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EFFECT OF PHOSPHATE ON GROWTH OF DIATOMS

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ABSTRACT

Diatoms (Bacillariophyceae) are microscopic unicellular algae found in every aquatic habitat and contribute a large percentage of global carbon budgets through photosynthesis. A study was conducted to assess the effect of phosphate concentration on diatom growth. The freshwater diatoms were cultured in modified SAG media supplemented with different concentration (0.01 mM to 0.07 mM) of K_2HPO_4 as phosphate source and incubated at 20.0 ± 2.0ºC and 4000 ± 10 lux (=lumem/m^2) cool white bulb with 16 h of light and 8 h of darkness. The external supplement of K_2HPO_4 caused the phosphorus availability to be key factor in cell-division rate with increasing concentration of phosphate in medium. In this present study, it was found that Growth responses of diatoms showed gradual increase in the growth at 0.01, 0.02, 0.03, 0.04, 0.05 mM and gets maximum at 0.05 mM concentration of K_2HPO_4 whereas diatom growth was declined when concentration increases more than 0.05 mM.

KEYWORDS: Diatom, phosphate SAG media

Diatoms are the dominant life form in phytoplankton and probably represent the largest group of biomass producers on earth. It is estimated that more than 100,000 species exist (Mc Hugh, 2003).The main storage compounds of diatoms are lipids (TAGs) and a β-1, 3-linked carbohydrate known as chrysolaminarin and are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods and feeds (Walter et al., 2005). Diatoms are known to synthesize a silicified cell wall (frustules) through the intracellular transport of silicic acid (Hecky et al.,1973). Phosphorus is an essential element for all life forms. It is a mineral nutrient. Orthophosphate is the only form of P that autotrophs can assimilate. Extracellular enzymes hydrolyze organic forms of P to phosphate. The effect is studied of different phosphorous concentration on changes in growth rate of diatom species isolated from Tones River. All diatoms examined increasing cell division rate with increasing phosphorous concentration in the medium. Diatoms, however, were not able to dominate when phosphate was deficient, although silicate and nitrate were in excess. More conclusive evidence for the effect of phosphorous on growth rate has been reported using culture of certain algal species (Fuhs et al.,1971).

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF SAMPLE

Fresh water diatoms were collected from Tones River (The Gangetic fresh water) on the Rewa road side which is away from 40 Km from Allahabad. For site characterizations by culturing, fresh water samples (10-50 cm deep) were taken from approximately 10 positions in each site of interest. These samples are typically distributed along transects of interest or are collected from random positions. Sample identification was performed according to the morphology of diatoms. Diatom analysis, sediment treatment and slide preparation followed the method by Battarbee in 1986. About 0.1 g of the sample was taken in a 200 ml beaker and 20 ml hydrogen peroxide (H_2O_2) was added. All the organic matter was removed by heating the contents on a hotplate. Few drops of hydrochloric acid (50%) were added subsequently to remove the remaining H_2O_2 as well as any carbonates present. After cooling, the contents were transferred to centrifuge tubes and were centrifuged at 1500 rpm for 5 min. The supernatant solution was decanted and the washing process repeated four times. Clay was removed in the final wash by adding few drops of very weak ammonia solution (1%) to the sample. The diatom suspension was diluted to a suitable concentration

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and a few drops of the above suspension were allowed to settle overnight in cover slips. Proper care was taken to avoid the contamination by dust and other foreign particles into the cover slips. Once the cover slips were dried, they were mounted on a glass slide with a drop of DPX. The slides were kept over a hotplate to dry off the mountant and allowed to cool. All slides were analyzed at microscope for diatom identification and quantification (Hasle et al., 1978).

CULTURE CONDITION

The fresh water diatoms were cultured in SAG media (Guillard and Lorenzen,1972).We have prepared solid media with addition of 1.5% agar (non-nutrient) w/v were grown for 6-7 days at 20.0 ± 2.0ºC and 4000 ± 10 lux (=lumem/m2) cool white bulb with 16 h of light and 8 h of darkness measured adjacent to the flasks at liquid level as recommended by Miller et al, 1978 and for sub-culture, sterilized the media and using sterile technique, transferred the diatoms from stock culture to a tube containing fresh media. As, diatom growth observed by cell counting method showed, that in SAG media growth was better compared to other growth medium. Therefore, SAG media was preferably selected for this study. Diatoms were cultured in SAG media supplemented with different concentrations (Control, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06 and 0.07mM) of K_2HPO_4 as phosphate source to observe its effect on growth.

