

CONSTRUCTION OF VERTICAL TUBULAR PHOTOBIOREACTOR FOR MICROALGAE CULTIVATION

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ABSTRACT

Mass cultivation of microalgae is gaining importance nowadays, because of its bioprospecting property. In this study laboratory scale tubular photobioreactor was constructed for marine microalgae cultivation using cheapest materials such as wooden sheets, wooden reapers, aquarium light, glass tube, and UPVC, PVC couplings. The two marine microalgae *Nanochloropsis occulata* and *Chaetoceros calcitrans* were studied. Effect of growth medium, pH was determined in normal culture technique and mass cultivation was carried out in tubular photobioreactor. The growth curve of marine microalgae with various inoculation volume and days of incubation were also determined. F₂ medium and pH 8 is suitable for the growth of the two marine microalgae. The biomass production is reached maximum level in 8 days of cultivation at 1.5 OD of inoculum volume. The biomass productivity is higher in tubular photobioreactor than the normal culture system.

Keywords: Tubular photobioreactor, Biomass, pH, *Nanochloropsis sp.* and *Chaetoceros sp.*

INTRODUCTION

Microalgae are eukaryotic photosynthetic microorganisms, which are used to produce highly valuable compounds.

Marine microalgae are the fastest growing organism, that are less than 2mm in diameter floating in the upper 200 M of the ocean where sunlight is available for

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photosynthesis. Microalgae are sunlight driven cell factories that convert CO₂ to potential biofuels, foods, feeds and high value bioactive metabolites (Spolaore *et al.*2006). It also produces carotenoids, which are used as colouring agents in nutraceuticals, pharmaceuticals, cosmetics, foods, scavenger and/or quencher of reactive oxygen species (ROS) and an enhancer for antibody production (Singh *et al.*2005).

The production of microalgal byproducts requires large quantities of algal biomass. Biomass is one of the better sources of energy and hence large scale biomass production getting significance. Large scale production of biomass energy could contribute to sustainable development on several fronts, environmentally, socially and economically. Several cultivation techniques are used for mass production of microalgae. We can cultivate microalgae in open system and also in closed system.

Closed systems generally have higher production rates than open system. Major limitations in open system include poor light utilization by the cells evaporative losses diffusion of log to the atmospheres and requirement of large areas of land. Furthermore contamination by predators and other fast growing heterotrophs have restricted the commercial production of microalgae.

In comparison with open culture system a closed photobioreactor is easy to control with regard to environmental parameters and can achieve high growth rates (Pulz, 2001; Sierra *et al.* 2008). A photobioreactor maximizes the growth conditions through its design the use of clear glass tubing for efficient and volumetric distribution of light; efficient delivery of light from the source to the algae. In addition bioreactor based photosynthetic microalgae cultures were being considered

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as a part of the closed ecological support system (Li J *et al.*2003). Fully closed photobioreactors provide opportunities for monoseptic culture of a greater variety of algae than in open system. Higher biomass of microalgae productivity is obtained in closed photobioreactor cultivation system and contamination is also easily prevented.

Photobioreactor is a bioreactor which incorporates some type of light source. Virtually any translucent container could be called a photobioreactor; however the term is more commonly used to define as a closed system. Algae can also be grown in a photobioreactor. In photobioreactor algal culture can be illuminated by artificial light, solar light or by both. Some of the photobioreactors include bubble column (Degen *et al.* 2001), airlift column (Kaewpintong *et al.*2007), stirred tank (Petkov, 2000) and tubular (Hall *et al.*2003) photobioreactors. Tubular photobioreactor is

one of the most suitable types for mass cultivation of algae because they have large illumination surface (Chisti, 2006). Aeration and mixing of the cultures in tubular photobioreactors are usually done by air-pump or air-lift systems (Traviso *et al.*2001). Furthermore, long tubular photobioreactors are characterized by gradients of oxygen and CO₂ transfer along the tubes.

