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EFFECT OF NITROGEN SOURCE, VITAMINS AND LIGHT PERIOD ON THE GROWTH OF MARINE DIATOM PHAEODACTYLUM TRICORNUTUM BOHLIN UNDER INDIAN CONDITIONS.

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ABSTRACT

The earth is considered a huge repository of microbes with immense diversities, both in terms of quantity and qualitative characteristics of microorganisms. Diatoms are considered among the highest contributors of primary productivity in the ocean by virtue of their photosynthetic efficiency and accumulation of photosynthates, mainly in the form of lipids. Laboratory scale cultivation of diatoms has long been considered as challenging under Indian conditions due to various factors like temperature , nutrition, photoperiod and specificity of genera chosen for the studies. In temperate regions, P.tricornutum normally flourishes under marine conditions. Successful cultivation of P.tricornutum has been reported with the help of G/f2 medium. But greater insights into the nature of growth of P.tricornutum, biomass accumulation etc was lacking. Standard nutritional requirements along with optimized photoperiod studies could only be the technological imperatives needed for the development of mass cultivation techniques. The present study *focused on the growth of P.tricornutum with different doses of nitrate as N-source, variable amounts of vitamins, when supplemented with the G/f2 medium. Effects of photoperiod was also studied in the growth of the said diatom. The highest growth was obtained with medium supplemented with 2X nitrate , however highest biomass was resulted with 4X nitrate. The choicest photo-period obtained was 14:10 hours of light : dark period. Vitamins had a passive effect with the initiation of growth activities*.

Key words: diatom, growth, g/f2-medium, light-period, nitrate, p.tricornutum

1. Introduction

The mother nature has bestowed the earth with enormous population of microbial genera with diverse species, both on the terrestrial surface and within the aquatic regions. Some are distinct in terms of shape, size, colour, still some others have different biochemical characteristics such as utilization of sulfate, nitrate, silica for their growth and multiplication. Marine diatoms are one such group with huge variations in metabolic characters than their terrestrial counterparts. Nutrient enriched marine areas are mostly crowded with diatoms.Temperature based laboratory scale experiments were limited within the low temperature range up to 25°C. However, shift in the rate of photosynthesis had been also observed(Li and Morris,1982). The photosynthetic efficiency is one of the most important criteria that separates this group from other algal members. The cause of variations in photosynthetic efficiency is mostly related to the nitrogen nutrition of the diatom.Alterations in nitrogen supply is due to the changes or content of the CO_2 fixing enzyme Rubisco (Ribulose bisphosphate carboxylase/ oxygenase)(Osmond,1983). Effects of nitrogen limitation on the growth of *P.tricornutum* was also reported (Osborne & Geider,1986). These views seem to be inadequate on nitrogen nutrition in *P.tricornutum* under Indian conditions principally due to its subtropical temperature regime and high PAR(Photosynthetically active radiation).Thus the present study attempts to find those answers in a lucid yet experimental manner.

2. **Materials & Methods**

P.tricornutum was grown with the help of medium prepared with seawater, supplied with necessary vitamins and trace elements(Guilard & Ryther,1962). The amount of sodium metasilicate added was 3% (v/v). The medium(without vitamins) was first autoclaved at 121°C and 15psi.Then filter- sterilized vitamin solution was added to it.

Growth experiments were performed by inoculating 30ml of inoculum in 120ml of medium with an Erlenmeyer's flask(500ml) in five replicates. Growth was monitored at 625nm spectrophotometrically(UV-VIS, Shimadzu) with samples diluted five time from each flask. The growth temperature was maintained at 25± 0.5°C.

Variations in Growth was monitored with differential doses of nitrogen in the form of nitrate(zero , X and 2X), vitamins (with or without) and mineral solution(with or without). The variation of light : dark period was 10:14 ; 12:12 and 14 :10 hours respectively. The light source was cool white fluorescence tube which gave an mean incident photon density of 200µmol m^{-2} s^{-2}.

Biomass estimations were done after the growth monitored by centrifugation at 5000rpm for 15 minutes. Dry biomass was obtained by drying at 60°C overnight.

3. **Results**

All the nutrient treatments (nitrate,vitamins and minerals) have showed the following individual effects in terms of growth of *P.tricornutum* as described in the fig 1,2, 3 and 4.

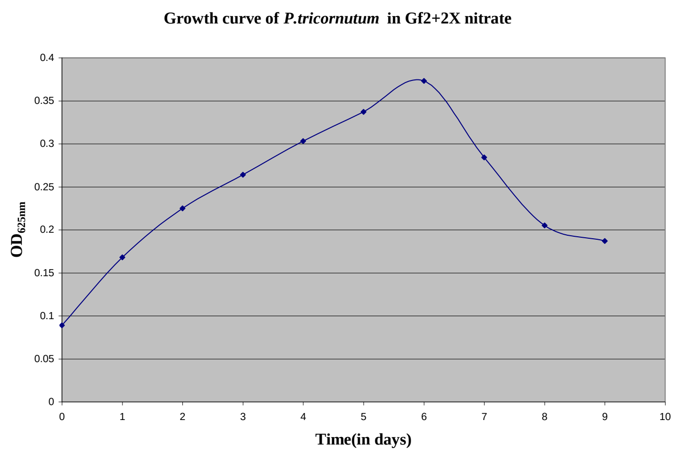

Fig 1.Growth with 2X nitrate.

