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EFFECTS OF CARBON DIOXIDE AND PHOSPHATE SUPPLIED DURING GROWTH, ON PHOSPHORUS CONTENT AND PHOTOSYNTHETIC RATES OF SOME UNICELLULAR MARINE ALGAE*

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ABSTRACT

Amphidinium carterae, Cylindrotheca closterium, Dunaliella tertiolecta, and *Phaeodactylum tricornutum* were grown under acid and alkaline conditions produced by varying the CO_2 supply. Increasing the phosphate in the growth medium from 2 to 20 μg P. ml^{-1} had only small effects on the rates of photosynthesis and respiration and on the photosynthesis: respiration ratio, unless inocula were so large that the subsequent cell concentrations used more than 2 μg P. ml^{-1} culture medium in the 48 hour growth period. Such a rate of phosphorus utilization was not found for *Amphidinium*. With the other algae, such high utilization gave cells of greatly decreased phosphorus content, with small decreases in the photosynthetic rates of *Cylindrotheca* and *Dunaliella* and reduction to half with *Phaedactylum*. When inocula were smaller so that less than 2 μg P. ml^{-1} culture medium were used, tenfold phosphate increased cell phosphorus only with *Amphidinium*.

The mean content of cell phosphorus as μg P. μl cell volume was: *Amphidinium* 1.4, *Cylindrotheca* 5.3, *Dunaliella* 14.0, and *Phaeodactylum* 8.3 when grown in media containing 2 μg P. ml^{-1}. In tenfold phosphate, corresponding figures were 1.7, 5.2, 14.1, and 8.5.

INTRODUCTION

IT was previously shown that the photosynthetic rate and the ratio photosynthesis: respiration of *Cylindrotheca closterium* decreased with age of culture (Humphrey and Rao, 1967). During the 14 days of culture, the medium contained excess nitrate, but inorganic phosphate decreased to zero in seven days and the pH rose to 9.2 at three days indicating that the carbon supply also was decreasing markedly. This study was extended to include *Amphidinium carterae* (Humphrey, 1973) and it was shown that if cultures of either alga were grown under the same conditions of stationary batch cultures as used by Humphrey and Rao (1967), disappearance of phosphate was caused mainly be precipitation brought about by the medium becoming increasingly alkaline as growth proceeded. Even when the initial phosphate concentration was increased tenfold, most of it was removed by precipitation rather than being taken up by the algae. The total phosphorus content of the algae was slightly increased as compared with algae grown in the usual medium with only 2 μg P. ml^{-1}, but the rates of photosynthesis and respiration were slightly decreased.

The effects of carbon dioxide supply on these metabolic rates were studied in several algae (Humphrey, 1975), and it was found that certain levels of carbon dio-

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xide supply produced large increases in photosynthetic rate and in the ratio photosynthesis : respiration. Further it was found that with some algae (e.g. *Amphidinium*) photosynthesis decreased markedly when the final culture pH was less than 7, but with others (e.g. *Cylindrotheca*) photosynthesis was still high below pH 7. Therefore these two algae were chosen as contrasting types in order to study the effects of increasing the phosphate concentration in the culture medium at both acid and alkaline pH.

It was also shown in the previous study (Humphrey, 1975) that *Phaeodactylum* behaved differently from all the other algae used, in that it gave maximal rates of photosynthesis only at the acid and alkaline extremes of its growth range. Probably *Phaeodactylum* suffered from extreme nutrient limitation in the middle of the pH range because there, cell concentrations as high as 38×10^6 cells. ml^{-1} were obtained. Therefore *Phaeodactylum* was included in this present study of the effects of phosphate. In order to provide a more direct comparison for *Phaeodactylum*, some experiments were carried out with *Dunaliella* which is also a commonly-used, rapidly-growing alga.

Methods

Bacteria-free cultures were grown as described by Humphrey (1975). The level of carbon dioxide supply was adjusted to give acid or alkaline growth conditions. Phosphorus in the culture supernatant, the washed cells, and the wash fluids was determined as in Humphrey (1973). Photosynthesis and respiration were determined as in Humphrey (1975). Nitrate was determined by the method of Harvey (1929).