The aim of this work was to construct the vertical tubular bioreactor at low cost and to determine the suitable growth medium, optimum pH, optimum inoculum level for the mass cultivation of marine microalgae.

MATERIALS AND METHODS

Tubular Photobioreactor Construction and Fabrication

The fabrication of photobioreactor was start by drilling a 45 mm hole in the

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wooden sheet of 0.25" thickness around the edges. There are about six holes were made. 1.25" couplings (MTAPL) fixed in the each hole from the bottom side. Six numbers of couplings (FTAPL) screwed into the bottom couplings by turning the coupling clockwise. The six hollow glass tube (75 cm X 45 mm) which is bottom closed (Flat bottom) were inserted into the UPVC couplings in a specific position. To avoid shacking of glass tube aquarium sealant was spread on sides of the coupling if necessary.

The top edge of all the six hollow glass tube were coupled by 1.25" UPVC slip and thread adaptor. The four corners and also the centers of the bottom wooden sheets screwed with wooden reapers (30") for the purpose of mechanical support. The same size of the wooden sheets which is in the bottom was also been fixed at the top. Both the bottom and top wooden sheets were linked by 30" wooden reapers by using

screws (2.50"). The center wooden reaper was used to fix the fluorescent light (40 watts) along the sides by using 1" plastic brackets.

15 cm of six PVC tubes were fixed with the six PVC threaded plugs and inserted to the holes of the top wooden sheets. This assembly was considered as the top part of the photobioreactor. The air pumps, O₂ and Co₂ supplied nutrient inlets were to be provided through the top assemblage for the aeration aquarium air pump were used for Co₂ supply.

Collection and maintenance of marine microalgae

Two different groups of marine microalgae such as *Nanochloropsis occulata* and *Chaetoceros calcitrans* were obtained from Central Marine Fisheries Research Institute (CMFRI), Tuticorin, India. For the cultivation filter sterilized seawater was used along with the required nutrients.

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About 10 – 20% of the inoculum in the growing phase was transferred to the culture flask and those were placed under the white fluorescent light (1000 lux) up to 4-5 days for attaining log phase. The time required for the maximum cell growth varies depending on the species, almost most of the species require two weeks for completion of the growth. The flagellates can be kept for two months in their stationary phase in the stock culture room under the controlled light and temperature condition.

Cultivation of marine microalgae in different growth medium

Marine microalgae such as *Nanochloropsis oculata* and *Chaetoceros calcitrans* were grown in three different culture media such as F₂ medium, MN III medium and ASN III medium depending upon the requirement of micro and macro nutrients. The three different marine microalgal samples were inoculated in 250

ml conical flask (containing three different medium) as separate manner. The algal cultures were incubated at 25°C±1°C under 1.2±0.2 klux light intensity with proper aeration for 3 weeks. After incubation, the growth of microalgae and the total biomass were estimated. Among the three, good yielding medium was selected for the further study.

Biomass estimation

Biomass concentration (g l⁻¹) was calculated by measuring dry weight. For dry weight measurement, the microalgal cells were harvested after 21 days of incubation by centrifugation at 5000 rpm for 15 min. at room temperature and the cells were washed with distilled water. Then the cell was placed petri plate and oven dried at 40°C for 4 to 6 hours. The dried biomass was cooled and weighed. The difference between the initial and final weight were taken and the

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biomass weight was calculated. The dry weights were expressed in g/l.

Effect of pH on marine microalgal biomass in F₂ medium

The effect of pH on growth of the marine microalgae was studied using F₂ medium with the pH range of 6, 7, 8, 9, and 10. The experiments were carried out in conical flask containing 100 ml of F₂ medium. The pH was adjusted with the help of 8 M NaOH and 1M HCl solution before autoclaving. All the flasks were incubated for 3 weeks at 25°C±1°C under 1.2±0.2 klux light intensity with proper aeration. After incubation, the growth of microalgae and the total biomass were estimated.

