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Phyton (Horn, Austria)	**Vol. 35**	Fasc. 1	139–151	28. 7. 1995

Effect of Phosphorus Starvation on Growth, Photosynthesis and Some Metabolic Processes in the Unicellular Green Alga *Chlorella kessleri*

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With 3 Figures

Received March 17, 1994

Accepted July 19, 1994

Key word: *Chlorella*, Lipid metabolism, Photosynthesis, phosphorus starvation.

Summary

EL-SHEEK M. M. & RADY A. A. 1995. Effect of phosphorus starvation on growth, photosynthesis and some metabolic processes in the unicellular green alga *Chlorella kessleri* – Phyton (Horn, Austria) 35 (1): 139–151, with 3 figures. – English with German summary.

The effect of phosphorus starvation on growth and several physiological parameters of the unicellular green alga, *Chlorella kessleri* have been examined. Increase in dry weight production and decrease in optical density of the algal suspension, chlorophyll concentration, photosynthetic activity (measured as oxygen evolution) and dark respiration are dominating features for phosphorus starvation. The lipid content of the alga was separated into five classes depending on their mobilities on thin layer chromatography. The five classes identified are: Digalactosyl diacylglycerol (DGDG), monogalactosyl diacylglycerol (MGDG), phosphatidyl choline (PC), phosphatidyl glycerol (PG), and sulfoquinovosyl diacylglycerol (SQDG). The differences in fatty acid composition of the total lipids and each individual lipid class was estimated by gas liquid chromatography. The phosphorus content of total lipids of Pi-starved and nutrient sufficient cells showed a large difference in the exponential and stationary growth phase.

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Zusammenfassung

EL-SHEEK M. M. & RADY A. A. 1995. Einfluß von Phosphormangel auf Wachstum, Photosynthese und einige Stoffwechselprozesse in der einzelligen Grünalge *Chorella kessleri*. – Phyton (Horn, Austria) 35 (1): 139–151, 3 Abbildungen, Englisch mit deutscher Zusammenfassung.

Der Einfluß von Phosphormangel auf Wachstum und verschiedene physiologische Parameter wurde an der einzelligen Grünalge *Chlorella kessleri* untersucht. Ein Anstieg in der Trockengewichtsproduktion und in der Verminderung der optischen Dichte der Algensuspension, Chlorophyllkonzentration, Photosyntheseaktivität (als Sauerstoffausstoß gemessen) und Dunkelatmung sind die Hauptkennzeichen für Phosphormangel. Der Lipidgehalt der Alge wurde in fünf Klassen entsprechend ihrer Wanderungsgeschwindigkeit in der Dünnschichtchromatographie eingeteilt. Diese sind Digalactosyl-Diacylglycerol (DGDG), Monogalactosyl-Diacylglycerol (MGDG), Phosphatidyl-Choline (PC), Phosphatidyl-Glycerol (PG) und Sulfoquinovosyl-Diacylglycerol (SQDG). Die Unterschiede in der Fettsäurezusammensetzung der Gesamtlipide und in jeder Lipidklasse wurden mit der Gas-Flüssigkeits-Chromatographie bestimmt. Der Phosphorgehalt der Gesamtlipide von Pi-unterversorgten und -ausreichend-versorgten Zellen zeigte einen großen Unterschied in der exponentiellen und stationären Wachstumsphase.

Introduction

The investigation of environmental influences on organisms is an area of increasing importance. In recent years, detailed information has been obtained from unicellular organisms by combining their structural, physiological and biochemical data.

The growth of microalgal cultures is basically controlled by the composition of the culture medium and operational variables such as temperature, intensity of illumination and pH. But there are other factors which should be considered as microalgal growth-determining factors too, i. e. the physiological conditions of the microalgae and the O_2 and CO_2 available to the culture medium (MOLINA & al. 1991). For microalgal cultures which are basically aimed to obtaining high productivity in biomass, optimum phosphorus concentrations are in the range 0.001–0.179 g litre^{-1} (SHELEF & SOEDER 1980).

