

Laboratory - scale high density culture of the marine diatom *Chaetoceros* sp.

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ABSTRACT

A laboratory scale continuous and semi-continuous system for culture of the marine diatom *Chaetoceros* sp. was devised. Using 2.5 times the normal nutrient concentration and limited supply of CO_2 , the culture density could be increased from an average 1.5 million cells / ml concentration in traditional batch culture systems to 13.7 million cells/ml in continuous system. Besides the increased biomass production, the duration of culture was enhanced to thirty days without crashing. The semi-continuous system was up-scaled to 60 l capacity with an internal illumination system to yield 528 litres of average 3.2 million cells/ml within 36 days. The velocity of cell growth during the logarithmic phase in doublings/day ranged from 0.21 in control (batch culture) to 2.51 in the semi-continuous system. Furthermore, the cost of production, even with additional inputs, are comparable or even less than the traditional batch cultures. The economic efficiency was highest in continuous systems as compared to semi-continuous systems (Rs. 0.03 and 0.017/billion cells as against Rs. 0.082 and 0.037/billion cells).

Introduction

Unicellular marine algae are widely used as food in the hatchery production of commercially valuable fish and shellfish. Bivalves and their larvae feed by filtering them from seawater. Rotifers and brine shrimps also ingest microalgae, which in turn form the food for larval fish and prawns. In some systems algae are added to the water containing fish or prawns to improve quality.

Microalgae can be cultured from closely controlled methods on laboratory bench top, with a few litres of algae, to less predictable methods in outdoor tanks, containing thousands of litres. Coutteau (1996) described three basic

types of phytoplankton culture systems.

a) Batch culture system; where the total culture is harvested and used as food. b) Semi-continuous culture; where part of the culture is harvested and used as food and the quantity taken is replaced with fresh culture medium. After allowing 2-3 days for the remaining cells to grow and divide, the process is repeated. c) Continuous culture, in which the number of algal cells in the culture is monitored and as the cells divide and grow an automatic system keeps the culture density at a pre-set level by diluting the culture with fresh medium.

Although batch culture is a relatively easy method, its efficiency is very poor

and the cultures are prone to crashes. Considering the advantages of continuous and semi-continuous culture systems, several designs for continuous production of algae in high densities were developed (Persoone and Sorgeloos, 1975; Boussiba *et al.*, 1988; James and Al-Khars, 1990; Fabregas *et al.*, 1996). Published works on microalgal culture in India are sparse. Gopinathan (1982) has described the batch culture method for culturing marine phytoplankton for use in shellfish hatcheries. In batch culture technique adopted presently in various Indian hatcheries and laboratories, production of microalgae is highly inconsistent, with frequent collapse of cultures due to ciliate infestation.

The present study therefore, was planned to devise a laboratory scale method to culture the diatom, *Chaetoceros sp.*, in high densities consistently over longer than usual duration and its comparison with batch culture

Kerala. Seawater (25-28 ppt) used for the experiments was chlorinated and subsequently dechlorinated.

Experimental set-up and treatments

Experimental details are given in Table 1. The experiments (Exp. 1-5) were conducted inside an air-conditioned algal culture laboratory of FHL as well as in covered outdoor conditions (Exp.6). All treatments were provided with 14 h light: 10 h dark regime to obtain synchronous cultures. The marine diatom *Chaetoceros sp.* (2-4 μ) was used in the experiments. Stock cultures were procured from the algal culture facility of FHL of CMFRI and 10% inoculum was used to start all cultures.

For control (Exp.1 - batch culture) standard procedures were followed. Flasks (3 litre capacity) were filled with de-chlorinated and filtered seawater upto 1.8 litre mark. The flasks were

TABLE 1. Details of experimental treatments

Exp. No	Particulars	Container Volume (litres)	Culture Volume (litres)	pH Range	Temp Range (°C)
1	Batch culture (Control)	3	2	6.5-9.2	23.4-28.0
2	Continuous culture	3	2	7.0-8.7	21.3-28.7
3	Continuous culture with CO ₂	3	2	5.5-8.5	22.3-28.7
4	Semi-continuous culture	5	4	7.0-9.0	22.4-28.0
5	Semi-continuous culture with CO ₂	5	4	6.2-8.1	20.9-27.6
6	Semi-continuous outdoor culture, with CO ₂	83	60	5.8-7.9	25.7-31.9

systems. The study also envisaged comparison of coasts involved in the production of microalgae using the batch, continuous and semi-continuous methods.