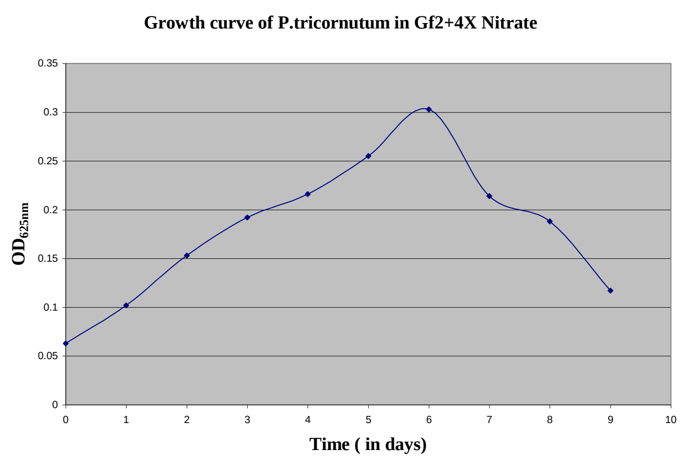

Fig 2. Growth with 4X nitrate.

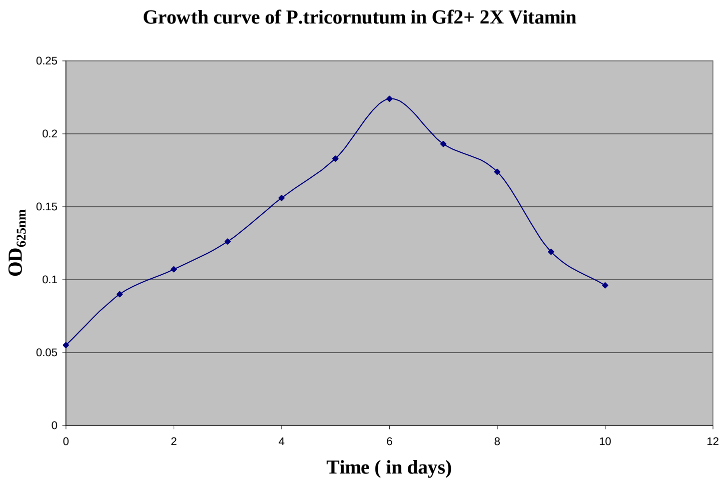

Fig 3. Growth with 2X vitamins.

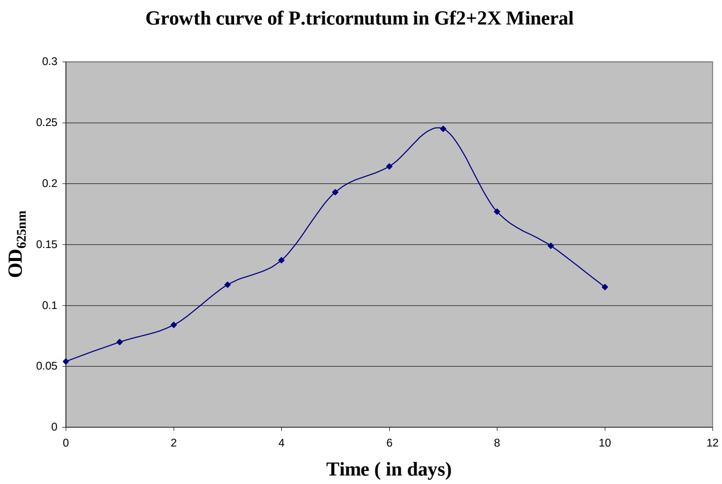

Fig 4. Growth with 2X minerals.

The growth and biomass responses of the different treatments are summarized below in table 1.

Table 1. Comparative growth and biomass responses against the treatment of nutrients .

Treatment	Highest OD_{625nm}	Sp. Growth rate(day^{-1})	Dry Biomass /litre obtained (g)	Biomass / day(g)
2X nitrate	1.805	0.312	1.033±0.0021	0.032
4X nitrate	1.55	0.2561	1.056±0.0034	0.0334
2X vitamins	1.153	0.1995	0.925±0.0011	0.0115

2X minerals	1.2	0.211	0.744±0.0002	0.0102

The effects of photoperiod on growth and biomass accumulation of *P.tricornutum* has been summarized in table 2.

Table 2. Effect of photoperiod on growth and biomass responses in *P.tricornutum*.

Treatment of Nutrients	**Type of Photoperiod (Light : Dark hours)**					
	10:14		**12:12**		**14:10**	
	Biomass(g)/lit	**OD_{625nm}**	**Biomass(g)/lit**	**OD_{625nm}**	**Biomass(g)/lit**	**OD_{625nm}**
2X nitrate	1.033±0.0021	1.805	0.926±0.0023	0.961	0.624±0.0031	0.772
4X nitrate	1.056±0.0034	1.55	0.873±0.022	0.977	0.565±0.0013	0.773
2X vitamins	0.925±0.0011	1.153	0.443±0.001	0.967	0.384±0.004	0.669
2X minerals	0.744±0.0002	1.2	0.552±0.021	0.966	0.486±0.0021	0.724

Both the highest OD_{625nm} and biomass were obtained under a photoperiod of 10: 14 of light: dark hours.

4. Conclusion

The successful growth of *P.tricornutum* under Indian conditions has been achieved which once again proved that nutrient conditions are one of the most predominant factors in the adaptation of a given algal member, here it is the marine diatom *P.tricornutum*. It is also confirmed that the organism can utilize the nitrate as nitrogen source with availability of vitamins and minerals at usual doses of G/f2 medium. Dry biomass recovery was more in case of higher nitrate dose which gave the inference that rate of cellular multiplication is directly linked to the nitrogen nutrition of the organism. These findings can also function as a stepping stone towards the future development of mass cultivation technique of *P.tricornutum* in India.

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