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Research Article

Growth Studies of *Dicrateria inornata* Parke. M. under Different Physical and Chemical Parameters

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Abstract

The experiment was performed to evaluate culture and growth performance of genus *Dicrateria inornata*Parke. M. Three different growth media i.e., Natural Sea water, f/2 and Walne's were selected for the comparative analysis. The culture flask containing 250ml media were inoculated in 50ml of axenic culture of *D. inornata*Parke. M. The culture flask were incubated at 24 ± 2°C for 12 hours light and12 hours in dark photoperiod. The media at 5 different pH ranging from 5 to 9 were observed. The growth rate of *D. inornata*Parke. M was analyzed by cell count method with the help of haemocytometer at an interval of 48 Hours. The maximum growth was observed at the end of 15th day in Walne's media (23.2 cells/100µl), in F/2 media (18.1 cells/100µl) and in sea water media (12.4cells/100µl) on 11th day at pH 8. The Effect of different photoperiod on the growth of *D. inornata* Parke. M.in natural sea waterat 16hrs light and 8 hrs dark (11.8cells/100µl) maximum growth achieved on 11th day. The maximum growth achieved on 15th dayin F/2 media (18.1cell/100µl) and Walne's media (24.9cells/100µl) at 16hrs light and 8hrs dark condition. Thus it can be concluded that at different pH the influence of chemical compounds on the growth of *D. inornata* Parke. M. varies.

Keywords: *D. inornata* Parke. M., f/2 Media; Walne's; Sea Water Media; Microalgae

Introduction

Microalgae play an important role in the marine ecosystem as a primary producer which contribute to maintain the biological production and eventually to enhance the fishery production. By considering the increasing demand for energy the microalgae base biofuel can replace fossil fuel [1]. The concentration of three different medium were used to determine the effect on growth of *D. inornata*Parke. M. viz sea water, F/2 and Walne's medium [2] studied that the marine microalga *D. inornata*Parke. M when enriched with F/2 medium shows different growth rate. Microalgae need a light as well as dark regime for productive photosynthesis, and light conditions and Temperature affect directly the growth rate of microalgae. Photoperiod strongly influence the cellular chemical composition, uptake of nutrient, $CO_2$and the growth rate of microalgae [3]. The pH is one of the most critical environmental condition in microalgal condition since it determine the solubility and availability of CO_2 and nutrient and has significant influence on microalgal metabolism [4]. During the daytime as the pH of microalgal cultures rises then uptake of inorganic carbon by microalgae increases. The availability of CO_2 gets lower as there is increasing pH so that inhibition in cell growth occurs [4].

Materials and Methods

Algal cultures

The axenic culture of marine cyanobacterium *D. inornata* Parke. M. obtained from algal collection center, Anna University, Chennai (India) used for the experiment. During this study three different *mediai. e.* Natural sea water, F/2and Walne's [5] medium were used to analyze the growth of *D. inornata* Parke. M. 1 liter medium containing 900 ml natural sea water was taken and required volume of each stock was added serially in the filtered Natural sea water as per the composition of the medium. The final volume was made up to 1 liter by using natural sea water. Final medium was distributed in 500ml of conical flask each containing 250 ml medium. pH was maintained by using 0.1N NaOH and or 0.1N HCl as per requirement. The media was sterilized and after cooling for 24 hours the definite value of axenic culture of microalgae (*D. inornata*) was inoculated in all conical flasks. In sterilized conical flasks 250ml of media and 60 ml axenic culture of *D. inornata* inoculated under aseptic conditions. The present work shows the effect of three different growth media, pH and Photoperiod on the growth of *D. inornata*. The flasks were incubated in growth room at temperature 20-25°C and at 1000 lux light intensity. The growth of *D. inornata*in the flasks were observed at each day. Microalgae was observed under (Leica DM 2500) microscope and cell concentration was monitored after every 48 hours using haemocytometer.

Sr. No.	Reagents	Per litre Sea Water
01	Solution A (Nutrient solution)	1.0 ml
02	Solution C (Vitamin solution)	0.1 ml

Table 1: Composition of Walne's medium.

Walne's medium composition: (Andersen.,2005).

Sr. No.	Solution A	Quantity (gram/litre D.W.)	Concentration in final medium
01	$FeCl_3.6H_2O$	1.3	4.81×10^{-6}
02	$MnCl_2.4H_2O$	0.4	1.82×10^{-6}
03	H_3BO_3	33.60	5.43×10^{-4}
04	Na_2EDTA	45.00	1.54×10^{-4}
05	$NaH_2PO_4.2H_2O$	20.00	1.28×10^{-4}
06	$NaNO_3$	100.00	1.18×10^{-3}
07	Solution B (Trace metal solution table no.3)	1 ml	-

Table 2: Composition of Solution A (Nutrient solution) for Walne's medium.