Results

None of the metabolic effects of tenfold phosphate was large. The results show that there is little point in increasing the phosphate in the growth medium except where the cell concentrations produced are so high, that they exhaust the phosphate already supplied. With the culture conditions used, such concentrations in millions of cells. ml^{-1} are about 2 for *Cylindrotheca*, 1 for *Dunaliella*, and 4 for *Phaeodactylum*.

Amphidinium

Under acid conditions, tenfold phosphate decreased growth, slightly increased the content of total phosphorus, increased photosynthesis and respiration, and decreased the photosynthesis : respiration ratio. Under alkaline conditions, extra phosphate decreased growth, increased the content of total phosphorus, usually increased photosynthesis, increased respiration, and decreased the photosynthesis: respiration ratio. Table 1 shows the types of changes obtained. In no experiment was the nitrate or phosphate in the growth medium completely used.

Cylindrotheca

In the experiments where cell concentrations rose to about 3×10^6. ml^{-1}, the phosphate in the 2 μg P. ml^{-1} medium was completely used but nitrate was always in excess; phosphorus in such cells was less than 1 μg. 10^{-6} cells and photosynthesis decreased as compared with cells grown to lower concentrations in media containing

TABLE 1. *Amphidinium carterae. pH is the value at the end of the 48 hr growth period and the cell concentration obtained is given as* 10^{-6} *cells* ml^{-1}; P_t *is the total cell phosphorus as* μg *P.* 10^{-6}; *cells; photosynthesis (Ps) and respiration (R) are given as* $\mu l\ O_2 \cdot 10^{-6}$; *cells.* hr^{-1}.

	ACID CONDITIONS		ALKALINE CONDITIONS	
Phosphate supply	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1}	20 μg P. ml^{-1}
pH ..	6.50	6.45	8.00	8.06
Cell concentration ..	0.6	0.4	1.1	0.9
P_t ..	1.03	1.21	1.01	1.30
P_s ..	6.5	8.7	11.1	12.5
R ..	2.1	4.4	3.2	3.9
Ps/R ..	3.2	2.0	3.5	3.2

2 μg P. ml^{-1} (Table 2). Tenfold phosphate maintained the usual cell phosphorus content even at high cell concentrations. When cell concentrations were kept below 2×10^6. ml^{-1} so that less than 2 μg P. ml^{-1} was used, under acid conditions tenfold phosphate slightly increased growth, had no effect on cell phosphorus, usually decreased photosynthesis, had a variable effect on respiration, and usually decreased the photosynthesis : respiration ratio ; under alkaline conditions tenfold phosphate decreased growth, had no effect on cell phosphorus, usually decreased photosynthesis, increased respiration, and decreased the photo-synthesis-respiration ratio.

Dunaliella

In some experiments the phosphate in the 2 μg. P ml^{-1} medium was completely used but nitrate was always in excess. Tenfold phosphate increased growth irrespective of the initial phosphate (Table 3). Cell phosphorus was greatly decreased only when cell concentrations were high. Tenfold phosphate sometimes decreased photosynthesis slightly, decreased respiration a little more, and consequently increased the photosynthesis: respiration ratio. Cells with a deficiency in phosphorus had a slightly lower rate of photosynthesis.

Phaeodactylum

Tenfold phosphate had only small inconsistent effects unless cell concentrations were so high that they used more than 2 μg P. ml^{-1}. When only 2 μg P. ml^{-1} was supplied to large inocula, growth continued after phosphate was exhausted and cell phosphorus fell to 0.2 – 0.4 μg P_t. 10^{-6} cells (Table 4) instead of the normal range of 0.5 – 0.7, (Table 5). Under these conditions tenfold phosphate increased cell phosphorus, photosynthesis, and the photosynthesis: respiration ratio. The highest rates of photosynthesis and the highest values for the photosynthesis: respiration ratio were found under alkaline conditions with final cell concentrations less than 4×10^6 ml^{-1} (Table 4). These rates and values were up to twice those obtained if cultures were grown to higher concentrations.

