



Optimum Nutritional Requirement for the growth of *Chaetoceros Calcitrans*

Bindhu K.B.

Department of Botany, Carmel College, Mala, Thrissur, Kerala, INDIA

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Abstract

An attempt was made to study the optimum concentration of culture media required for the culture of *Chaetoceros calcitrans* under laboratory conditions so as to achieve a viable and economical method of culture. The aim of the study was determination of the effectiveness of hydrological factors especially nutrients which was supplied externally to the seawater while culturing the diatom, and to determine its optimum concentration for ideal growth of the culture. The cell count, percentage of transmittance and the chlorophyll a values are significantly higher for the group in which the medium was provided on the initial day. Almost similar values were also noted for the 5th day's enrichment, indicating that the culture can grow upon the nutrients present in the seawater up to the 5th day and enrichment is needed only afterwards.

Keywords: Diatom, culture medium, *Chaetoceros*, nutrients.

Introduction

As diatoms are known to be a good source of food for marine invertebrates, supplying protein there is an increased trend of culturing them in mass scale¹. Research on mass culture of diatom has been carried out in many parts of the world for the past fifty years. The larvae of prawns and fishes prefer diatoms as basic food. The success of any hatchery operations depends mainly on providing the required food species of micro algae suitable for the larvae. Even after the two decades of research on the formulation of the micro diets to replace live food in larviculture, there is limited success². Eventhough alternate feeds are available in market, being costly; the micro algal culture is most economical live feed in hatcheries.

There are many culture medium for diatom culture such as Erd-Schriber's³ and Miquel's⁴ Walne's or Coway⁵ etc. These media incorporate trace metals and several inorganic and organic salts. Although most algae are photo autotropic and can grow in purely inorganic medium many require organic compounds, the requirement of which may be either absolute or stimulatory.

Popularization and commercial application of photosynthetic biomass production systems like cultivation of algae are more relevant now than ever before in the international context of energy shortage, water disposal problems, environmental protection, alternative food additives and cheaper sources of feed proteins⁶. Production of microalgae for more varied and newer applications like aquacultural practices has come of age. There have been very few attempts in the past to study the nutritional requirements of microalgae under laboratory conditions. In this account an attempt was made to the optimum concentration of culture media required on suitable period of culture of *Chaetoceros calcitrans* under controlled conditions so as to achieve a viable and economical method of culture. Here the study was carried out to determine the influence of

hydrological parameters especially nutrients which was supplied externally to the seawater while culturing the diatom under laboratory conditions, and to determine its optimum concentration for ideal growth of the culture, in such a condition of increased rate of pollution due to the industrialization and others.

Material and Methods

The material for this investigation was the culture of *Chaetoceros calcitrans*, which is widely used in aquacultural practices as live feed. The diatoms were grown in enriched seawater. Seawater collected from offshore of Cochin was transported to marine hatchery complex of CMFRI and kept 2-3 days in a settlement tank. Further, it was chlorinated by active chlorine and dechlorinated by aeration. Chlorinated seawater was filtered through absorbent cotton and boiled in 5 litre flask at 100^oC, cooled and kept in the culture room before further process. The culture room was being a controlled environment of temperature 25^oC, fluorescent light was provided from four ft. tube light above the culture flask and photoperiod was maintained in 16:8 hours of light and dark period. The experiments were conducted to determine the growth of *Chaetoceros calcitrans* in different concentration of enrichment. The experiment was conducted for a period of one month. In the first concept the enrichment was made by Walne's (1974) medium at different concentration such as 25%, 50%, 75% and 100% added on different days of culture. The 100% enrichment was taken as control. Duplicate samples were taken for all the treatments. The stock was maintained in the laboratory under controlled conditions of temperature (25^oC), salinity (30 ppt) and the photo period of 16: 8 hours total darkness. An initial inoculum of 15x10⁶ cells/ml of culture was added to all the flasks.

Culture conditions: One litre of sterilized water was put in each 34 flasks containing different concentration of Walne's medium constituting 25%, 50%, 75% and 100% of enrichment. Further enrichment was given on initial, 5th, 10th and 15 days of culture period. All the 34 flasks were inoculated with 10 ml of stock culture of *Chaetoceros* having concentration of 14×10^6 cells/ml. Two flasks without any addition of enrichment were also kept for observing the growth without providing additional enrichment.