Culture media

Macronutrients

Components	Stock Solution (mg/100 ml)	Nutrient Solution Applied (ml/litre)
$Ca(NO_3)_2.4H_2O$	200 mg	20 ml
K_2HPO_4	*	*
$MgSO4.7H_2O$	100 mg	25 ml
Na_2CO_3	100 mg	20 ml
$Na_2SiO_3.9H_2O$	200 mg	50 ml
Fe-Citrate	100 mg	10 ml
Citric acid	100 mg	10 ml
Micronutrients	–	5.0 ml
Distilled Water	–	820 ml

* Varying concentration of Phosphate

Micronutrients

Components	Stock Solution (Mg/100 ml)	Nutrient Solution Applied (ml/litre)
H_3BO_3	200 mg	5.0 ml
$MnSO_4 \cdot 4H_2O$	100 mg	2.0 ml
$ZnSO_4 \cdot 7H_2O$	100 mg	1.0 ml
$Co(NO_3)_2 \cdot 6H_2O$	20 mg	5.0 ml
$Na_2MoO_4 \cdot 2H_2O$	20 mg	5.0 ml
$CuSO_4 \cdot 5H_2O$	5 mg	1.0 ml
FeEDTA	--	10.0 ml
Vitamin Solution		
Thiamine Hcl	100 mg	1.0 ml
Biotin	0.00025 mg	0.5 ml
B_{12}	0.0001 mg	0.5 ml

CELL COUNT

The growth of the investigated diatoms was measured during 8th day experiments the growth of the diatom population was monitored by microscope cell counts every other day. Cell densities were determined by 3 replicates of samples preserved in Lugol for each phosphate concentration variants in a haematocytometer. Cell number are expressed in the text as the mean ± 5% SE

RESULTS

Diatom culture experiments were carried out in media varying in initial phosphate levels from 0.01 to 0.07 mM per litre. Table.1 shows the result found the diatoms populations. According to our results, the diatom growth is sensitive to changes in phosphate concentration. From this we can conclude that phosphorous gives a good reflection of cell division. For determining the effect of phosphate on growth of fresh water diatoms, we were growing diatoms on various concentration of phosphate and analyzed its growth by cells count and found that the 0.05 mM concentration of phosphate the diatom growth maximum at 8th days interval. In this present study it was found that growth rate increases when phosphate rises up to an optimum around 0.05mM concentration. At higher phosphate concentration in media cell height seems to be reduced at faster rate.

DISCUSSION

The responses to phosphate by numerous representatives of the freshwater diatoms include significant physiological and biochemical changes in their growth characteristics, cell metabolism demonstrated by Correll, (1999).

Our observations of the freshwater diatoms support the hypothesis that phosphate exerts a significant influence on diatoms. According to the findings of the present study, the result of depressing the concentration the phosphate concentration was reducing in growth. The population of this diatom incubated at different phosphate concentrations demonstrated reproducible growth, however the cell number declined with decreasing phosphate concentration in the medium.

Table 1: Cell number in culture of diatoms during incubation

Phosphate concentration (milimolar)	Cell number × 10^4						
	2 Days	4 Days	6 Days	8 Days	10Days	12 Days	14 Days
T1(0.01mM)	0.50±0.029	1.23±0.026	2.38±0.072	3.15±0.069	2.78±0.078	2.35±0.035	2.03±0.046
T2(0.02mM)	0.56±0.028	1.55±0.064	2.78±0.049	3.46±0.038	3.04±0.069	2.65±0.072	2.24±0.014
T3(0.03mM)	0.57±0.020	1.68±0.017	3.05±0.072	3.68±0.087	3.19±0.046	2.79±0.015	2.37±0.049
T4(0.04mM)	0.68±0.032	1.86±0.061	3.58±0.084	4.29±0.040	4.05±0.075	3.69±0.090	3.28±0.089
T5(0.05mM)	0.87±0.017	3.48±0.029	6.03±0.026	6.23±0.049	5.89±0.046	5.66±0.029	5.48±0.015
T6(0.06mM)	1.02±0.026	2.67±0.049	3.85±0.072	4.29±0.061	3.29±0.104	2.88±0.078	2.28±0.031
T7(0.07mM)	1.12±0.028	2.23±0.061	3.37±0.078	3.87±0.115	3.98±0.066	2.40±0.040	1.90±0.066

