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Changes in Levels of Cellular Constituents of *Dunaliella parva* Associated with Inorganic Phosphate Depletion

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Abstract: The effect of phosphorous limitation on some physiological and biochemical parameters of the green alga *Dunaliella parva* have been examined. Algal cells were cultured under conditions of either Pi limitation or nutrient sufficiency. At the end of the experiment and under phosphorus limitation conditions the percent of decrease in number of cells and growth rate reached 58.4% and 36.3% respectively, while the generation time increased by 2.21 days compared to nutrient sufficient medium. The content of chlorophyll fractions, glycerol, β-carotene, total soluble carbohydrates and proteins were also significantly reduced in response to phosphorus limitation. The photosynthetic and respiratory activities in nutrient sufficient control medium increased gradually till the 12th days of culturing where at the 16th day they decreased by 8.9% and 17.8% for photosynthesis and respiration, respectively. Phosphorous limitation resulted in 36.8% lower rate of respiration and 65.5% decline in rate of photosynthesis at the 16th day of culturing compared to control. The results cleared also that *D. parva* when cultured under phosphorous starvation conditions, growth of the alga was the most sensitive parameter followed by photosynthesis then respiration.

Key words: Phosphorous limitation · Photosynthesis · Respiration · Growth rate · *Dunaliella parva* · Algae

INTRODUCTION

Inspite of its absence in nature phosphate is frequently the limiting nutrient in many environments since it is mainly found in forms not readily available such as insoluble salts [1, 2]. However, Keasling *et al*. [3] found that synthesis and degradation are influenced by the energy state of the cell and extracellular phosphate level. Tillberg and Rowley [4] studied the effect of phosphorous starvation on morphology, intracellular structure and on reactions related to the energy metabolism of the unicellular green alga *Scenedesmus obtusiusculus*. Increase in cell size, shape and cell wall thickness and disorganization in the internal structure are the dominating features of phosphorous starvation. In addition, Theodorou *et al*. [5] found that *Selenastrum minutum* cultured under conditions of phosphorous limitation resulted in noticeable decrease in rate of respiration, rate of photosynthesis and significant increase in activities of phosphoenol pyruvate (PEP), carboxylase and PEP phosphates. Also, El-Sheikh and Rady [6] obtained the same results for the effect of phosphorus starvation on growth, photosynthesis and some metabolic processes in the unicellular green alga *Chlorella kessleri*.

Phosphate appears also to be required for the active transport of metals into the cell [7]. Phosphate limitation should thus directly reduce metal uptake as has been observed in studies of nickel uptake by *Phaeodactylum tricornutum* [8]. Phosphate may complex or precipitate metals making both toxic metal and phosphate unavailable to the cell [9]. When the phosphate concentration is increased, excess metal is complexed reducing toxicity and the surplus phosphate produces growth stimulation.

This work was designed to assess the effect of phosphorous nutrition on some physiological and biochemical parameters of the green alga *Dunaliella parva* cultured under conditions of either phosphorous limitation or nutrient sufficiency.

MATERIALS AND METHODS

Biological Material: The biological material used in this work was the unicellular marine green alga *Dunaliella parva*, obtained from UTEX at Austin, USA. The axenic culture of *D. parva* was grown on MH medium [10].

Culture Conditions: The axenic inoculum was grown in 100 ml MH medium with or without phosphate in 250 ml

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Erlenmeyer Pyrex-glass flasks under controlled laboratory conditions (temp. at 25±3°C and light at 4000 lux) in a controlled culturing chamber under a regime of 16 h light / 8 h dark. Cultures were lasted for 16 days old and harvested first after 2 days then after 4 days by centrifugation at 10000 rpm for 20 minutes using angle rotor centrifuge. The supernatant were discarded and the remaining pellets were used for determination of chlorophyll fractions, β-carotene, glycerol, total soluble carbohydrates and proteins.

Growth Measurements: The growth of the investigated alga was determined by cell number by using the hemacytometer slide at least 10 replicates were taken and the mean number of cells / ml culture was calculated. From the obtained number of cells; the growth rate, mean growth rate, relative growth rate and generation time were determined.

Chlorophyll Fractions and β-carotene: The acetone extract of known weight of algal pellets was spectrophotometrically analyzed for estimation of chlorophylls a and b as mg l^{-1} by using the equation proposed by Jeffrey and Humphrey [11]. While β-carotene (mg l^{-1}) was measured by the formula proposed by Jaspers [12].

Glycerol Determination: Glycerol was determined by the method recommended by Chitlaru and Pick [13].

Total Carbohydrates Determination: Total carbohydrates content were estimated according to the method described by Dubois *et al*. [14].

Total Protein Determination: Total protein was determined by the method described by Hartree [15] which is the modification of the original folin-phenol method of Lowry *et al*. [16].

Photosynthesis and Respiration: The photosynthetic activity was measured polarographically as oxygen evolution using Clarke type electrode (VSI, model 53). The actinic white light was obtained from a 150 W. tungsten lamp. Respiration activity was measured in dark as oxygen uptake. Measurements of both photosynthesis and respiration were carried out by using 3 ml of the algal suspension at room temperature.