Mass cultivation and growth studies

Marine microalgae such as *Nanochloropsis oculata* and *Chaetoceros calcitrans* were grown in the selected culture medium (F₂ medium, pH-8) for mass cultivation. The experiment was carried out

in constructed tubular bioreactor. Inoculum concentration is also very important in growth studies and hence four inoculum concentrations were used for the cultivation of both marine microalgae *Nanochloropsis oculata* and *Chaetoceros calcitrans*. The four inoculums concentration used for the study were OD₆₂₀ - 0.05, 0.10, 0.15, 0.20 respectively. Then the photobioreactor was kept in 25°C±1°C under 1.2±0.2 klux light intensity with proper aeration. Growth curve for two microalgae was also studied.

RESULTS AND DISCUSSION

The mass cultivation of marine microalgae require a appropriate culture system with consistent cultivation parameters which will be suitable for producing algae based products which has pharmaceutical, biotechnological and marketed commercially. Most of the large scale cultivation of microalgae has been success with open system of cultivation.

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However major short coming at the open culture system contamination with bacteria and other contaminants as well as changes in local climatic conditions, cost of the land and water. Further perspective of mass culture of microalgae will required closed system because of the algae and algal products must be grown free of potential contaminants such as heavy metals and microorganisms. Continuous culture and good control over the growth environment result in a consistent product quality and highest operating cell density. In this present investigation a small scale tubular photobioreactor with the capacity of 4L was constructed using low cost material and has been used for the mass cultivation of marine microalgae.

After 21 days of incubation the conical flasks have the greenish bloom of algal biomass. The amount of total biomass

was calculated, which was higher in F₂ medium than ASN III and MN III medium. So F₂ medium was preferred for further experimental analysis. Among these microalgae *Chaetoceros calcitrans* have 4.6g/l of dried biomass and *Nanochloropsis oculata* contain lower amount (i.e.) 3.4g/l of dried biomass in F₂ medium (Figure.1).The present study found that the significant effect of pH on biomass yield of marine microalgae. When compared to all pH ranges pH 8 yield higher amount of marine microalgal biomass (Figure.2). *Chaetoceros calcitrans* could produce higher amount of dried biomass (4.9g/l) compared with *Nanochloropsis oculata* (4.0g/l) at pH 8. Dayananda *et al.* (2005) reported the effect of media and culture conditions on growth and biomass production of *Botryococcus braunii*.