Sr. No.	Solution B	Quantity (gram/litre D.W.)	Concentration in final medium
01	$ZnCl_2$	21.0	1.54×10^{-7}
02	$CoCl_2.6H_2O$	20.0	8.41×10^{-8}
03	$(NH_4)\ Mo_7O_{24}.\ 4H_20$	9.0	7.28×10^{-9}
04	$CuSo_4.\ 5H_2O$	20.0	8.01×10^{-8}

Table 3: Composition of Trace metal solution for Walne's medium.

Sr. No.	Solution C	Quantity (gram/litre D.W.)	Concentration in final medium
01	Thiamine HCl (Vitamin B_1)	1.00gm	2.96×10^{-10}

Table 4: Composition of Solution C (Vitamin solution) for Walne's medium.

F/2 medium composition (Andersen.,2005).

Sr. No.	Component	Stock solution gram/litre D.W.	Quantity	Concentration in final medium
01	$NaNO_3$	75	1 ml	8.82×10^{-4}
02	$NaH_2PO_4.H_2O$	05	1 ml	3.62×10^{-5}
04	Trace Metal solution	(See the table no.6)	1 ml	_
05	Vitamin solution	(See the table no.7)	0.5 ml	_

Table 5: Composition of F/2 medium.

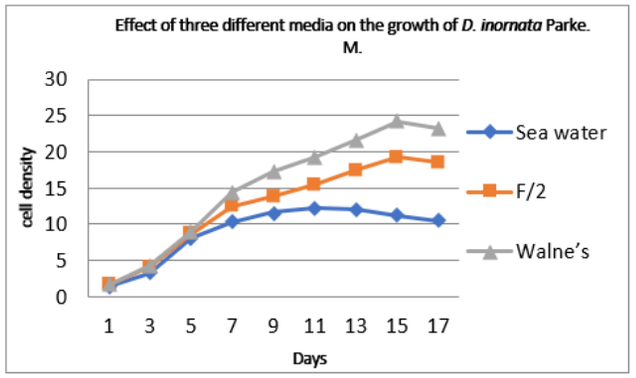
Sr. No.	Component	Stock solution gram/litre D.W.	Quantity	Concentration in final medium
01	$FeCl_3.6H_2O$	-	3.15 gm	1.17×10^{-5}
02	$Na_2EDTA.2H_2O$	-	4.36 gm	1.17×10^{-5}
03	$MnCl_2.4H_2O$	180.00	1 ml	9.10×10^{-7}
04	$ZnSO_4.7H_2O$	22.00	1 ml	7.65×10^{-8}
05	$CoCl_2.6H_2O$	10.00	1 ml	4.20×10^{-8}
06	$CuSo_4.\ 5H_2O$	9.8	1 ml	3.93×10^{-8}
07	$Na_2MoO_4.2H_2O$	6.3	1 ml	2.60×10^{-8}

Table 6: composition of Trace Metal solution for F/2 medium.

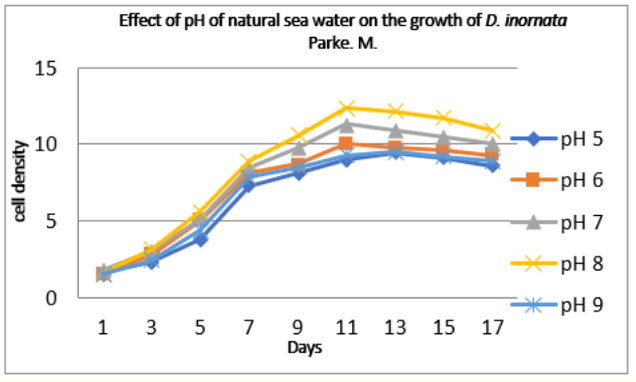
Sr. No.	Component	Stock solution gram/ litre D.W.	Quantity	Concentration in final medium
01	Thiamine HCl (Vitamin B_1)	-	200 mg	2.96×10^{-7}
02	Biotin (vitamin H)	1.0	1 ml	2.05 × 10-9

Table 7: Composition of Vitamin solutionfor F/2 medium.