In each treatment the medium is added at every 5 days interval. Each treatment group is taken for growth study immediately after adding the medium, in a spectrophotometer at λ 430 nm. The readings of all treatments were taken in alternate days also. All the cultures were illuminated with light of fluorescent tubes.

Aeration was not provided to the cultures; instead cultures were shaken manually to give three to four rotations every now and then to keep them in uniform suspension. Growth study was carried out by three methods. i. by measuring the cell count using haemocytometer ii. by measuring the growth by percentage of transmittance iii. by estimating the chlorophyll *a* content by spectrophotometry.

Measurement through cell count: The cells were counted under the microscope at 100x using a calibrated haemocytometer and expressed in number of cells/ml.

Measurement through percentage of transmittance: Cultures were taken for growth study at every alternate day using Genesys spectrophotometer. The transmittance rate was measured at 430, 530 and 678 nm with reference to the seawater.

Measurement through estimation of chlorophyll *a* content: Quantity of chlorophyll *a* is also used as an index of physiological activity. The concentration of chlorophyll *a* was estimated by spectrophotometric analysis of acetone extracts⁷.

20ml of culture was filtered and pigments were extracted by adding 10 ml of 90% acetone to each residue. The extraction was carried out at low temperature for 20 hrs. The extracts were centrifuged and the supernatant was measured with Genesys spectrophotometer at 630, 645 and 663nm.

Data analysis: Data were analyzed using (ANOVA). When a significant deviation was found, the mean values were tested for the significance⁸. Statistical analysis was performed using the SPSS 10.00 version for Windows and results were tested for significance at 1% level.

Results and Discussion

Growth study by estimating the cell concentration: In the flask with 100% enrichment treated as control of the experiment indicated active transmittance reading up to 20th day, after that the transmittance rate reading indicated declination in the

growth rate. Minimum value indicating maximum growth was obtained in the 20th day (36%) with reference to sterilized seawater.

Upon adding the enrichment on the initial day it was observed that there was not much difference in the cell concentration up to 5th day. On the 10th day the cell concentration was more prominent in the 100% concentration followed by the 75, 50 and 25% containing 168, 159, 142 and 130×10^6 cells/ml respectively. In the 100% enrichment the growth was found to be 19% less than the control. There was not much difference between the treatments. On the 15th day also the higher concentration was observed for the 100%, followed by the 75, 50 and 25%. Not much difference was observed between the 100, 75 and 50 %, but the 25% showed a decline of growth by 16% from the 50%. On the 20th day it was 276, 296, 393 and 400×10^6 cells/ml for 25, 50, 75 and 100% respectively. On the 25th day wide variation was observed between the control and 100% concentration. But there was not much difference between the 25 and 50% concentrations, and between the 75 and 100% concentration. The difference between 75 and 50% was 11.5% on the 30th day at the same time it was almost same for 100% and control (figure 1).

Enrichment on the 5th day also showed a similar growth pattern up to 5th day. The growth pattern and rate was almost similar to that on the initial day. From 10th day onwards the growth increased with increase of enrichment showing a growth rate of $130, 142, 157$ and 163×10^6 cells/ml for 25-100% concentrations respectively. Control has a concentration of 200×10^6 cells/ml. There was a difference of 22.6% between the control and the 100% concentration. On the 15th day the control has 315×10^6 cells/ml while that for 25-100% was 250, 290, 318 and 330×10^6 cells/ml respectively. On the 20th day also more prominent growth was observed for the 100% followed by the 75, 50 and 25%, ($330, 318, 290$ and 250×10^6 cell/ml respectively). Control has 400×10^6 cells/ml; there was difference of 3.7 % between the 100 and 75%. On the 25th day wide difference in the cell concentration was observed between the 100% and the control (17.6%), control has high value, then between the 75 and 50% there was a difference of 10.7%. On the 30th day there was reduction in the cell concentration indicating a declination phase. The observed values were 224, 223, 247 and 294×10^6 cells/ml for 25-100% concentrations. Control has 300×10^6 cells/ml. The difference between 100 and 75% was 19% while that for the 50 and 75 was 10.7%. The acceleration phase was from 5-10th day. The rate of acceleration on the 5-10th day for the control was 23.35 while all the treatment groups showed more than 2 fold increase. From 10-15th day it was 57.5% for control, which was almost similar for the 100 and 75% and least for the 25%. On the 25th day the highest declination rate was observed for the treatments than the control (figure 2).