Statistical Treatment: The obtained data were analyzed statistically using two ways ANOVA (analysis of variance).

RESULTS

Data of *D. parva* growth measured as No. of cells (Table 1) cleared that under both Pi availability and Pi deficiency, the number of cells increased gradually till the end of the experiment (16 days), but maximum rate of growth and the least generation time were recorded at the 8th day. However, the number of cells under Pi deficiency was less than that obtained under Pi availability. At the 8th and 16th day of culturing the number of cells under Pi deficiency decreased by 35.6% and 58.4% respectively compared to those obtained at Pi availability (control).

The results obtained for chlorophylls content under Pi deficiency and availability (Figure 1) followed nearly the same trend as for cell number although under Pi deficiency a slight decrease was recorded at the end of the experiment. The results cleared also that chlorophyll a is more sensitive to Pi deficiency than chlorophyll b. At the 8th and 16th day of culturing the percent of decrease in chlorophyll a content in *Dunaliella* cells cultured under Pi deficiency reached 35.6% and 47.4% respectively. While chlorophyll b decreased by 31.7% and 35.2% compared to those obtained under Pi availability. At the same time total chlorophylls under Pi deficiency decreased by 34.6% and 44.2% compared to those obtained at normal culture medium.

Table 1: Effect of Pi deficiency on growth parameters of *Dunaliella parva* cultured for 16 days

	No. of cells x10^6/ml		Growth rate		Mean growth rate		Relative growth rate		Generation time	
Time (days)	+P	-P	+P	-P	+P	-P	+P	-P	+P	-P
0	0.22	0.22	-	-	-	-	-	-	-	-
2	0.25	0.25	0.092	0.092	0.092	0.092	0.028	0.028	10.843	10.843
4	0.36	0.342	0.263	0.226	0.178	0.159	0.053	0.048	5.629	6.248
8	1.04	0.670	0.383	0.243	0.280	0.201	0.084	0.060	3.569	4.979
12	2.19	0.921	0.269	0.115	0.276	0.172	0.083	0.052	3.619	5.809
16	2.91	1.211	0.103	0.099	0.233	0.154	0.070	0.046	4.294	6.502

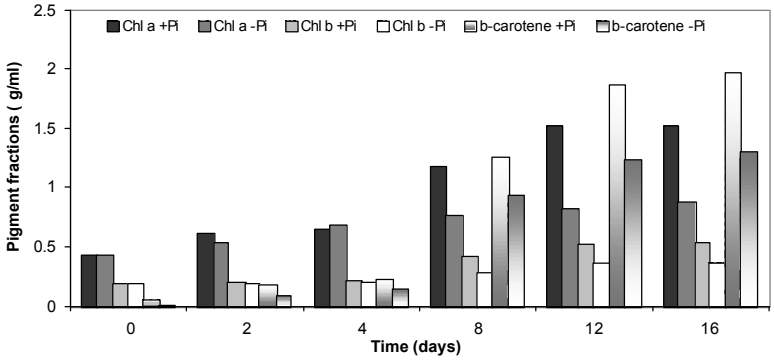

Fig. 1: Effect of Pi deficiency on content of chlorophylls (a and b) and β-carotene in *Dunaliella parva* (µg/ml) cultured for 16 days

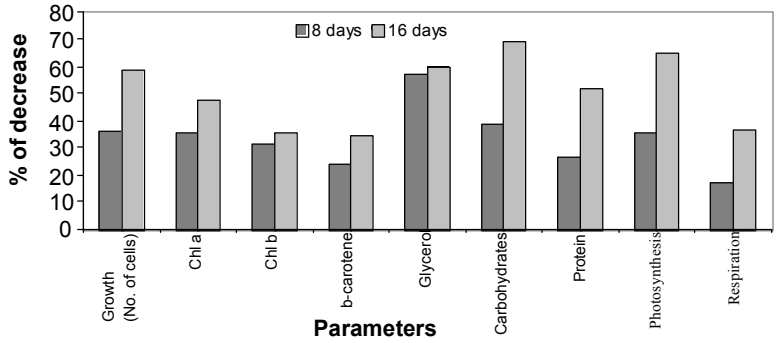

Fig. 2: Percent of decrease at the 8th and 16th day of culturing on growth and content of some metabolites in *D. parva* under the effect of Pi deficiency compared to control

Table 2: Effect of Pi deficiency on content of glycerol, total carbohydrates and total proteins of *Dunaliella parva* (mg/l) cultured for 16 days

	Glycerol		Carbohydrates		Protein	
Time (days)	+ Pi	- Pi	+ Pi	- Pi	+ Pi	- Pi
0						
2	2.434	2.131	10.274	8.741	6.501	4.712
4	5.454	3.517	21.258	18.524	10.149	7.815
8	14.593	6.248	52.742	32.414	14.262	10.541
12	23.367	12.473	73.828	30.211	17.489	10.431
16	31.952	12.791	83.148	25.432	19.241	9.301