The data are the mean of 3 replicates± SE

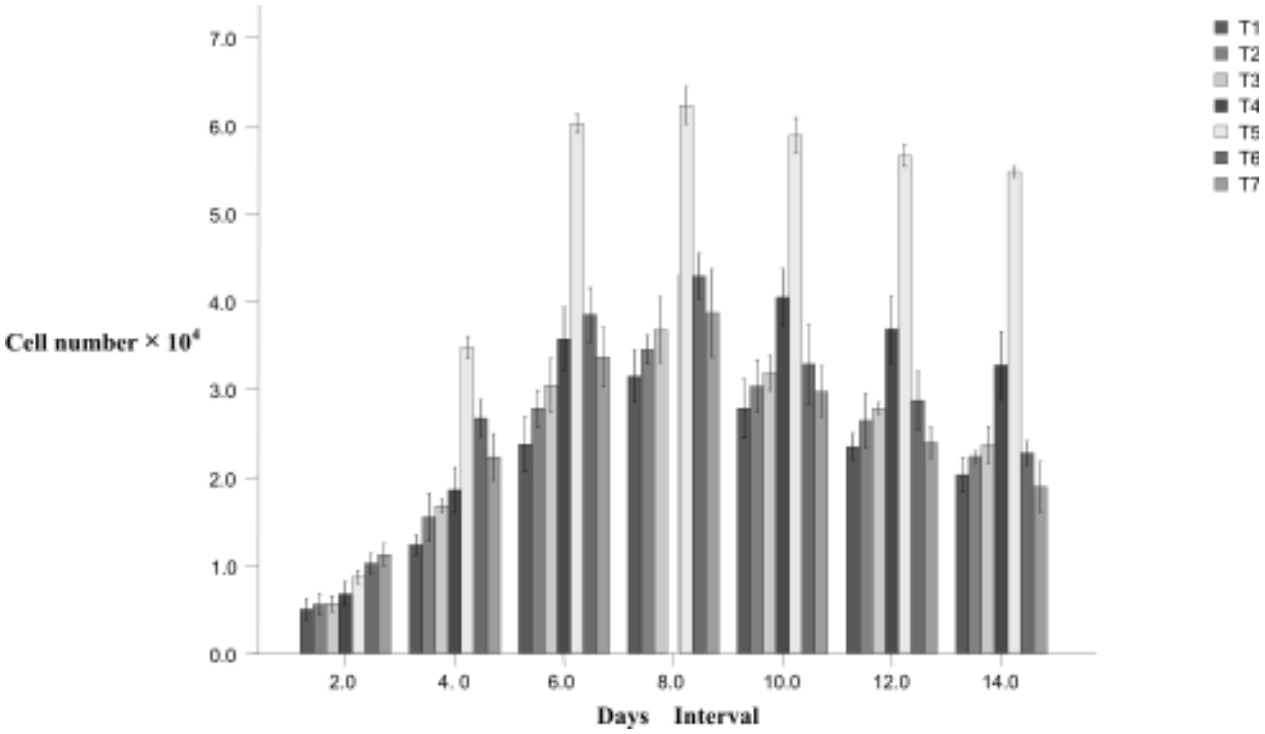

Fig.1: Growth graph of diatom cells in relation to different phosphate concentrations 1×10^{-3}, 2×10^{-3}, 3×10^{-3}, 4×10^{-3}, 5×10^{-3}, 6×10^{-3} and 7×10^{-3} at different days intervals

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