Enrichment on the 10th day showed not much difference for all concentration upto 10th day. But wide variation from control was observed from 5th day onwards. Compared to the control

there was much reduction in the cell concentration for all the treatment groups. Among the four treatment groups the higher concentration was noted for the 100%, followed by the 75, 50 and 25% respectively. On 5th day the control showed more or less 2 fold increase from the treatment groups. On 10th day it was three fold increase for control. On 15th day 1.5 fold increase was there for the control over the 100% concentration, while the 25, 50 and 75% has almost same cell concentration. On 20th day the maximum cell concentration was observed for the control and the treatments. The 75 and 100% concentration have same cell concentration, at the same time 50 and 25% have the same concentration. The difference between the 50 and 75% was 6.34%, while that for 100% and control was 44.75%. Almost same reading was observed for the 25th day's concentration. In short the values of cell concentration are much lesser than that on the 5th day's enrichment (figure 3).

Addition of the medium on the 15th day indicates not much difference between the treatments up to the 10th day. On the 15th day the 25 and 50% showed not much difference between them. The values were 127, 130, 142 and 147 for 25-100%

concentration respectively. Between 50% and 75% there was a difference of 9.2%. On the 20th day maximum value was obtained for all. But the 25% showed the lower cell concentration, indicating that there was increase in cell concentration with increase in the media concentration. The values on 20th day were 200, 205, 218 and 221 for 25-100% concentration. On the 20th day the control was 131.1% higher over the 100% concentration indicating that the addition on the 15th day did not bring much increase in the cell concentration (figure 4).

The ANOVA indicates that the cell concentration was significantly influenced by the day in which the enrichment was provided concentration of medium and the age of culture. The DMRT indicated that the control has significantly ($P < 0.01$) higher concentration of cell than the other. Among the treatment groups the 100% showed significant cell concentration followed by the 75, 50 and 25%. It was also revealed that on 20th day the cell concentration is significantly higher over the other and addition of medium on the 15th day showed significantly lower concentration than the others.