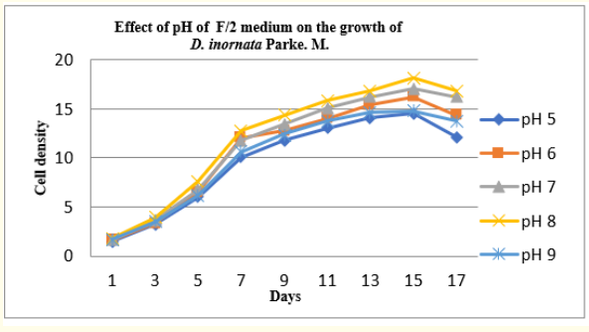
Results



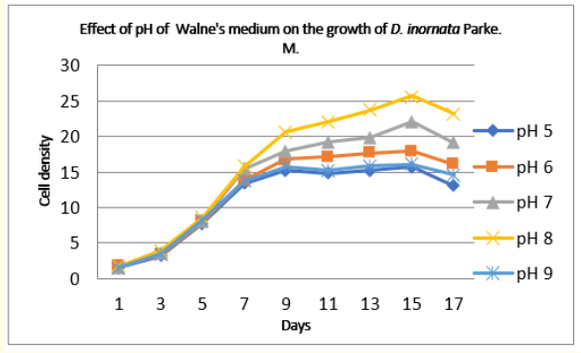
Graph 1: Effect of three different media on the growth of *D. inornata* Parke. M.



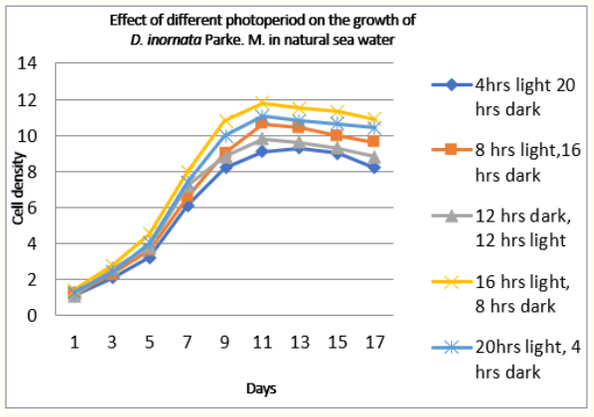
Graph 2: Effect of pH of natural Sea water on the growth of *D. inornata* Parke. M.



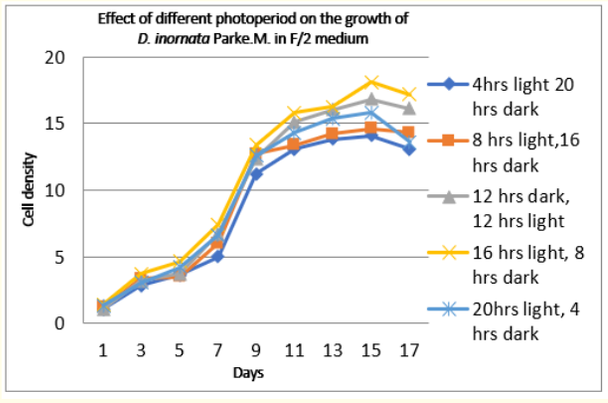
Graph 3: Effect of pH of F/2 medium on the growth of *D. inornata* Parke. M.



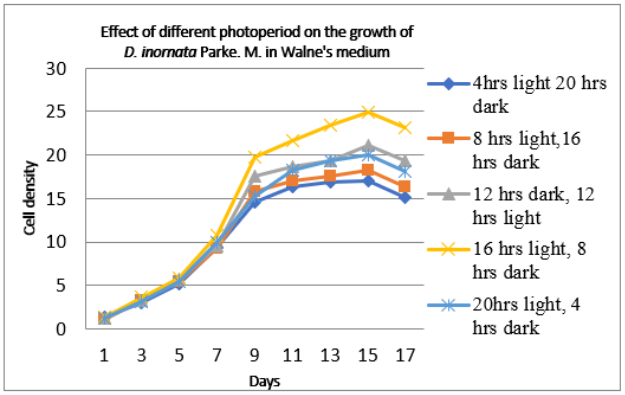
Graph 4: Effect of pH of Walne's medium on the growth of *D. inornata* Parke. M.



Graph 5: Effect of different photoperiod on the growth of *D. inornata* Parke. M.in natural sea water.



Graph 6: Effect of different photoperiod on the growth of *D. inornata* Parke. M. in F/2 medium.



Graph 7: Effect of different photoperiod on the growth of *D. inornata* Parke. M. in Walne's medium.