Inorganic phosphate is believed to be one of the most important nutritional factors which regulates plant growth and metabolism. Phosphorus plays a significant role in most cellular processes, especially those involved in generating and transforming metabolic energy. It is thus indispensable for the growth and reproduction. The phosphorus requirements for optimal algal growth differ considerably from species to species even if no other external factors are limiting (KUHL 1974). Phosphorus has a key role in the conveyance of metabolic energy and as an essential structural component of nucleotides and phospholipid molecules in all living cells (TILLBERG & ROWLEY 1989). The aforementioned authors found

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that, phosphorus – starved *Scenedesmus sp.* cells increased in size, shape and cell wall thickness. They indicated also that starch granules and lipid globules size increased during the studied period. As a strategy for securing the supply of phosphorus for maintenance of vital metabolic functions, many lower plants are known to accumulate and store large quantities of phosphorus as polyphosphates when growing under conditions of phosphate availability (KULAEV & VAGABOV, 1983).

In order to obtain more information about the importance of phosphorus starvation, the following physiological and biochemical study was initiated to compare qualitative and quantitative aspects of the unicellular green alga *Chlorella kessleri,* when it exposed to phosphorus starved conditions.

Abbreviations: chl, chlorophyll; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; PC, phosphatidyl choline; PG, Phosphatidylglycerol; SQDG, sulfoquinovosyl diacylglycerol; UI, unsaturation index: Un/S, polyne index B; 14 : 0, Miristic acid; 16 : 0, Palmitic acid; 18 : 0, Stearic acid; 18 : 1, Oleic acid; 18 : 2, Linoleic acid; 18 : 3, linoleinic acid.

Materials and Methods

Experimental material

Cells of the unicellular green alga *Chlorella kessleri* 211–11 h (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen, Germany) were cultured in a medium described by KUHL 1962. This nutrient medium containing (in 1L) 1.011 g KNO_3, 0.089 g $Na_2HPO_4 \cdot 2H_2O$, 0.621 g $NaH_2PO_4 \cdot 2H_2O$, 0.0294 g $CaCl_2 \cdot 2H_2O$, 0.2465 g $MgSO_4 \cdot 7H_2O$, 1 ml iron complex (0.7 g $FeSO_4 \cdot 7H_2O$ + 0.93 g Triplex EDTA in 100 ml stock, each ml of this solution contains 6.95 mg Fe), 1 ml trace mineral solution (50 mg $CuSO_4 \cdot 5H_2O$, 0.1 gm H_3BO_3, 0.1 gm $ZnSO_4$, 169 mg $MnSO_4 \cdot H_2O$, 130 mg $NaMoO_4$, 100 mg $Co(NO_3)_2$ in 100 ml stock solution). When the phosphorus was omitted, the medium was buffered with 20 mM TRIS-HCL to pH 6.3 and supplemented with sodium chloride to keep the ratio of sodium the same as in the sufficient medium. To obtain cells starved for phosphorus (Pi), 10 ml aliquots from the exponential growth phase (approximately 2×10^6 cell/ml) were transferred to 500 ml culture vessel containing 200 ml of a Pi – free medium. The cultures were incubated at 27° C under continuous light (5000 Lux) and supplied by a mixture of 95% sterile air and 5% CO_2 (v/v). Samples were removed at different time intervals under sterile conditions parallel to the control for different analyses.

Growth parameters measurements

Growth of the cultures was monitored daily either by measuring the optical density of the cell suspension spectrophotometrically at 560 mm (WETHERELL 1961) or as the increase in dry weight production as reported by LEGANES & al. 1987. For determination of the dry weight, 10 ml were removed from the culture washed twice with dist. water and incubated for 24 hours at 95° C. For determination of chlorophyll concentration, the method recommended by MACKINNEY 1941 was used. This method is more convenient to the whole algal cells than any other method. Total

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proteins were estimated colorimetrically by the method described by LOWRY & al. 1951.

Oxygen evolution measurements

Oxygen evolution or uptake measurements were made with a Clark – type oxygen electrode in 3 ml samples in a thermostated closed perspex cuvette at 25° C under saturated light.

Analysis of lipids

Lipids were extracted from 10 ml aliquots of culture by the method of BLIGH & DYER 1959. The lipid classes were separated by thin – layer chromatography on precoated silica – gel plates (5721 – Merck, Darmstadt, Germany) with a mixture of benzol: acetone: dist. water, 30 : 91 : 8 (v/v) as the mobile phase. After development, the plates were dried in a stream of CO_2 and the lipid classes were identified using 8 – anilinonaphthalin-sulphate (ANS) fluorescence elution and phosphatidyl choline, phosphatidyl glyecerol, sulphoquinovosyl diacylglycerol, digalactosyl diacylglycerol and monogalactosyl diacylglycerol (Seradary Research Lab) as standards. The separated lipids were taken into ampoules, containing 5% HCl in dry methanol and transesterified at 80° C within 2–3 hrs under CO_2 or N_2. The methylesters of the fatty acids were extracted from esterification mixture after dilution with an equal volume of dist. water by n-hexane.