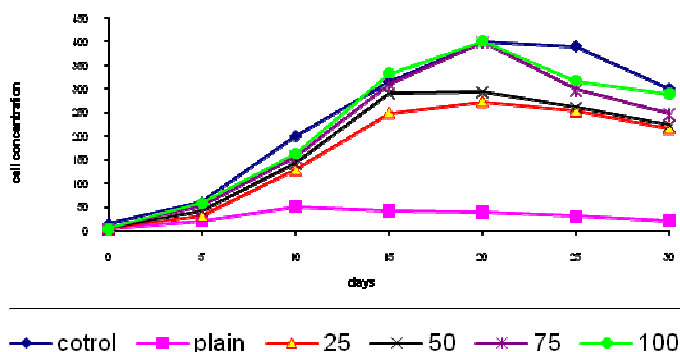


Figure-1
 Cell concentration on initial day of enrichment

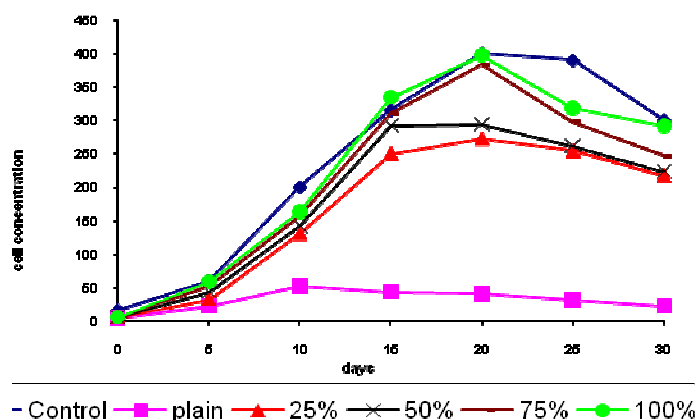


Figure-2
 Cell concentration on 5th day enrichment

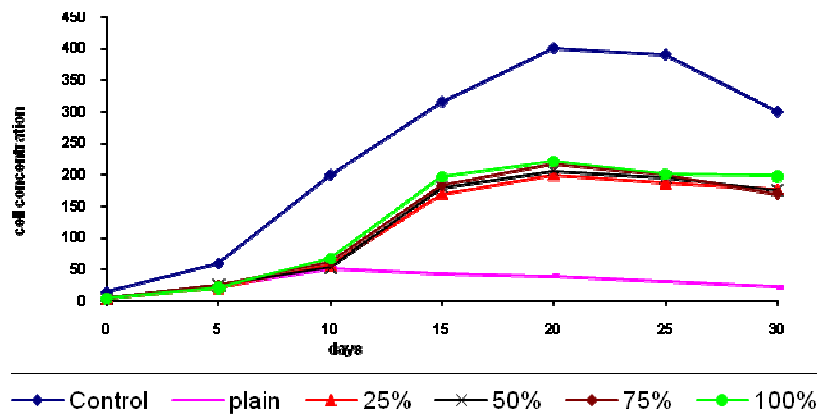


Figure-3
Cell concentration on 10th day enrichment

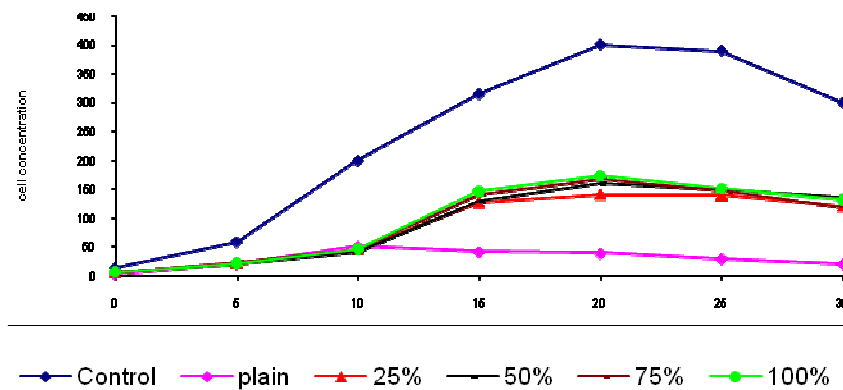


Figure-4
Cell concentration on 15th day enrichment

Growth study by estimating the percentage of transmittance: Enrichment of seawater on the initial day period did not showed wide variation of growth among treatments of 25.50 and 75%. Where as there was a named increase in growth in the 100% enrichment, which was very prominent from 10th to 20th day of growth. On 25th and 30th day there was declination indicated by increased transmittance values. The culture without enrichment showed growth only up to 10th day and there after declination. For all the concentration there was active phase from 5-10th day. Then from 15th day onwards only slow increase was noted and remains unchanged after the 20th day corresponding to the death phase of the growth (figure 5).

Addition of enrichment on 5th day showed a pattern of growth which was similar to that of initial day's enrichment. Here the group without enrichment showed growth up to 10th day followed by decline phase. For all other treatment groups the growth rate was similar without much difference between them throughout the culture period and the difference between the treatment and control was also negligible even on the exponential day constituting transmittance of 54.9, 53.9, 53.73

and 53.2% respectively for concentration ranging from 25-100%. The value of the control was 53.6%. In the decline phase also almost same value were observed for all the concentrations. The acceleration phase was between 5-10th day (figure 6).

The 10th day enrichment showed similar growth rate up to 5th day. But from 10th day onwards slight difference in growth was noted in which the lower transmittance was for the 100% followed by 75, 50 and 25%. The values were 76, 74, 73.4 and 71.6% for 25-100% concentration. The value for control was 68.9%. The control showed an increase of 2.7% over the 100% concentration. While that for 100% over the 25% concentration was 17.1%. On 15th day also almost similar values were noted for the concentration ranging from 25-100%. On 20th day the maximum value was observed for all treatment groups. There was wide variation between the control and 100% (22.6%). The declination phase was from 25th day. The acceleration rate was higher for the control than the treatments (figure 7).

The enrichment on 15th day showed a transmittance almost similar up to 10th day with a decrease of 9.1% from the control.

On 15th day also there was not much difference between the treatment groups 76, 74, 72 and 72 for 25-100% concentrations, while that for control was 55.8 showing a high rate of reduction from the control. The 100% showed 16.4% lesser growth rate than that of the control. On 20th day minimum value of transmittance was noted for all 74, 72, 70 and 69.8 for 25-100% concentrations with more reduction in growth for 100% from the control (16.2%). The acceleration phase was from 5th day onwards. The rate of acceleration was higher for the control than the treatments. The declination phase was noted from 25th day onwards and the rate of declination was lower for the control than the treatments (figure 8).

The three way ANOVA indicated that the transmittance was significantly affected by the concentration of medium, the day on which the enrichment was provided and the age of culture. From the DMRT it was revealed that the 100% concentration produce significantly (P>0.01) lower values of transmittance indicating higher growth over the 25% concentration, at the same time enrichment on 15th day produce significantly higher transmittance indicating lower growth rate than the initial day. It was also clear that transmittance on 20th day was significantly lower compared to that on the initial day.