TABLE 2. *Cylindrotheca closterium*

	ACID CONDITIONS				ALKALINE CONDITIONS			
Phosphate supply	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1} *	20 μg P. ml^{-1}	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1} *	20 μg P. ml^{-1}
pH ..	6.82	6.71	6.41	6.39	7.31	7.25	7.92	8.15
cell concentration ..	1.2	1.3	3.0	2.7	1.6	1.5	2.7	2.5
P_t ..	1.49	1.45	0.83	1.22	1.22	1.31	0.91	1.39
Ps ..	8.6	7.7	5.8	6.5	8.0	7.1	5.7	6.1
R ..	1.1	1.2	1.0	1.1	0.8	0.9	1.2	1.2
Ps/R ..	7.6	6.7	5.8	6.1	10.5	7.8	4.8	5.1

Symbols as in Table 1.

*indicates phosphate exhausted during growth period.

TABLE 3. *Dunaliella tertiolecta*

	ACID CONDITIONS				ALKALINE CONDITIONS			
Phosphate supply	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1} *	20 μg P. ml^{-1}	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1} *	20 μg P. ml^{-1}
pH ..	6.20	6.30	6.84	6.86	7.21	7.20	8.08	8.02
cell concentration ..	0.8	0.9	0.9	1.2	1.0	1.2	2.6	2.7
P_t ..	3.51	3.65	2.90	3.37	3.31	3.89	1.18	2.83
P_s ..	16.5	16.0	14.2	14.5	17.1	14.4	12.5	13.2
R ..	1.7	1.5	1.6	1.2	1.4	1.0	1.0	0.9
P_s/R ..	9.8	11.0	9.1	12.1	12.4	14.4	12.5	14.3

Symbols as in Table 1.

* indicates phosphate exhausted during growth period.

TABLE 4. *Phaeodactylum tricornutum*

	At least 2 μg P. ml^{-1} used				Less than 2 μg P. ml^{-1} used			
	ACID CONDITIONS		ALKALINE CONDITIONS		ACID CONDITIONS		ALKALINE CONDITIONS	
Phosphate supply	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1}	20 μg P. ml^{-1}	2 μg P. ml^{-1}	20 μg P. ml^{-1}
pH ..	6.73	6.90	7.41	7.27	6.77	6.77	8.00	7.94
Cell concentration ..	12.3	11.9	12.5	12.0	2.2	2.6	3.0	2.6
P_t ..	0.28	0.45	0.28	0.56	0.61	0.57	0.53	0.64
Ps ..	1.4	1.7	1.2	1.5	1.6	1.8	2.4	2.2
R ..	0.3	0.4	0.4	0.3	0.4	0.4	0.3	0.4
Ps/R ..	4.2	4.4	3.4	5.8	4.3	5.1	7.5	5.2

Symbols as in Table 1.

In one experiment where a range of acid conditions was used (pH 6.15 to 6.97) for some unknown reason no growth was obtained. After inoculation the tubes contained 1.5×10^6 cells ml^{-1} and after the 48 hr growth period, cell concentrations were from 1.4 to 1.59 $\times$ 10^6 cells ml^{-1}. Microscopic examination showed the cells were about to divide; in the 2 μg p. ml^{-1} medium each million contained 1.10 to 1.32 μg pt, and in the tenfold phosphate 0.80 to 1.43 μg P_t i.e. twice the normal amount.