Gas chromatography:

Methylesters were separated using a (Hewlett Packard 5890 series II) equipped with a capillary column coated with SP 2330 of 0.25 µm thickness (0.25 mm I.D × 30 mi CPS – Li Quadrex, New Haven, CT. U.S.A.). High purity nitrogen was applied at a flow rate of 230 KPa, hydrogen 100 KPa and oxygen 280 KPa). The dual column system was programmed from 160° C to 200° C to give partial separation of 18 : 3 at the rate of 2.5° C min^{-1}. The detector temperature and injector temperature was 220° C. Identification of the peaks was made using linoleonic standard and by plotting log relative elution temperature versus the number of carbon atoms (SCHMIDT & WYNNE 1967). To calculate the percentage composition using Hewlett-Packard 3396 A integrator all peaks emerging between the myristic (14 : 0) and Linolenic (18 : 3) were included in calculations.

Total phosphorus

Phosphorus content from the total lipids extract was determined according the method of ROUSER & al. 1970, spectrophotometrically at 790 nm. The amount of phospholipids was derived from lipid phosphate as $\mu mol.g^{-1}$ dry weight. *Data* were subjected to student's t-test.

Results and Discussion

Effect of phosphorus starvation on growth

Pi-starved *C. kessleri* had lower growth determined by optical density of the algal suspension (Fig. 1A). However, the dry weight of the Pi-starved

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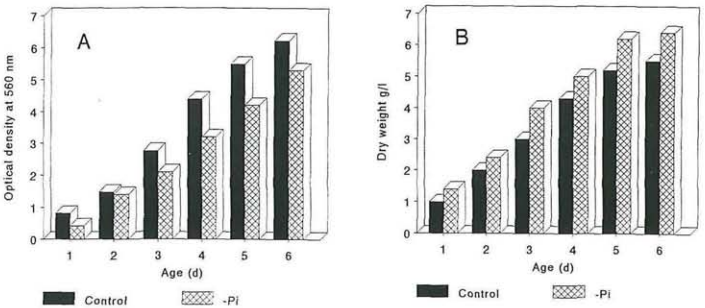

Fig. 1: Effect of phosphorus starvation on growth of *Chlorella kessleri* (A) optical density of the cell suspension, (B) Dry weight production.

cells was higher than that of control, during the studied period (Fig. 1B). PIRSON & al. 1952 found that in phosphate deficient *Ankistrodesmus* cultures, dry weight production, cell division are inhibited. Recently TILLBERG & ROWLEY 1989 showed that the dry weight increased all through 96 h of phosphorus starvation and they found a correlation between the dry weight, starch, lipids and cell wall thickness. Our results indicated that, optical density of the cell suspension decreased under Pi-starvation as compared with control.

Physiological changes resulting from Pi-starvation

Table 1 presents the effect of Pi-starvation on some physiological processes. Chlorophyll content was reduced under Pi-starvation. The reduced chlorophyll content of Pi-starved cells implies that, as has been demonstrated for Pi-starved *Scenedesmus obtusiusculus* (TILLBERG & ROW-

Table 1: Physiological changes resulting from pi starved cells of the green alga *Chlorella kessleri.* Chlorophyll (a+b) concentration measured as ($\mu g \cdot ml^{-1}$), oxygen evolution or uptake as (μ mol $O_2 \cdot$ mg $chl^{-1} \cdot h^{-1}$). Values are the mean ± SE of three measurements.

