-40	20-24
Light intensity (Lux)	1,000-10,000	2,500-5,000
Photoperiod Hrs (light: dark)	16:8 (minimum)	24:0 (maximum)
pH	7-9	8.0-8.5

i. 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. 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 (E.g. Erlenmeyer flasks - 2,000 Lux; Larger volumes - 5,000-10,000 Lux). Fluorescent white lights are mostly used in indoor microalgal culture facilities, which can provide 2,500 Lux, while outdoor systems and greenhouses gets ambient sunlight, and 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 (minimum) of light per day, although cultivated phytoplankton develops normally under constant illumination.



Illumination in indoor algal culture

ii. Temperature

The optimal temperature for microalgae 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 30°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.

iii. 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.

Temperature, light, and salinity range for culturing selected microalgae species.

Species	Temperature (°C)	Light(Lux)	Salinity(ppt-‰)
<i>Chaetoceros calcitrans</i>	25 - 30	2,000-10,000	20 - 35
<i>Isochrysis galbana</i>	25 -30	2,000-10,000	10 – 30
<i>Skeletonema costatum</i>	10 - 27	2,500-5,000	15 – 30
<i>Nannochloropsis oculata</i>	20 - 30	2,500-8,000	12 - 30
<i>Pavlova</i> sp.	15 -30	4,000-8,000	10 – 40
<i>Tetraselmis</i> sp.	20 -28	5,000-10,000	20 - 40
<i>Chlorella</i> sp.	10 -28	2,500-5,000	26 - 30
<i>Thalassiosira</i> sp.	25 - 30	2,000-10,000	20 - 35

iv. pH

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.0-8.5. Changes in the pH and culture condition due to precipitation of certain nutrients may lead to complete culture collapse. Reviving of the cultures affected by pH changes can be accomplished by aerating the culture. Carbon dioxide plays a dual role in microalgal cul-ture. It provides a source of carbon to support photosynthesis, and it helps maintain pH at optimum levels. In the case of high-density algal culture, the addition of carbon dioxide allows to correct the increased pH, which may reach up to pH 9 during algal growth.

v. 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 microalgae for a few days more. Air circulation is important to avert sedimentation of 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 microalgae. 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 pH changes by maintaining the $\text{CO}_2/\text{HCO}_3^-$ balance. Based on the scale of the culture system and type of algal species, mixing of culture media can be done by daily handstirring (test tubes, Erlenmeyer's), 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.
- ✓ Total ammonia and nitrite 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 pH are interrelated while maintaining pH, alkalinity will also remain under safe limits in most cases. Generally, the total alkalinity level of $80 - 120 \text{ mg l}^{-1}$ is maintained in hatcheries.
- ✓ Water quality parameters like salinity, temperature, pH 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.

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