Materials and methods

All experiments were conducted at the Fisheries Harbour Laboratory (FHL) of C.M.F.R.I. at Thoppumpady, Cochin,

plugged with cotton and heated to boil the water inside and then allowed to cool. When flasks were properly cooled, nutrients (Liang, 1991) were added at 1 x concentration (Table 2). After inoculation, the flasks were kept on a rack with illumination (3000 lux). No aeration was provided in the flask. The cultures were harvested when the density started declining.

TABLE 2. Concentration of solutions A, C and D used in preparation of nutrient enriched seawater (Liang, 1991)

Solution	1 x ml/I (Control)	Cost (Rs)	2.5x ml/I (Experiments)	Cost (Rs)
A	1.0	0.03152	2.5	0.0788
C	0.1	0.0084	0.25	0.021
D	2.0	0.0192	5.0	0.048

A 3-tier system was set up for Exp. 2 and 3 (continuous culture system). In the top, a reservoir flask was kept and nutrient salt solutions (Liang, 1991) were added at 2.5 x concentration. The culture flask was kept in the middle tier. Gravity flow was used for transferring media from reservoir to culture flask. In the third tier a harvest flask was kept. The culture flask was connected to the reservoir flask with tubing having a control to maintain the constant level of culture (2 litre) in flask by adding nutrients from reservoir bottles. All flasks were autoclaved at 115 psi for 20 min. In this three-tier system, air/CO₂ connections were provided to the culture flask and harvest flask. Air supply was provided from a rotary blower and passed through an activated charcoal filter. CO₂ was provided from a portable CO₂ cylinder with an improvised flow valve. The CO₂ was bubbled for a minute a day through the culture to bring down pH to 6.5 - 7.5. Fluorescent lighting (3000-7400 lux) was provided on the backside of culture flask and harvest flask.

In the continuous culture system, harvest of 500 ml was taken daily after an initial culture period of 5-6 days. The daily harvest was retained in the harvest flask for 24 h during which cell multiplication was very fast. Thus the final harvest was after a one-day lag period.

In semi-continuous culture system (Exp.4 and 5), a 5 l conical flask was filled with de-chlorinated and filtered seawater

upto 3.6 l mark. The nutrient solutions (Table 2) were added at 2.5 x concentration. Air, CO₂ and illumination was provided into the cooled autoclaved flask as described earlier. Once the cell concentration reached 3.5 million cells / ml within 4-5 days, a part of the culture was harvested and the rest diluted with newly autoclaved cooled nutrient added seawater to make a cell concentration of approximately 1 million cells / ml in the 4 litre water. The harvest was repeated every 2-3 days since the 3rd day when the culture regained a concentration of about 3.5 million cells / ml. When the concentration of cells was not increasing even after 3-4 days total harvesting was done and the experiment was closed.

Experiment 6 (up scaling) was done in a rectangular plexiglass tank of 83 litre capacity placed outdoors. The tank was filled with de-chlorinated seawater upto 60 l capacity and charcoal - filtered air supply was provided through two air stones.

An internal illumination system was prepared by fixing a compact fluorescent lamp in a 5 l capacity plexiglass jar fixed at the centre of tank in such a way that 3/4 of jar would be immersed in the water without water entering the jar. The tank was covered completely with a white paper and plastic sheet so as to keep all the illumination reflected back in the tank. The nutrients were added to the culture tank at 2.5 x ml/ litre. CO₂

was bubbled for a min/day from a portable cylinder.

Culture monitoring

The following parameters were noted daily between 0800 and 1000 h. 1) Temperature of air and water, 2)Salinity 3) pH. Algal cell concentrations were determined with a hemocytometer with improved Neubauer ruling. To obtain measures of growth, velocity doublings / day were calculated as per Herrero *et al.* (1991):

$$\text{doublings / day} = \frac{\ln N(n) - \ln N(i)}{\ln 2 (t_n - t_i)}$$

where t_i and t_n are initial and final time of the logarithmic phase, both expressed in days, and $N(i)$ and $N(n)$ are the initial and final algal cell densities, respectively.

All chemicals were sourced from M/s HiMedia, Qualigens and Citra Chemical Co. for which the input costs were worked out (Table 2).

Comparative economics of culture systems

To investigate the cost efficiency of the different algal culture systems, the costs of chemicals and CO₂ gas were included. Expense on containers, illumination, treated seawater, autoclaving/boiling and aeration was considered as equal in all experiments and therefore accounted. Costs were calculated as cost (in Rupees) of production per billion algal cells.

Results and discussion

The growth curve of *Chaetoceros* using 1 x nutrient concentration (control) is shown in Fig. 1. The exponential phase was observed upto day 12, when the cell concentration was 3.44 million cells/ml.