Figure 1. Total biomass of two marine microalgae in different growth medium

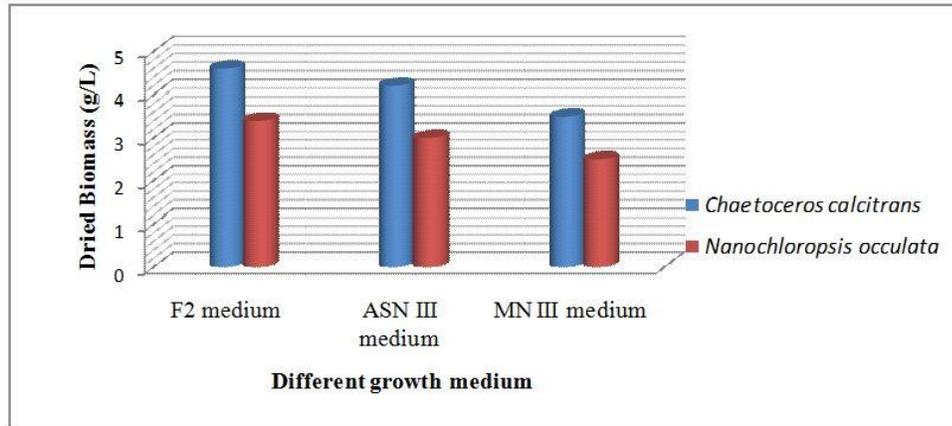
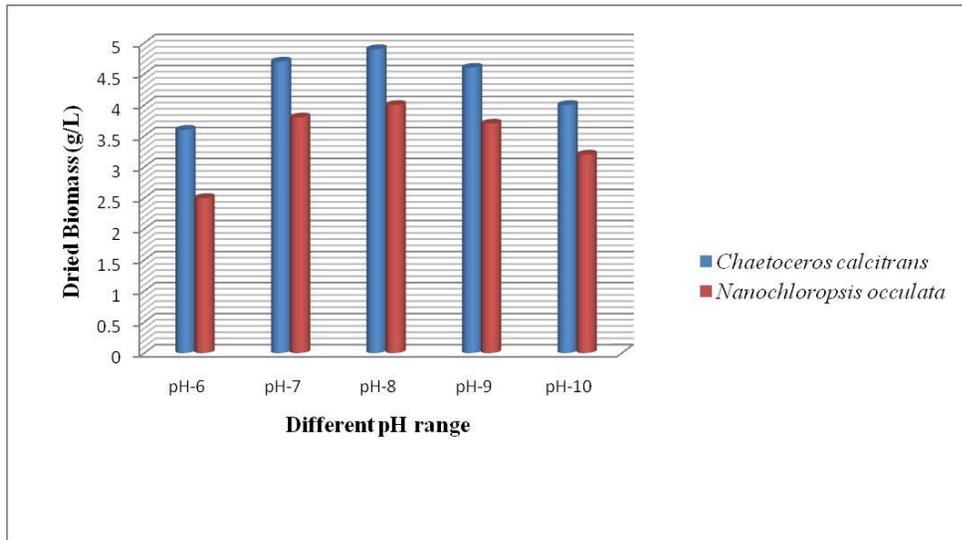


Figure 2. Effect of various pH range on growth of two marine microalgae in F₂medium



The array of long and low diameter polythene tubing designed in a clearly deficient scaling up of the original pilot scale device enable the accumulation of new algae and algal products. In recent years

they have been major advantages in operation of closed tubular bioreactor for *Dunaliella salina* culture, Hejazi and Wiffels (2003). Cultivation of microalgae in suitable inoculum concentration is necessary

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which can effectively shorten the lag phase of cell growth and allowed to go into logarithmic phase very earlier. OD₆₂₀-0.15 inoculum produces maximum amount of biomass at the 12th day of cultivation. *Chaetoceros calcitrans* could produce 8.82g/l dried biomass (Figure.3) compared with *Nanochloropsis oculata* (Figure.4). The inoculum volume and days of cultivation, will determine the sufficient biomass of any microalgal culture system. In this present study the four different

inoculum volume and 8 days of cultivation period were maintained to determine the optimum requirements for getting higher biomass of both microalgae in closed tubular photobioreactor. Yue-Hui Zhu (2008) was reported that inoculum volume of 0.15 for *Dunaliella salina*. Continuous cultivation was used to study the growth curve of the microalgae. Both *Chaetoceros calcitrans* and *Nanochloropsis oculata* reached steady state from 7th day of cultivation in photobioreactor.