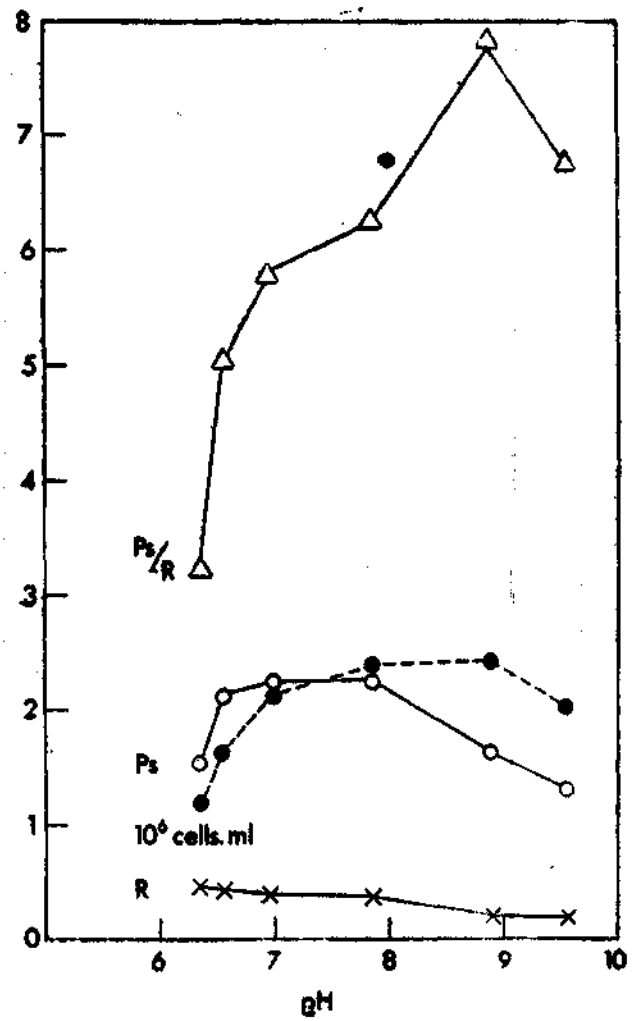

Fig. 1. Growth and metabolic rates of *Phaeodactylum tricornutum*: symbols and units as in Table 1.

Tenfold phosphate was never exhausted even when initial cell concentrations of 7 $\times$ 10^6 ml^{-1} were used (final concentration 36 $\times$ 10^6 ml^{-1}). However, at these high cell concentrations, nitrate was usually exhausted or almost completely so. Therefore the data and curves presented previously (Humphrey, 1975) for *Phaeodactylum* grown in media containing 2 μg p. ml^{-1}, to cell concentrations of 38 $\times$ 10^6

ml^{-1}, were for cells grown under nitrogen-and phosphorus-deficient conditions. Curves similar to those presented previously for other algae, were obtained with *Phaeodactylum* if cell concentrations were kept below 4×10^6 ml^{-1} (Fig. 1).

Discussion

In stationary cultures of *Amphidinium* (Humphrey, 1973), tenfold phosphate decreased photosynthesis. The present results show that the changed culture conditions brought about by supplying carbon dioxide during growth, produced cells which increased their photosynthesis when grown in tenfold phosphate. Such cells also had twice the phosphorus content of cells from stationary culture. Even so, cell phosphorus (Table 5) was less than that found by Sakshaug, Myklestad, Krogh and Westin (1973) who used stationary cultures grown for several days in the presence of penicillin and streptomycin ; 2.6 μg P_t. 10^{-6} cells was found at a medium concentration of 0.2 μg P. ml^{-1} and 4.5 μg P. ml^{-1} at 1.2 μg P. ml^{-1}. Cell concentrations were never more than 0.6×10^6 ml^{-1}.

Table 5. *Cell phosphorus*

The values are for cells grown for 48 hours without exhausting the medium phosphate ; values in brackets are means ; cell volume was calculated from algal dimensions.

Organism	μg P_t. 10^{-6} 2 μg P. ml^{-1}	cells 20 μg P. ml^{-1}	μm^3 cell volume	μg P_t. μl^{-1} 2 μg P. ml^{-1}	cell volume 20 μg P. ml^{-1}
Amphidinium	0.9-2.2 (1.46)	1.1-3.6 (1.73)	1050	0.9-2.1 (1.4)	1.1-3.4 (1.7)
Cylindrotheca	1.1-2.3 (1.78)	1.2-2.2 (1.74)	334	3.6-6.9 (5.3)	3.6-6.6 (5.2)
Dunaliella	3.2-4.2 (3.91)	2.5-4.6 (3.95)	281	11.3-14.9 (14.0)	8.9-16.3 (14.1)
Phaeodactylum	0.5-0.7 (0.62)	0.4-0.7 (0.64)	75	6.7-9.3 (8.3)	5.3-9.3 (8.5)

In general the effects of tenfold phosphate on *Cylindrotheca* were slightly adverse. Supplying carbon dioxide during growth gave cells with twice the amount of cell phosphorus previously obtained in cells from stationary cultures (Humphrey, 1973).