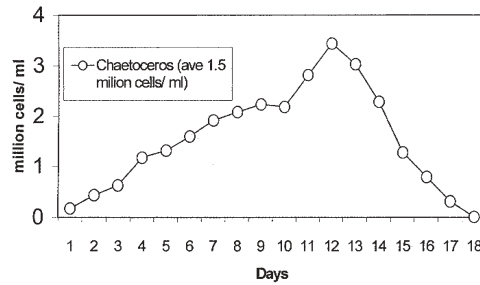


Fig.1. Culture cell density of control batch culture (Exp.1) of *Chaetoceros* sp.

After this, the culture started declining and collapsed on 18th day. Gopinathan (1986) also reported that the exponential period of algal cultures normally last for 9 days under these conditions. The culture gave, an average 1.5 million cells / ml.

The continuous culture (Exp.2) was initiated with an initial cell concentration of 0.96 million cells/ml. On 5th day it reached 5.01 million cells/ml, when the first harvesting was done (Fig.2) and the

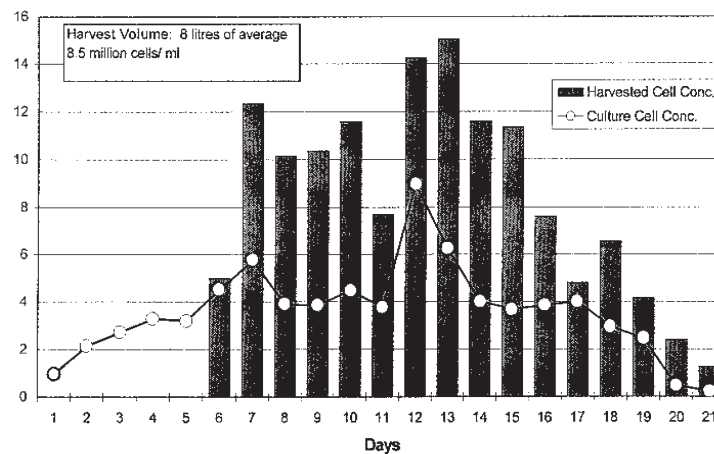


Fig. 2. Culture cell and harvested cell density in Exp. 2, (Continuous system without CO₂).

harvest was retained until next day. The cell concentrations in the harvest flask almost doubled after 24 h incubation. This could be due to the presence of additional nutrients for growth. From 7th to 14th day the harvest flask showed a cell concentration of more than 10 million cells / ml with the exception on 11th day. After 14th day, declining phase

was observed and on 21st day complete harvesting was done. The temperature difference between 15th and 16th day was about 7-8°C of both air and water due to air-conditioning failure, which may be the main reason for the culture to collapse. The pH and temperature range of the culture is shown in Table 1. During the culture period a total of 8 litres of harvest was obtained with an average cell concentration of 8.5 million cells/ml. It is thus very clear that the increase in nutrient concentration together with continuous harvest helped in attaining higher cell densities and sustain the culture for longer periods. Liang (1991) also reported similar results in fully automated culture systems. Using 4 m M NaNO₃ instead of the normal 2 m M also resulted in maximum cell density in the case of the flagellate *Isochrysis galbana* (Fabregas *et al.*, 1986).

In Exp.3, daily harvest of 500 ml was initiated from day 4 when the cell concentration reached 4 mil-

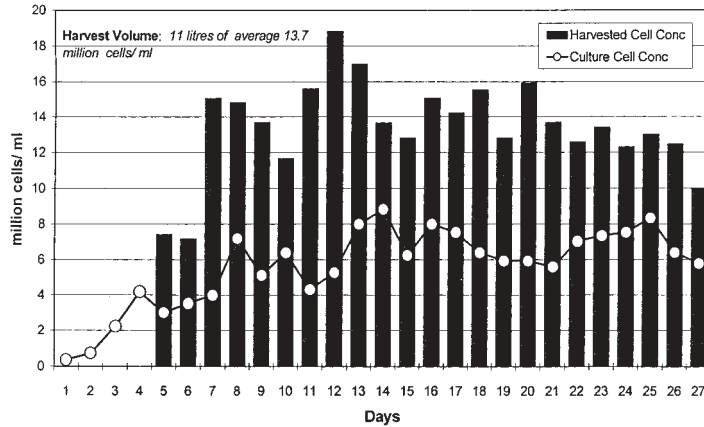


Fig. 3. Culture cell and harvested cell density in Exp.3, (Continuous system with CO₂).

lion cells / ml (Fig.3). After incubation of the harvest for 24 h, further increase in cell concentration was noted. This daily harvesting was continued upto 27 days after which culture showed declining phase when complete harvesting of culture was carried out. Altogether 23 harvests were made resulting in harvest of 11 litres with an average density of 13.7 million cells/ml.