Table 3: Effect of Pi deficiency on activities of photosynthesis and respiration of *Dunaliella parva* cultured for 16 days

	Photosynthesis (O_2 evolution) µ mol O_2 mg chl^{-1} h^{-1}		Respiration (O_2 uptake) µ_mol O_2 h^{-1}	
Time (days)	+ Pi	- Pi	+ Pi	- Pi
4	473.00	432.00	1.67	132
8	502.00	322.00	193	1.61
12	516.00	220.00	2.31	1.50
16	470.00	162.00	1.90	1.20

The content of β-carotene and glycerol increased also till the end of the experiment under both deficiency and availability of phosphorous, but the increase in case of Pi deficiency was less than in case of Pi availability. It must be mentioned that under Pi deficiency glycerol from the 8th to the 16th day of culturing (Figure 2) greatly decreased compared to those obtained under Pi availability (57.2% and 60.0% respectively).

Total carbohydrates and proteins content (Table 2) under normal cultures increased gradually till the end of the experiment. On the contrary, in case of Pi deficiency the contents of these two metabolites are the only ones

that began to decrease after the 8th day of culturing. However, the percent of decrease in carbohydrates content under Pi deficiency was higher than in case of protein. At the 8th and 16 thday of culturing, carbohydrate content decreased by 48.3% and 69.4% respectively; while in case of protein they decreased by 26.1 and 51.7% respectively compared to those obtained at normal culture medium (Figure 2). This may indicate that carbohydrates are greatly affected by Pi deficiency than proteins.

Anent effect of phosphorus on oxygen uptake (respiration) by *D. parva* the results recorded in Table (3) cleared that oxygen uptake at normal culture conditions increased gradually till 12th day of culturing, while in case of Pi deficiency it increased only till the 8th day of culturing. At the end of the experiment, the rate of O_2 uptake under Pi deficiency decreased by 36.8% compared to normal medium. Concerning the results obtained for O_2 evolution (photosynthesis), the results cleared that photosynthesis is greatly affected under Pi deficiency than respiration (Figure 2). At the end of the experiment, it decreased by 65.5% compared to those obtained at normal culture medium.

Nearly all of the metabolites that have been analyzed in *D. parva* under Pi deficient cultures showed rapid decreased and degradation. For chlorophylls content in *D. parva* the results cleared that lack of phosphorous in the medium led to a marked reduction of chlorophyll fractions in specially chlorophyll a.

DISCUSSION

Aquatic algal communities are often affected by authropogenic disturbances to the environment [17]. Microalgae are considered to be the first organisms affected by any disturbances in aquatic environment [18]. The results of this work cleared that:

- Maximum values of growth parameters in *D. parva* cultured on basal medium (nutrient sufficient medium) or on Pi-deficient medium was recorded nearly at the 8th day of culturing [19-22]. Also, rate of growth of *D. parva* in Pi starved cells was lower than control. These results are in agreement with those obtained by El-Sheikh and Rady [6] for *Chlorella kessleri*; Kozolowaska-Szerenos *et al*. [23] for *Chlorella vulgaris*; El Agawany [22] for *Dunaliella tertiolecta*. In addition, Egge [24] showed that diatoms were unable to dominate when phosphorous was deficient.
- Anent effect of Pi deficiency on the synthesis of the analyzed metabolites in this work, the results cleared that under Pi- deficient nearly most of the cellular constituents decreased. The rate of decrease depended mainly on type of the metabolite, its role for the cell and length of the culture period [22, 25]. Lack of phosphorous in the medium led to a marked reduction of chlorophyll fractions in *D. parva*. The reduction was more prominent in chlorophyll a than b. These results are similar to those reported by Bekoura and Dauta [26] and Theodorou *et al.* [5]. Also, Zachleder and Tukaj [27] reported that a rapid degradation of all pigment fractions was recorded in Pi starved cells of *Scenedesmus axmatus*. β-carotene is the only pigment fraction that slightly affected in Pi starved cultures.
- Glycerol, total carbohydrates and total proteins were the metabolites that steeply decreased under conditions of Pi starvation. The decrease in total carbohydrate and total proteins content in Pi starved cultures may be due the fact that most newly fixed carbon appears to be directed toward respiratory metabolism and other biosynthesis pathways [28, 29]. Theodorou *et al*. [5] reported that soluble proteins content was reduced in response to Pi limitation. The photosynthetic and respiratory activities were high during incipient Pi starvation and then decrease with increasing period of exposure to Pi deficiency.
- Pi limitation of the green alga *D. parva* resulted in significant decline in cellular photosynthesis and respiration rates. However, the rate of decrease in photosynthesis was more prominent than in respiration. Parasad and Kashyap [30] found that Pi deficiency has been correlated with lower photosynthesis. It can be concluded from the obtained results of this work that growth of *D. parva* grown under Pi deficiency was the most sensitive parameter followed by photosynthesis and then respiration. This conclusion was also ascertained by Lumsden and Florence [31] for *Chlorella pyrenoidosa*, *Astionella gracilis* and *Nitzschia closterium* cultured under Pi starvation conditions.

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