On the basis of cell volume, *Dunaliella* accumulated phosphorus to a greater extent than the other algae (Table 5). The phosphorus-deficient cells produced by growing cultures to between 1 and 3×10^6 cells ml^{-1} showed slightly decreased photosynthesis. The cells used previously (Humphrey, 1975) must have been very deficient since concentrations up to 6.4×10^6 cells ml^{-1} were used. Such cells also had slightly lower photosynthesis. Previously a maximum rate of photosynthesis of 14.7 μl O_2. 10^{-6}. hr^{-1} and a maximum value of the photosynthesis:respiration ratio of 10.9 were found, each at a cell concentration of 5.3×10^6 cells ml^{-6}. The maxima found in the present study were 17.1 μl O_2. 10^{-6} cells. hr^{-1} at 1.0×10^6 cells ml^{-1}, and a ratio of 15.0 at 1.1×10^6 cells ml^{-1}.

Kuenzler and Ketchum (1962) showed that *Phaeodactylum* would continue to grow after exhausting phosphate from the medium, and that this growth lead to decreased cell phosphorus. This has been confirmed (Table 4). The cells used previously (Humphrey, 1975) would have had a very low cell phosphorus content, probably about 0.1 μg 10^{-6} cells. They would also have been suffering nitrogen deficiency. They had half the photosynthetic rate (and consequently half the photosynthesis : respiration ratio) of cells grown at concentrations less than 4×10^6 ml^{-1} where there is no nitrogen or phosphorus deficiency (Table 4). This table also shows that even cells grown to 12×10^6 ml^{-1} (phosphorus-deficient but not nigrogen-deficient) had a higher photosynthetic rate than those used previously which had been grown to concentrations as high as 38×10^6 ml^{-1}. The maximum rate of photosynthesis now found is 2.6 μl O_2. 10^{-6} cells. hr^{-1} (260 mol O_2. mol^{-1} chl *a*.h^{-1}) at 2.9×10^6 cells ml^{-1} and the maximum value of the photosynthesis-respiration ratio is 7.8 at 2.4×10^6 cells ml^{-1}.

The high range of values for cell phosphorus found in the cells about to divide are twice the normal range (Table 5). The cells were larger than normal and the μg P_t. μl^{-1} cell voume would have been within the normal range. The only previous values for phosphorus in cells together with values for the volume of those cells, are those of Kuenzler and Ketchum (1962) and Parsons, Stephens and Strickland (1961). The *Phaeodactylum* used by Kuenzler and Ketchum had a volume of 133 μm^3 and a phosphorus content of 0.8 μg P_t. 10^{-6} cells when grown 48 hours in a medium containing 2 μg P. ml^{-1} (same growing time and normal amount of P supplied in the present study). Thus Kuenzler and Ketchum found 6.0 μg P_t. μl^{-1} cell volumes, a value with which the results in Table 5 agree. The *Phaeodactylum* used by Parsons, Stephens and Strickland (1961) had a volume of 120 μm^3 and a phosphorus content of 0.44 μg P_t. 10^{-6} cells when grown to within the exponential phase in a medium containing 1.6 μg P. ml^{-1}. These values show 3.62 μg P_t. μl^{-1} cell volume, a value below those given in Table 5. Parsons, Stephens and Strickland (1961) also analysed *Amphidinium* grown as above and found 2.8 μg P_t. 10^{-6} cells with a cell volume of 740 μm^3 thus giving 3.74 μg P_t. μl^{-1} which is higher than the value given in Table 5.

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