Age (d)	Chl (a+b)		Oxygen evolution		Dark respiration		soluble proteins	
	control	–pi	control	–pi	control	–pi	control	–pi
1	15±1	17±1.2	139±3.2	105±3	22±1	12±0.5	50±0.8	48±1
2	44±1	39±1.3	153±2.5	141±3.5	28±1	18±1	79±0.8	79±0.9
3	77±1.5	61±2	178±2.1	153±4	43±1.2	33±1	130±2	128±3
4	105±2	86±2	192±2.3	187±4	47±2	39±3.2	200±4	216±2
5	117±2.5	98±3	264±4	171±3.5	60±1.2	38±2.2	235±4.1	235±4
6	123±3	112±3.3	198±2.5	172±3.2	59±3	42±1.5	256±3.5	265±4

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LEY 1989), *Chlamydomonas reinhardii* (BALL & al. 1990) and *Selenastrum minutum* (THEODOROU & al. 1991), low Pi-treatment is associated with a decline in photosynthetic activity. Measurements of rates of O_2 and dark respiration (Table 1) indicated that, on the fifth day of growth the O_2 evolution is reduced by about 35% under Pi-starvation and this decrease is corresponded with similar decrease in chl concentration. The total soluble protein has not affected by Pi-starvation. However, THEODOROU & al. 1991 found that Pi-limited *Selenastrum minutum* had much reduced concentrations of protein. They suggested that the synthesis of nonessential proteins may be repressed during Pi-limitation.

When the unicellular green algae or higher plants are subjected to Pi-limitation, most newly fixed carbon appears to be partitioned toward the synthesis of nonphosphorylated storage polyglucans (i. e. starch) or sucrose with less photosynthate directed toward respiratory metabolism and other biosynthetic pathways (PREISS 1984, WALKER & SIVAK 1986, THORSTEINSSON & TILLBERG 1987, SICHER & KRAMER 1988, BALL & al. 1990; DIETZ & HELIOS 1990). This is probably the case with Pi-starved *C. kessleri* where the dark respiration is inhibited and it seems to be constant all through the experimental period.

Lipid analysis

Quantitative analysis of total lipids in *C. kessleri* after 6 days incubation indicated that, lipid yield increased in Pi-starved cells (0.095 mg/mg dry weight) as compared with the control cells (0.074 mg/gm dry weight). This increase in total lipids was companied by an increase in dry weight, 22 and 15%, respectively.

Our results also indicated that five major glycerolipid components, MGDG, DGDG, SQDG, PG and PC were identified in *C. kessleri* on the basis of their mobilities during thin layer chromatography as previously reported elsewhere (NICHOLS 1965, SHAW 1966, NICHOLS & APPLEBY 1969).

Fatty acids of total lipids and its classes

Fatty acids from sufficient and Pi-starved *C. kessleri* were determined by gas chromatography and presented in Table 2 and 3. Saturated fatty acids were identified as 14 : 0, 16 : 0 and 18 : 0 and monounsaturated fatty acids as 16 : 1 and 18 : 1 and diunsaturated fatty acids as 16 : 2 and 18 : 2 and triunsaturated as 16 : 3 and 18 : 3. The data presented in Table 2 show that total lipids fatty acids of nutrient sufficient cells contain higher proportion of unsaturated fatty acids especially, (linoleic and linoleinic) acids. These acids increased with the increase in growth and then decrease after the exponential growth phase. The same results are obtained with Pi-starved cells, but its quantity was much higher if it compared to the nutrient sufficient cells. Any change in lipid composition of the algae by growth in

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Table 2: Fatty acid composition (mol %) of the total lipids and individual lipid Phosphatidyl choline (PC) and Phosphatidyl glycerol (PG) of control (C) and phosphorus starved (P) *Chlorella kessleri*