Growth study by estimating chlorophyll a content: Upon adding the enrichment on the initial day there was not much difference in the chlorophyll concentration among the treatments of 25, 50, 75 and 100% (control) concentrations, which was varied from 0.034 to 0.039 µg/ml. The 25% concentration has a chlorophyll content which is 12.8% lesser than that of the 100% which is the control. Same pattern was noted up to the 30th day. Maximum chlorophyll values were observed on 20th day, but it was increased with increase in concentration constituting chlorophyll values of 0.135, 0.136, 0.139 and 0.141µg/ml for 25-100% concentration. Here not much difference was noted between the chlorophyll values of the four concentrations. On the 25th and 30th day also not much difference was noted between the chlorophyll values of all the groups. The lag phase was from the 0-5th day and active growth phase from 5-10th day, and then there was slow and steady increase in the chlorophyll value, followed by a declination from 25th day onwards. The rate of acceleration was higher for the 100% and least for 25%. On the 30th day not much difference was noted for the chlorophyll values of the four concentrations, 0.128, 0.130, 0.131 and 0.131µg/ml for 25-100% concentrations respectively (figure 9).

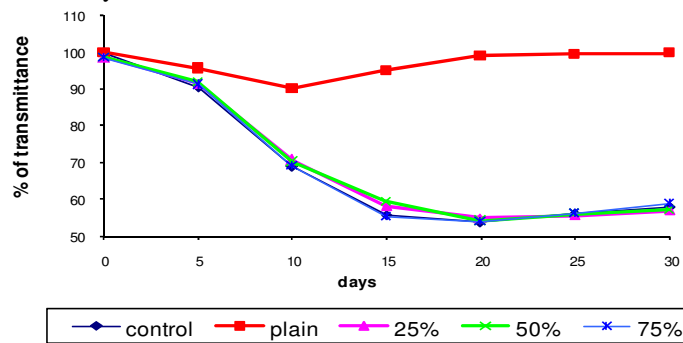


Figure-5
 Percentage of transmittance on initial day of enrichment

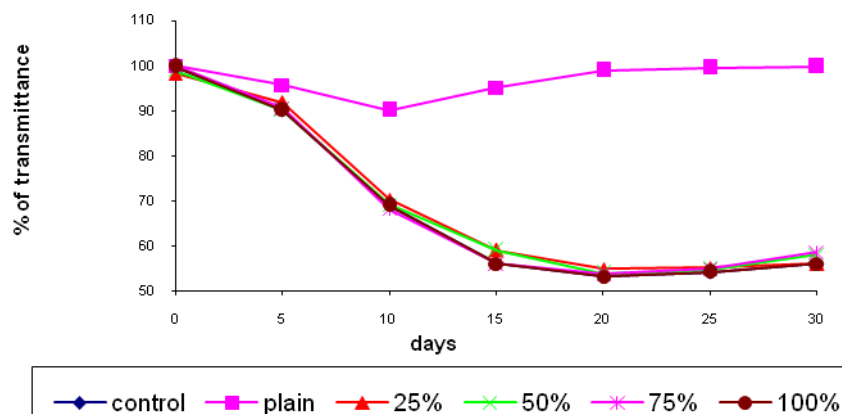


Figure-6
 Percentage of transmittance on 5th day of enrichment

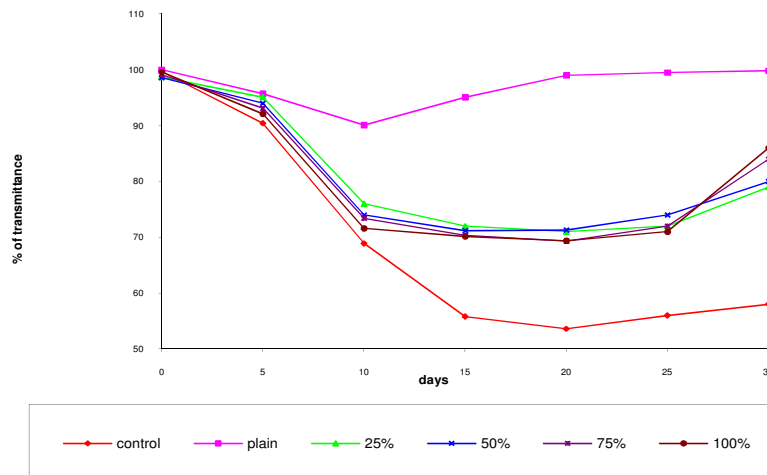


Figure-7
 Percentage of transmittance on 10th day of enrichment

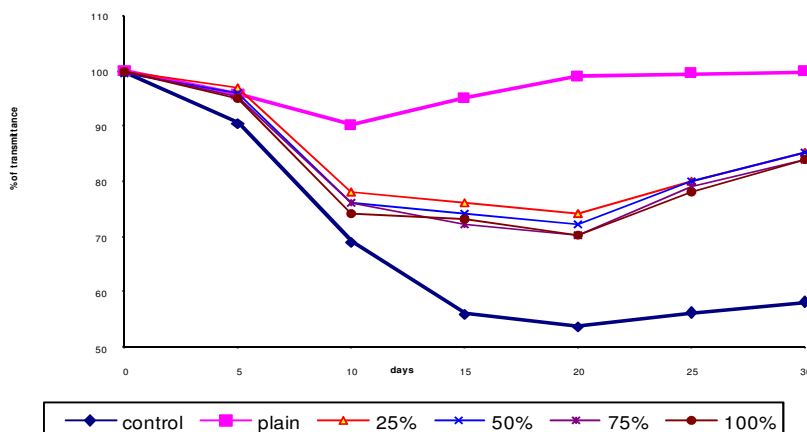


Figure-8
 Percentage of transmittance on 15th day of enrichment

Providing enrichment on 5th day almost similar values of the initial day's enrichment was observed. Here also not much variation up to 5th day was noted. On 5th day the values were 0.031, 0.333, 0.039 and 0.042 $\mu\text{g/ml}$ for the 25-100% concentrations respectively. Here the 100% has an increase of 7.7% over the 75%, at the same time 75% showed an increase of 18.1% over the 50%, and the 25% concentration was lesser than the 50% by 6.4%. On the 10th day there was marked difference (5%) between the 25 and 50% concentration was observed, 0.13, 0.129, 0.126 and 0.120 for 100-25% respectively. The same pattern was for 15th and 20th day. The maximum value of chlorophyll *a* was observed on 20th day 0.131, 0.135, 0.137 and 0.139 $\mu\text{g/ml}$ for 25, 50, 75 and 100% concentration respectively. There after, there was declination from 25th day onwards. Here also the lag phase was from initial to 5th day followed by an