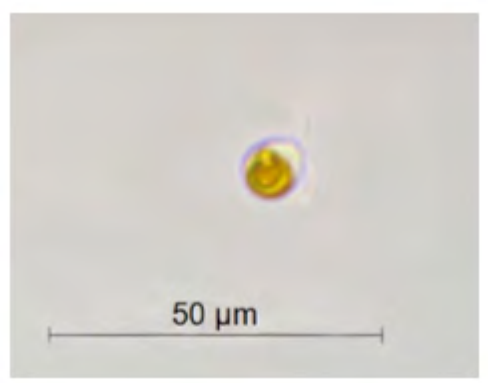


Figure 1: Microscopic view of *Dicrateria inornata* Parke. M.

Discussion

The present study revealed that *D. inornata* Parke. M. shows maximum growth on Walne's medium as compare to other two media. It can be prove that certain nutrient in appropriate quantity are requierd for growth of algae.Now [2] studied that in *Synechocystis salina* by changing physical condition and chemical composition of media the growth rate varies.

The results of three different media had observed which revealed that walne's medium showing maximum growth at 15th day while in case of f/2 medium shows lesser growth as compared to walne's medium at 15th day. The natural sea water had showed minimum growth. [2] also studied that F/2 medium the growth was maximum as compared to sea water. It clearly indicates the effect of different chemical composition of culture medium on the growth of *D. inornata* Parke. M.varies. The recent results are accordance with previous results which expalined different nitrogen sources could affect microalgal growth, as it is well known that in microalgae nutrient assimilation and transport is modulated by nitrogen sources [6].

[7] studied that effect of nitrogen and phosphorous supply on maximum yield in the algae *D. inornata* Parke. M.and *Cheotoceros debilis* to determine the sensitivity of growth to phosphorous limitation.It observed that growth of *D. inornata* Parke. M. decreases as phosphorous depleted in the medium.It had also observed that the growht recover after resupply of phosphorous. It clearly indicate the micralgal sensivity to nutrient limitaton in laboratory culture of microalgae.

Beside nutrient composition there are other factors that affect growth rate of *D.inornata* Parke, M. in laboratory culture for e.g. saliity, pH, photoperiod and temperature. In the present work the effect of range of pH in different culture medium on the growth of *D. inornata* Parke. M. was analysed. The major chemical stimuli are nutrient starvation, salinity and growth-medium pH [8]. An optimal culturing pH range was determined by taking considering not only the cell growth but lipid production by culturing *Chlorella sorokiniana*stain number DOE1412 [4]. From the experiment it had observed that Walne's medium pH 8 shows maximum growth at pH8 while F/2 medium shows at pH 8 and the natural sea water shows differential growth rate at different pH. The maximum growth was observed at pH 7 in natural sea water while the minimum growth was observed on pH 5.

Sunlight is ultimate source of energy.Plant and algae need light for photosynthesis. In case of microalage the photoperiod is one of the most important factor in the growth cycle. The *D. inornata* Parke. M. shows differential growth at different photoperiod. The growth of *Chlorella vulgaris* respond differently on biomass and fatty acid composition as there is change in irradiances and photoperiod [9]. In the present study the effect of five different photoperiod in three different growth medium on the growth of *D. inornata* Parke. M. was analysed. Among five different photoperiod the maximum growth was observed on 16 hrs light and 8 hrs dark condition in all three medium while minimum growth was observed on 4 hrs light and 12 hrs dark condition in all three medium.

Conclusion

The chemical composition of the medium and the physical factors affects the growth rate of *D. inornata* Parke. M.in laboratory cultures. The significant effect of various factors on the growth of *D. inornata*Parke. M. wasrecorded in this study. Among the different medium maximum growth was recorded on Walne's medium. This study has also investigated the significance of artificial medium with specific physical conditions for the maximum growth of the marine microalgae *D. inornata*Parke. M. in laboratory cultures. Among different pH of medium the *D. inornata*Parke. M. shows maximum growth on pH 8 of Walne's medium and on pH 8 of F/2 medium. Thus pH 8 is most suitable for the growth of the D*. inornata* in all the three media studied. The cell number doubles within three days (72hours), but the doubling time may varies depending on light intensity, pH and nutrient contain of the media. It has been prove that Thiamine HCl (Vitamin B_1), and$NaNO_3$having major role in cell growth in Walne's media. Thus at 16hrs light and 8hrs dark conditions shows maximum cell growth in all studied medias.

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