Lipid	Age (h)		FATTY ACID									Un/S	UI
			14:0	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3		
Total Lipids	24	C	4.9	17.5	5.2	9.3	5.9	2.2	5.8	21	28.2	3.1	174
		P	8.3	20.5	5.1	8.5	5.3	3.4	6.1	19	23.9	2.1	154
	48	C	5.4	12.0	4.5	16.5	6.6	–	4.8	28.6	21.6	4.7	184
		P	2.8	14.2	3.3	16.2	2.5	0.6	3.5	36.4	20.5	4.7	181
	72	C	3.2	15.0	4.2	14.4	3.6	1.5	6.9	34.9	16.3	4.1	169
		P	2.5	12.4	1.4	21	3.6	1.1	3.1	40.3	14.6	5.3	182
	96	C	1.9	14.8	3.8	14.8	3.6	1.2	6.4	38.5	15.1	4.6	173
		P	2.9	12.2	3.5	19.4	2.9	1.1	3.3	42.9	11.9	5.2	176
	120	C	3.3	15.6	4.2	13.9	3.0	1.2	5.6	39.3	13.9	4.0	167
		P	3.3	13.8	3.8	18.6	2.8	1.6	4.4	39.6	12.2	4.4	170
PC	24	C	–	25.9	5.2	–	–	4.6	7.9	39.3	17.1	2.3	143
		P	–	34.9	2.5	–	–	9.8	19.6	26.7	6.5	1.2	95
	48	C	2.3	23.1	4.0	0.6	-	5.8	10.5	49.7	4.1	2.2	127
		P	2.2	22.2	1.8	–	1.1	4.8	–	60.6	7.2	2.4	148
	72	C	1.3	20.1	1.9	2.6	0.4	2.6	6.1	60.1	4.9	3.16	149
		P	1.2	26.5	4.6	2.6	–	2.6	5.8	53.3	3.5	2.3	133
	96	C	1.3	16.9	4.3	2.0	0.7	2.6	9.1	56.7	6.5	3.8	152
		P	1.8	13.2	1.2	2.2	–	2.6	20.5	54.2	4.4	4.7	148
	120	C	–	20.0	3.6	1.9	–	4.5	7.9	56.2	5.9	3.1	145
		P	1.5	21.2	2.6	2.2	–	2.9	4.9	55.2	9.7	2.9	151
PG	24	C	1.7	38.4	7.9	–	–	2.4	5.8	35.1	8.7	1.4	110
		P	–	44	–	–	–	–	15.1	29.1	11.8	1.3	109
	48	C	6.6	34.8	20.9	1.9	0.9	6.1	8.6	19.2	1.1	1.1	78
		P	4.4	42.1	25.6	2.1	–	1.4	3.5	16.6	4.4	1.1	80
	72	C	5.6	36.1	–	–	2.8	3.7	5.6	46.3	–	1.2	107
		P	1.7	36.8	10.6	2.5	–	2.1	3.8	38	4.6	1.5	109
	96	C	1.1	34.5	–	0.7	–	1.4	3.6	53.3	5.4	1.7	128
		P	1.1	22.4	2.8	3.5	0.4	1.7	3.7	57.8	6.7	3	150
	120	C	1.4	51.7	–	1.5	0.4	18.8	11.6	13.2	1.6	0.39	47
		P	2.1	32.9	–	2.1	0.7	7.3	8.8	42.3	3.8	1.4	111

The values represent have average of two independent measurements. The deviation in the values was within ± 1.0%. UI, index of unsaturation = the sum of the percentage of the weight multiplied by the number of olefinic bond for each fatty acid in the mixture. Un/S, Polyne index = sum of unsaturated fatty acids/sum of 14 : 0, 16 : 0 and 18 : 0. DGDG, digalactosyl Diacylglycerol; MGDG, monogalactosyl diacylglycerol; SQDG, sulfoquinovosyl diacylglycerol.

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Table 3: Fatty acid composition (mol %) of individual lipid classes isolated from *Chlorella kessleri* cells, under control (C) and Phosphorus starvation conditions