Figure 3. Growth of *Chaetoceros Calcitrans* at different volume of inoculum

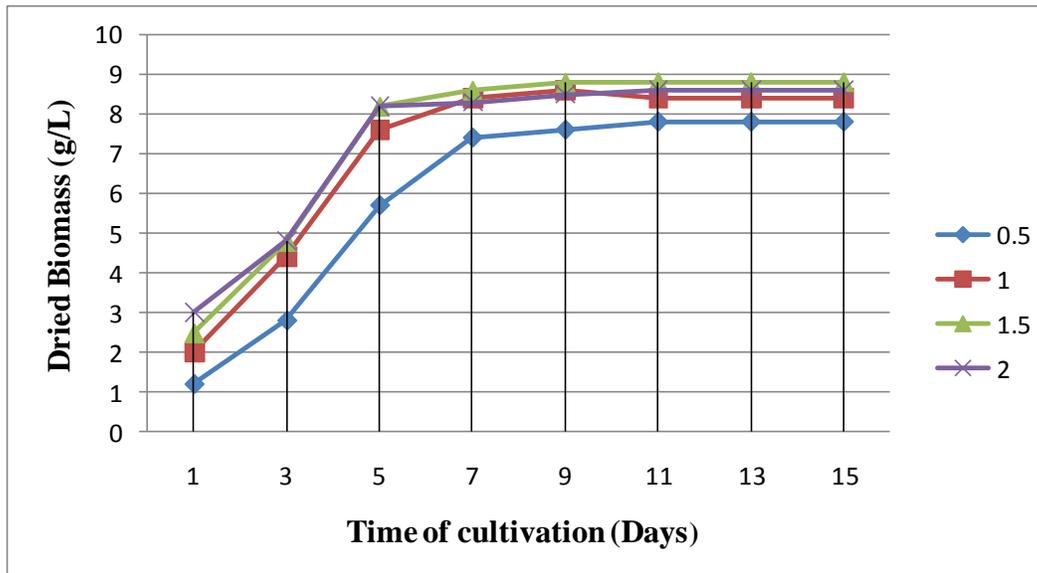
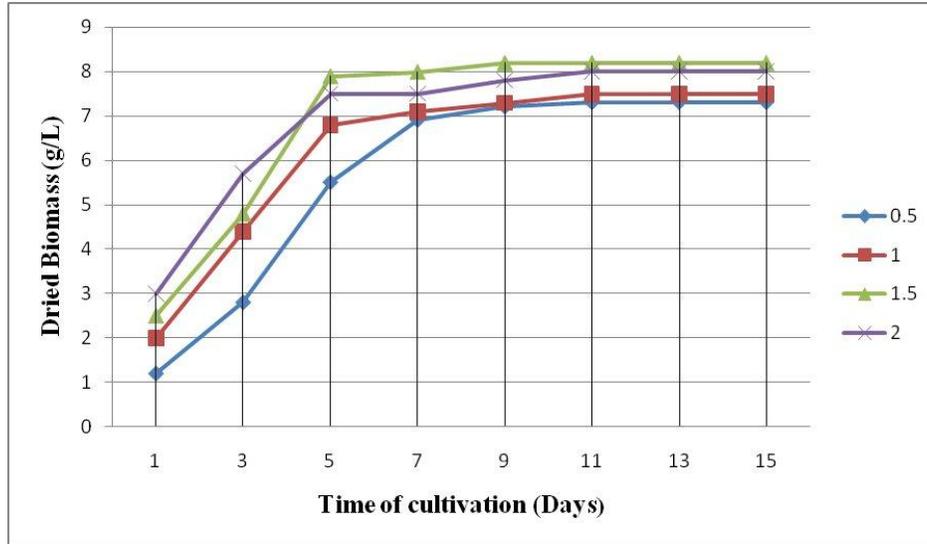


Figure 4. Growth of *Nanochloropsis oculata* at different volume of inoculum



CONCLUSION

Compare with other culturing system a tubular photobioreactor as a large illuminating surface area suitable for outdoor cultures fairly good biomass productivity relatively chief. The closed photobioreactor provided opportunity for monoseptic cultures of great variety of algae then is possible in open system. The closed photobioreactor will also overcome the major impact of solar radiation-

photoinhibition. Major advantage of tubular photobioreactor would be acclimated to high light and therefore the negative effect of photoinhibition would be minimal in such system. In future the optimization of the operating parameters and intrinsic properties of algae, biology of algae and the engineering requirements is still place left for further technological advances and improvement for growth performance and higher biomass quantity cultivation in closed

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photobioreactor which will enable the commercialization of new algae and algal products in future.

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