The use of CO₂ helped to maintain the pH of the culture near to 7.5 which is most ideal for phytoplankton cultures (Liang, 1991; Gopinathan, 1996). In the present study, the culture period was significantly prolonged in continuous cul-

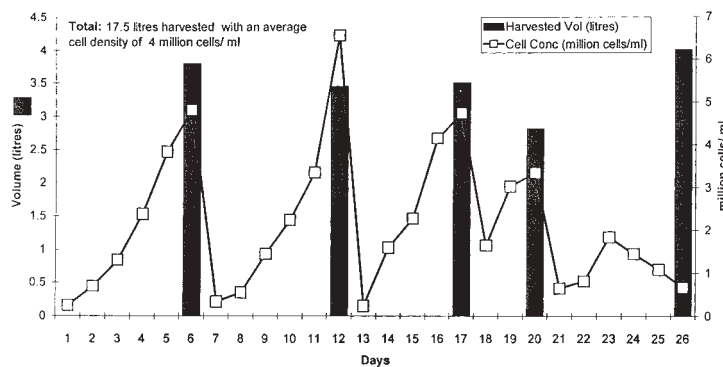


Fig. 4. Culture cell density and harvested volume in Exp.4; (Semi-continuous system without CO₂.)

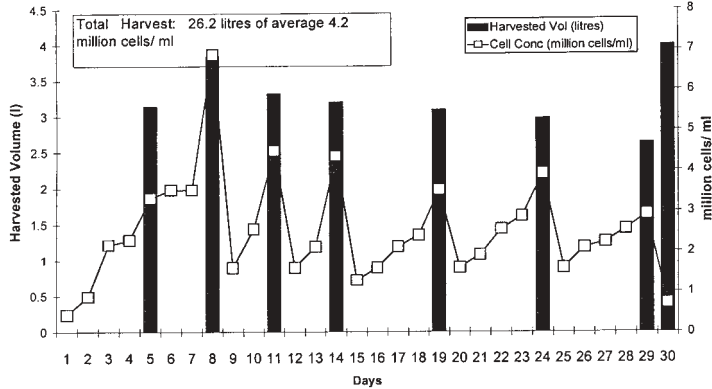


Fig. 5. Culture cell density and harvested volume in Exp.5, (Semi-continuous system with CO₂.)

ture system with the use of CO₂.

In semi-continuous culture (Exp.4), a cell concentration of 4.82 million cells / ml was observed within 6 days, when the first harvest was done. The culture was diluted to attain a cell concentration of about 0.33 million cells / ml with addition of fresh nutrient seawater (Fig. 4). In this manner a total of 5 harvests was made and complete harvest was made on the 26th day. Totally, 17.5 litres of culture was harvested with an average cell density of 4.0 million cells/ml.

In Exp. 5, a concentration of 3.32

million cells / ml was obtained on 5th day, when first harvest was carried out (Fig.5). After every 3-5 days, harvesting was carried out when culture attained a concentration above 3 million cells/ml. From 25th day onwards the culture showed decrease in cell concentration and on 30th day complete harvesting was carried out.

Significantly in-

creased culture densities were obtained with the use of semi-continuous culture systems as compared to traditional batch culture. The yield was further doubled with the use of continuous system. The advantage of the use of CO₂ was clearly evident in the continuous system and less so in the semi-continuous system. Taub

(1988) reported that although batch culture appears to be the simplest method, is not necessarily the most efficient, nor the least expensive as also seen from the present study.

The outdoor culture (Exp.6) was initiated with an initial cell concentration of 0.02 million cells/ml, which reached 3.73 million cells/ml within 5 days, when the 1st harvest was done (Fig.6). This process was continued after every 3-4 days when the concentration reached more than 3.0 million cells / ml. The culture was continued upto 36 days when complete harvesting was done. A total har-

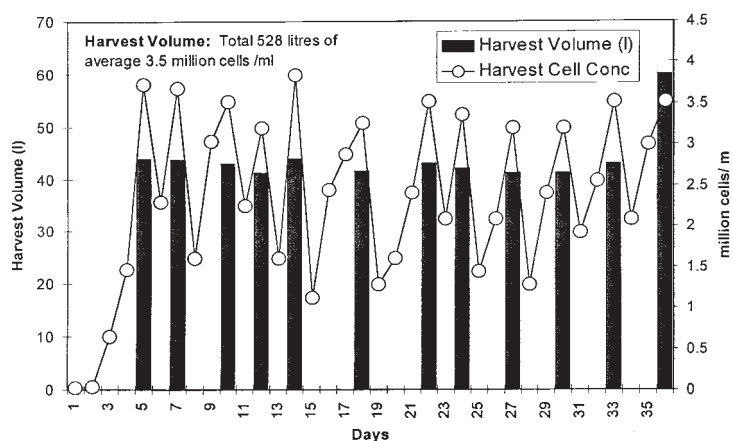


Fig. 6. Culture cell density and harvested volume in Exp. 6 (Up-scaled semi-continuous system with CO₂.)