Lipid	Age (h)		FATTY ACID									Un/S	UI
			14:0	16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3		
DGDG	24	C	5.5	25	4.9	6.5	2.8	6.8	9.8	17.0	22.5	1.71	36
		P	6.2	46.5	–	8.6	–	–	–	17.2	21.4	0.9	116
	48	C	5.8	29.9	2.1	5.8	0.8	14.8	10.9	19.7	10.2	1.0	97
		P	3.1	28.9	1.2	8.3	1.5	2.2	5.1	32.0	17.9	1.9	145
	72	C	1.7	21.5	3.6	6.5	1.5	2.9	9.6	38.5	14.2	2.81	150
		P	–	15.7	2.7	8.2	0.8	5.1	12.5	42.2	12.6	3.8	156
	96	C	1.5	14.8	2.9	7.7	0.7	0.9	5.1	51.2	15.2	4.8	174
		P	0.9	9.8	0.1	11.5	0.9	0.3	2.1	58.7	15.1	8.2	190
	120	C	1.4	12.6	3.2	6.2	0.7	0.7	5.0	55.1	15.1	5.8	172
		P	1.2	12.4	2.1	9.1	0.6	1.1	3.5	56.9	12.6	5.8	177
MGDG	24	C	1.5	3.2	2.3	18.2	11.4	1.4	1.8	8.5	51.7	15.6	247
		P	–	8.1	5.1	18.3	–	9.2	3.9	13.7	41.7	4.8	198
	48	C	1.7	9.6	5.1	21.4	6.1	2.8	9.8	23.7	19.9	6.1	183
		P	1.0	3.9	2.9	35.5	6.5	0.7	1.9	23.3	24.3	16.9	215
	72	C	4.4	7.4	7.2	31.1	5.9	1.5	6.2	12.3	24.0	6.5	190
		P	1.8	4.5	8.3	36.3	4.8	–	–	32.0	16.7	15	205
	96	C	0.8	8.1	5.0	23.1	4.2	5.3	7.3	29.4	16.8	6.0	180
		P	0.5	2.0	2.6	32.8	4.5	0.3	1.6	37.1	18.6	33.8	213
	120	C	0.4	4.4	5.1	25.3	4.3	1.0	5.6	35.6	18.3	16.1	200
		P	0.4	2.8	3.2	30.9	3.6	0.7	2.7	38.7	17.1	25.0	207
SQDG	24	C	4.6	43.1	15.1	–	–	7.4	7.4	14.8	7.6	0.8	75
		P	20.3	45.6	12.7	–	–	–	11.4	10.1	–	0.5	44
	48	C	8.0	45	11.1	–	–	2.0	15.2	15.4	2.8	0.8	66
		P	3.8	42.9	8.3	–	–	1.4	7.5	32.7	3.5	1.1	92
	72	C	0.6	58.6	9.3	–	–	3.1	12.1	14.9	1.3	0.6	55
		P	15.9	43.6	12.5	–	–	3.6	6.7	17.7	–	0.6	55
	96	C	–	50.8	4.2	–	–	3.5	15.1	23.3	3.1	0.84	75
		P	1.7	46.5	9.4	–	–	0.8	9.2	31.7	0.8	1.1	85
	120	C	1.0	45.8	2.0	–	–	2.5	22.1	24	2.6	1.0	80
		P	2.3	45.3	10.7	–	–	0.8	16.2	22.9	1.8	1.1	78

The values represent the average of two independent measurements. The deviation in the values was within ± 1.0%. UI, index of unsaturation = the sum of the percentage of the weight multiplied by the number of olefinic bond for each fatty acid in the mixture. Un/S, Polyne index = sum of unsaturated fatty acids/sum of 14 : 0, 16 : 0 and 18 : 0. DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; SQDG, sulfoquinovosyl diacylglycerol.

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control or Pi-starved cells may be due to greater production of chloroplast material (NICHOLS 1965). Thus acids may be involved chemically as well structurally in some part of photochemical processes. The most abundant of fatty acids of (PC) are palmitic, linoleic and linoleinic. The level of linoleic acid ranged between 40–60%. Higher UI and polyene index B may be is related to the observed significant rise of the ratio of linoleic and linoleinic, so indicated that in the Pi-starved cells was more accumulated of polyunsaturated fatty acids which consider as the major determinate of membrane fluidity as described for bacteria by several authors (OKUYAMA 1969, MCELHANEY 1974, SINENSKY 1974).

The fatty acid composition of (PG) shows no major changes in UI and Un/S ratio during the exponential growth phase (from 72–96 hrs), but significantly decreased in the last stage of alga growth and the more abundant fatty acid was palmitic acid and this may suggest that, a close relationship exist between phosphatidyl glycerol containing hexadecenoic acid and the primary photosynthetic processes in plants (ALLEN & al. 1964). The fatty acid composition of (MGDG) is given in Table 3. It can be seen that, linoleinic acid represents about 52% after 24 hrs growth then, it decreased with increase the age of the alga. Generally, the sum. of saturated fatty acids increased with growth except at the last of growth it decreased to reach its normal level. On the other hand, unsaturated fatty acids increased in Pi-starved cells as compared to the control. DGDG fraction shows that, the fatty acids 14 : 0, 16 : 0 and 18 : 0 increased only during exponential growth phase after that, they decreased and the same was with oleic acid. UI and the unsaturated ratio decreased during this period.