active growth phase from 10-15th day, then steady increase and declination (figure 10).

The chlorophyll *a* values on adding the enrichment on 10th day showed much reduction than that on 5th day. There was not much deviation up to the 5th day. In all the following days marked increase was noted for all concentration, and the increase was higher for the 100% concentration than any other treatments. The culture without enrichment showed active growth up to the 10th day then declination was observed. On 10th day the value of 100% concentration was 67% lesser than that of control. The 75% concentration was 25% greater than the 50%. Between the 50 and 25% there was a difference of 25%. The values for chlorophyll *a* on 10th day were 0.079, 0.075, 0.061 and 0.48 $\mu\text{g/ml}$ for 10-25% concentration, while

that for control was 0.132 µg/ml. On the 15th day the 50, 75 and 100% concentration showed almost the same reading without much difference, 0.086, 0.089 and 0.089 for 50-100% respectively, which was nearly 55% less than the control. For the 25% concentration a value of 0.062 was observed which was 38% lesser than the 100% concentration. In that group in which no enrichment was added there were lower chlorophyll *a* values than the 25% concentration. On the 20th day same pattern was observed but a difference of 33.8% was there between the 25 and 50% concentration. The control showed an acceleration phase from 5-10th day (221.9%) increase. Then there was lower rate of increase from 10-15th day (4.5%) and from 15-20th day (2.2%) then declination was observed. The rate of acceleration for 100 and 75% is lesser than that of the control (102%) while that for 50 and 25% again less (33.3 and 66.6 respectively) (figure 11).

Upon adding the different concentration of enrichment on 15th day no difference was observed up to the 10th day. On 15th and 20th day growth was more pronounced in the 100% concentration followed by the 75, 50 and 25%. On 15th day the 25 and 50% concentration has almost same values 0.041 and 0.042 µg/ml respectively, while 75 and 100% concentration showed similar values of 0.051 and 0.056 µg/ml respectively. Much difference was observed between the 50 and 75%

concentration on 15th and 20th day (21.4 and 20%). From the 25th day the growth was declined. In each day observation there was wide variation between the control and different treatments. Eventhough the value for control was higher among the treatment; it was lower in comparison with the control (figure 12).