vest of 528 litres with an average cell density of 3.2 million cells / ml was obtained from this experiment.

Up-scaling the semi-continuous system resulted in slightly lower algal production rate (average 3.2 as compared to 4.2 million cells/ml), but the duration of the culture was significantly enhanced. Compared with a hypothetical control batch culture, the yield (in cells and volume) and duration of culture was significantly higher. The rate of biomass production was not significantly affected in outdoor cultivation of *Isochrysis galbana* in open reactors, as compared to laboratory scale cultures (Boussiba *et al.*, 1988). The different culture systems affected the velocity of growth of *Chaetoceros* sp. The least growth rate in the logarithmic phase (0.21 doublings /

day) was observed in the control batch culture (Fig.7). The continuous system showed a five-time improvement in growth rate and with CO₂ it was further

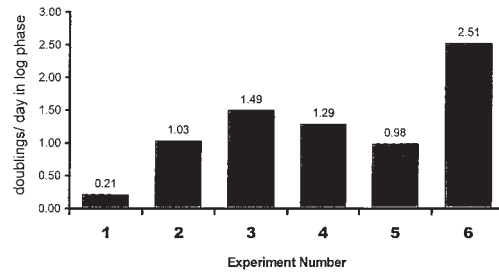


Fig. 7. Cell doublings /day in the logarithmic phase of different experiments indicating velocity of cell growth.

improved to seven times. The semi-continuous system showed less growth rate as compared to the continuous system, yet it was about five times more than

TABLE 3. Details of production, input costs and cost of production per billion cells of *Chaetoceros* in different treatments, assuming charges are same for facilities like container, treated seawater, aeration, illumination, etc.

Experiment/ Particulars	Batch culture (Control)	Outdoor batch culture (hypothetical - control)	Cont. with CO ₂	Cont.	Semi- cont. with CO ₂	Semi- cont.	Semi-cont. culture outdoor (60 litres)
Duration (Days)	30 (5 batches)	36 (6 batches)	27	21	30	26	36
Total volume harvested (litres)	20	360	11	8	26.2	17.5	530
Average cell density (million cells/ml)	1.5	1.0	13.7	8.5	4.2	4.0	3.2
Total cells harvested (Billion cells)	30.00	360.00	150.70	68.00	110.04	70.00	1696.00
Cost (Rs.) of production							
1) Chemicals	1.1824	21.78	1.6258	1.1824	3.8724	2.5865	78.334
2) Carbon dioxide	-	-	2.916	-	5.832	-	12.000
Total	1.1824	21.78	4.5418	1.1824	9.7056	2.5865	90.334
Cost (Rs.) of production per billion cells	0.039	0.059	0.030	0.017	0.082	0.037	0.053

control. The up-scaled 60 l culture showed the maximum growth rate of 2.51 doublings / day and this could be due to the internal illumination system provided which ensured more efficient light availability to the cells.

The economics of production of microalgal cells in different experimental treatments was made to ascertain the efficiency and advantages of high density culture (Table 3). For *Chaetoceros*, the cost of production in semi-continuous system with CO₂ was marginally higher than control, while the continuous systems with and without CO₂ were cheaper in production cost than the control batch cultures. In the outdoor semi-continuous culture the cost of production per billion cells was marginally cheaper as compared to a hypothetical control batch culture. However, the advantages of higher production and increased volume of harvest over a sustained period of time are much superior.

Samonte *et al.* (1993) estimated the economics of multi-step (batch) production of *Chaetoceros calcitrans* in Philippines. The production cost (inclusive of all facilities) during four days culture period was estimated as P 715.5/ tonne (1 US\$=25 Pesos). These results are not directly comparable to that obtained presently, however, the costs seem to be high for such batch cultures.

The results of the present study reveal that algal cultures in the laboratory can be carried out more efficiently using continuous and semi-continuous systems. The advantage of sustained higher biomass production and use of limited laboratory space are evident. Moreover, the costs of production, even with additional inputs, are almost same or even less than the traditional batch cultures.

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