These differences in fatty acid composition between DGDG and MGDG could be seen to be an argue against the hypothesis that MGDG is converted to DGDG by direct galactosylation (FERRARI & BENSON 1961). In SQDG class, the proportion of linoleic acid was ranged from 14% in the exponential phase and 24% during the stationary phase in control cells, while in Pi-starved cells it can reach 32% in the second day and fourth day of growth. In this time, the unsaturation ratio and UI decreased in the log. phase and increased from the 4th to 5th day as compared to the Pi-starved cells.

Phosphorus content of total lipids

The amount of phosphorus determined in the total lipids is presented in Fig. 2. It was found that, the amount of phosphorus was high at the beginning of growth, aferwards, it decreased drastically during the exponential growth phase (approximately 2×10^6 cells/ml) in which the organism needs high amount of energy to reproduce and this may be the reason for the gradual decrease of Pi during this phase of growth. After

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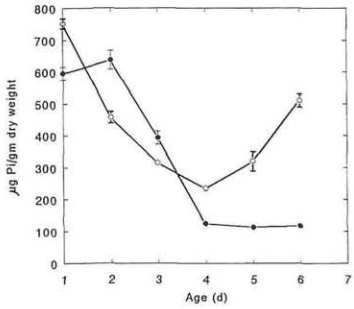

Fig. 2: Effect of phosphorus starvation on the phosphorus content of total lipids in *Chlorella kessleri*. Control (○), phosphorus starved cells (●).

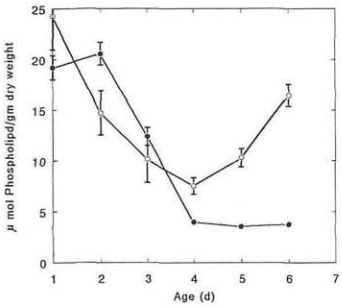

Fig. 3: Influence of phosphorus starvation on phospholipid synthesis in *Chlorella kessleri*. Control (○), phosphorus starved cells (●).

this phase the alga accumulates the secondary metabolites in the form of lipid globules and this was evident from the increase in Pi content after the third day of growth, while there was no increase in Pi-starved alga due to the lack of this nutrient in the growth medium. These results are in a good correlation with the results obtained on phospholipids (Fig. 3) in which Pi consider a major component.

In general, this work demonstrates that cell division, chlorophyll content and photosynthetic activity decreased during the incubation period of phosphorus starvation whereas there was an increase in the dry weight production which is pronounced when the phosphorus reached a minimum at the end of incubation. On the bases of our results on fatty acid, it can be concluded that changes in the proportion of fatty acid composition depend on the growth medium and Pi-starved cells contained much higher concentrations of unsaturated fatty acids than nutrient sufficient algae cells. This is an indicator for high membrane fluidity of the pi-starved cells. Our results indicated also that metabolic and physiological processes are slowed down by the low phosphorus status of the cells whereas dry weight appear to be unaffected and this may be due to the increase in cell volume and cell wall thickness and also the increase in storage compounds such as lipid globules (TILLBERG & ROWLEY 1989). Since phosphorus is often limiting for growth in the upper layers of water in lakes and ponds but may be found in abundance in lower strata. An increase in the weight and lipid yield due to phosphorus starvation of a nonmotile unicellular organisms such as *Chlorella* would make it sink towards strata in the lake with more favorable supply of nutrients. Our suggestions are in agreement with KOHATA & WATANABE 1986 and WATANABE & al. 1988 who reported on the diel migration of a unicellular organisms between phosphorus-poor surface water and phosphorus-rich bottom layers. In

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contrast to the increase in dry weight a drastic reduction of essential photosynthetic pigments and photosynthetic activity was observed. Reduced utilization of the light energy for photosynthesis will increase membrane damage caused by excessive photooxidation, this may be compensated for by a reduction of thylakoid membranes. This hypothesis is in agreement with the results obtained by WANNER & al. 1986 and as a consequence of this hypothesis, starvation conditions should generally lead to comparable changes in cellular parameters, such as accumulation of storage products e. g. lipid (TILLBERG & ROWLEY 1989) and degradation of cellular structures.

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Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 1995

Band/Volume: 35_1

Autor(en)/Author(s): El-Sheek Mostafa M., Rady Amal A.

Artikel/Article: Effect of Phosphorus Starvation on Growth, Photosynthesis and Some Metabolic Processes in the Unicellular Green Alga Chlorella kessleri. 139-151