The three way ANOVA indicated that there was significant relation between the chlorophyll value and the day on which the enrichment was provided, the age of culture and the concentration of the medium. From the DMRT it was clear that the addition of medium on 15th day has significantly less effect ($P>0.01$) on the chlorophyll *a* value than the initial day, and the chlorophyll *a* value on the 20th day is significantly higher than that on initial day. It was also observed that the 100% concentration provides significantly higher values of chlorophyll *a* than the 25%.

Discussion: The physical, chemical and biological factors of the aquatic ecosystem affect the species diversity of algae. The effect of nutrient concentration on *Cheatoceros calcitrans* under laboratory conditions, is studied to assess whether the nutrients present in seawater is sufficient for the entire growth of culture or addition is needed.

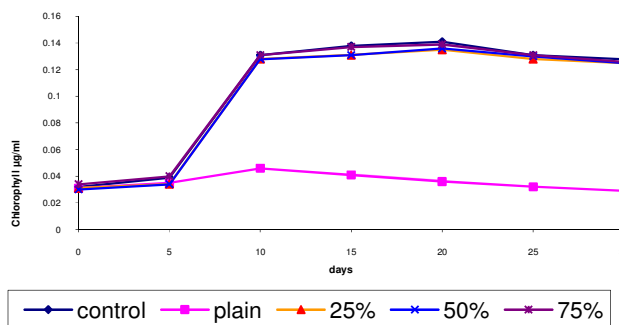


Figure-9
 Chlorophyll on initial day's enrichment

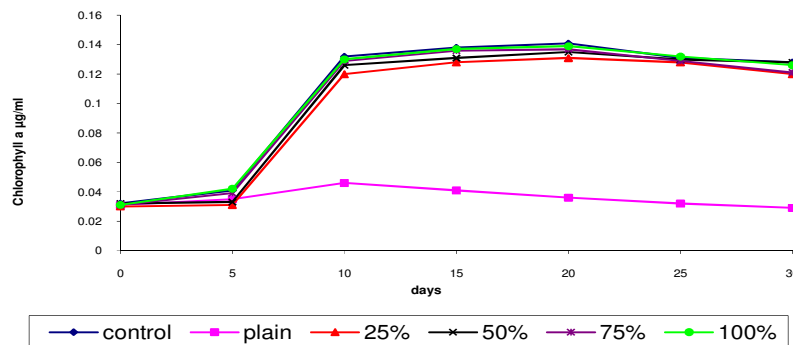


Figure-10
 Chlorophyll on initial 5th day enrichment

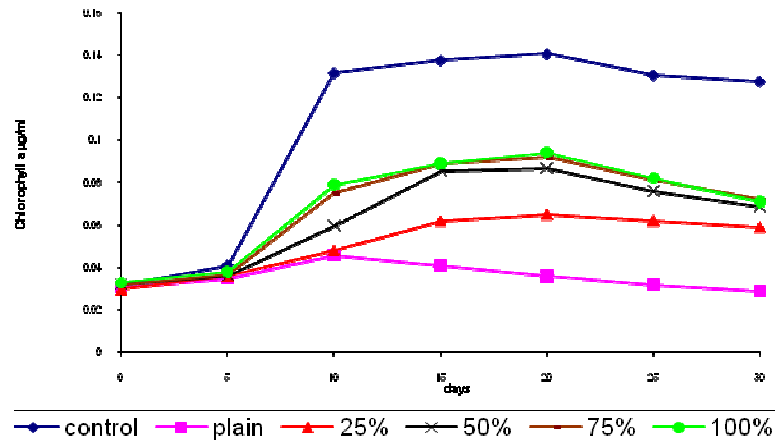


Figure-11
 Chlorophyll on initial 10th day enrichment

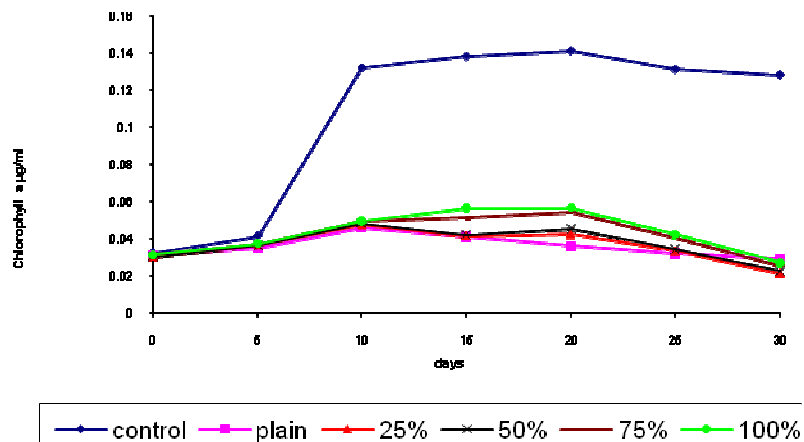


Figure-12
 Chlorophyll on initial 15th day enrichment

In natural seawater blooming may be due to the nutrient enrichment as a result of upwelling, land run off and man made sources etc. Nowadays increased rate of nutrients like NO₃, NO₂ and PO₄ was there in the seawater due to increased use of chemical fertilizers, the influx of water containing the residuals of fertilizers will cause the increased rate of these nutrients in the seawater. In addition to this, as a result of industrialization large amount of sewage were getting deposited in the seawater. Actually we are using the enriched seawater for culture experiment, by adding further chemicals. This experiment was conducted to study whether there is any need of additional amount of chemical enrichment for growth of microalgae. Gopinathan⁹ reported that the soluble fraction of hydro carbons at low concentration seem to enhance the rate of photosynthesis of microalgae.

The potentialities of algae as source of food, feed, fodder and manure has been further established by the extensive research carried out during the past few decades. The economic

utilization of algae necessitates the development of techniques for axenic culturing of these organisms in large scale. According to Venkataraman and Gopinathan¹⁰ no single medium can be said as the best one. Since the nutritional requirements of algae vary with species, the successful and long term culturing of any algal species demands a thorough understanding of its nutritional requirements which can be studied under controlled laboratory conditions using unialgal cultures. The works of Ammini Joseph¹¹ on *Isochrysis* and *Tetraselmis* and that of Sathi¹² on some phytoflagellates have shown that in higher concentrations of nutrients there was reduction in the chlorophyll *a* content than in the optimum¹⁴. Varying concentrations of nutrients influence the productivity in culture system.

From the present study, it is clear that there was increase in the rate of growth along with increase in concentration in each enrichment except in plain. In plain the maximum growth was up to 10th day. In all others it was up to 20th day. The

maximum growth was on 5th day enrichment flask. In all others it was reduced. The maximum utilization was found on 5th day. So we can conclude that up to 5th day there is no need of enrichment because the system can utilize the nutrients of the seawater itself up to the 5th day and we can save the chemicals up to the 5th day.

In the cell concentration study also eventhough there is increase in cell concentration along with the increase in the medium concentration in every day's addition there is much more increase in the 5th day's addition itself compared to that of 10th day and 15th day. This is due to the increased enrichment of seawater nowadays due to sewage and other effluents.

The chlorophyll *a* values of the 5th day's enrichment and initial day's enrichment showed similar growth pattern, indicating not much fluctuation upto the 5th day. As compared to the early days, the enrichment of seawater is very high in the south west coast of India due to the industrial effluents and the culture can survive upto the 5th day without any additional enrichment in the highly enriched seawater.

Form the present investigation it is evident that the increased rate of various nutrients in the seawater will definitely affect the growth of the *Cheatoceros calcitrans* under laboratory condition during the initial days of growth.

Conclusion

There have been very few attempts in the past to study the nutritional requirements of micro algae under laboratory conditions. In this account an attempt was made to study the optimum concentration of culture media required on suitable period of culture of *Chaetoceros calcitrans* under controlled conditions so as to achieve a viable and economical method of culture. The main objective of the study was primarily to determine the effect of hydrological parameters especially nutrients which was supplied externally to the seawater while culturing the diatom under laboratory conditions, and to determine its optimum concentration for ideal growth of the culture, in such a condition of increased rate of pollution due to the industrialization and effluents. The cell count, percentage of transmittance and the chlorophyll *a* values are significantly higher for the group in which the medium was provided on the initial day. Almost similar values were also noted for the 5th day's enrichment, indicating that the culture can grow upon the nutrients present in the seawater up to the 5th day and enrichment is needed only on 5th day. It was found that the addition of medium on 5th day have almost the same effect on

the growth of *Chaetoceros* as that on the initial day indicating that they can grow upon the nutrients present in the sea water up to the 5th day. So we can reduce the wastage of chemicals by providing the enrichment on the 5th day.

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