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PHOTOSYNTHETIC AND RESPIRATORY RATES, AND PHOSPHORUS CONTENT OF ALGAE GROWN AT DIFFERENT PHOSPHATE LEVELS

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ABSTRACT

Amphidinium carteri and **Cylindrotheca closterium** were grown for 42-65 hr in stagnant batch-cultures using media containing 2 μgP/ml (normal phosphate) and 20 μgP/ml (tenfold phosphate). Phosphorus content of the cells was determined after washing several times with 3.5% NaCl until no more P was removed.

After growth the pH of the media ranged from 8.57 to 9.96. In media containing 2 μgP/ml the total phosphorus in 10^6 cells ranged from 0.33 to 1.38 μg for **Amphidinium** and 0.35 to 0.83 μg for **Cylindrotheca**. The corresponding values for tenfold-phosphate were 0.87 to 1.53 μg and 0.63 to 1.23 μg. The ratios of respiration of tenfold-phosphate cells to respiration of normal-phosphate cells ranged from 0.59 to 1.16 **(Amphidinium)** and from 0.60 to 1.03 **(Cylindrotheca)**. Corresponding ratios for photosynthesis were 0.61 to 0.99 and 0.45 to 1.02. Therefore the supply of extra phosphate increased the total phosphorus of the cells but decreased, rather than increased, the rates of photosynthesis and respiration.

The NaCl washings contained large amounts of inorganic phosphorus, probably precipitated from the media by the high pH. Using many assumptions in recalculating data of other authors, the phosphorus content of these washed cells was compared with the phosphorus content of cells of other species.

INTRODUCTION

The photosynthetic and respiratory rates of phytoplankton are often used in models of marine productivity. It would be preferable to use cells reared under natural conditions when determining these rates, but it is not yet possible to culture unicellular algae under conditions even approximating to natural ones. The commonly used stagnant batch-cultures, shaking batch-cultures, chemostat cultures, and turbidostat cultures are therefore worth investigation to see what ranges of values are obtained for cells produced by these different methods.

It is already known that within each method, the values of the rates depend greatly on growth conditions and many other factors. For example, Humphrey and Rao (1967) using stagnant batch-cultures of *Cylindrotheca closterium* found that photosynthesis was greatest on the first day and decreased to about 50% of this at three days, and to about 10% at fourteen days. During these periods the cultures were depleted of much of their carbon source for photosynthesis as was shown by increases in pH to 9 or more. There was also considerable loss of phosphate, but not nitrate, from the culture medium.

In the present investigation, stagnant batch-cultures of *Cylindrotheca closterium* and *Amphidinium carteri* were used to determine the effect of extra phosphate on the magnitude of photosynthesis and respiration.

Methods

Bacteria-free cultures were grown for periods between 42 and 65 hours at 19-21° in 25 ml of culture medium (2 μgP/ml) or the same medium containing 10 times this normal amount of phosphate, in 50 ml conical flasks over white fluorescent lamps giving 1.2–1.3 mW/cm^2 (Humphrey and Rao, 1967). The flasks were shaken gently twice daily. Inocula were 5 ml of 2 or 3-day cultures. Duplicate cell-counts were made in a haemocytometer.

A Rank (Rank Brothers, Cambridge, England) oxygen-electrode was used at 0.65V to measure respiration and photosynthesis. The electrode was calibrated with N_2, 10% O_2 in N_2, and air. A sample of the culture was mixed with one-tenth of a volume of 0.1 M $NaHCO_3$ containing 0.01M K_2CO_3, and 3 ml of the mixture placed in the glass electrode-compartment. The stirrer was set on its slowest speed; water at 20° was circulated through the clear plastic jacket. Respiration was determined for a period of 5-10 minutes while the jacket was covered by black cloth. Photosynthesis was then determined for a period of 10-20 minutes after removing the cloth and while irradiating with white light from a tungsten lamp to give 16 mW/cm^2 inside the electrode-compartment. This intensity was slightly more than enough to saturate photosynthesis. After these determinations the cells were still whole, but their motility was considerably reduced; the pH of the suspensions after the determinations varied from 7.89 to 8.60.

Phosphorus was determined by the method of Murphy and Riley (1962), total phosphorus involving an extra stage of digestion with $HClO_4$. The culture supernatant was obtained by centrifuging 1 to 6 ml of culture for 15 minutes at 15,000 g. The cells were then suspended in 6 ml of 3.5% NaCl with the aid of mechanical vibration for a few seconds. The suspension was centrifuged as before.

This washing procedure was repeated several times to ensure removal of soluble phosphorus.

RESULTS

Tables 1 and 2 give the results of two of the experiments; in these two, cultures were grown for 48 hours. In the 17 experiments carried out, the culture pH on sampling ranged from 8.57 to 9.96 and the amounts of phosphate in the supernatants were, on the whole, lower at the higher pH values. In normal phosphate, the total phosphorus in 10^6 cells ranged from 0.33 to 1.38 for *Amphidinium* and 0.35 to 0.83 for *Cylindrotheca;* the corresponding values for tenfold phosphate were 0.87 to 1.53 and 0.63 to 1.23. The ratios of respiration of tenfold-phosphate cells to respiration of normal-phosphate cells ranged from 0.59 to 1.16 (*Amphidinium*) and from 0.60 to 1.03 (*Cylindrotheca*). Corresponding ratios for photosynthesis were 0.61 to 0.99 and 0.45 to 1.02.

TABLE 1. *Phosphate Concentration and Amphidinium*
P_i and P_t are the inorganic and total phosphorus values referred to 1 ml of the culture
(– signifies below the analytical limit)

	NORMAL PHOSPHATE		TENFOLD PHOSPHATE	
Culture pH	9.78		9.78	
Cell number	1.3×10^6/ml		1.0×10^6/ml	
	μg P_i / ml	μg P_t / ml	μg P_i / ml	μg P_t / ml
Culture supernatant	0.04	0.09	0.38	0.75
First washing	0.42	0.57	9.74	9.64
Second washing	0.33	0.44	7.52	7.44
Third washing	—	—	3.98	4.09
Fourth washing	—	—	2.00	2.06
Fifth washing	—	—	0.73	0.75
Sixth washing	—	—	—	0.34
Seventh washing	—	—	—	—
Eighth washing	—	—	—	—
Ninth washing	—	—	—	—
Washed cells	—	0.43	—	0.87
	μl $O_2/10^6$ cells/hr		μl $O_2/10^6$ cells/hr	
Respiration	−10.9		−10.8	
Photosynthesis	+12.7		+12.2	

TABLE 2. *Phosphate Concentration and Cylindrotheca*
P_i and P_t are the inorganic and total phosporus values referred to 1 ml of the culture
(– signifies below the analytical limit)

	NORMAL PHOSPHATE		TENFOLD PHOSPHATE	
Culture pH	9.3		9.3	
Cell number	1.1×10^6/ml		1.0×10^6/ml	
	μg P_i / ml	μg P_t / ml	μg P_i / ml	μg P_t / ml
Culture supernatant	0.91	0.96	1.92	2.07
First washing	0.21	0.27	8.56	8.25
Second washing	0.15	0.23	4.04	3.84
Third washing	0.11	0.13	1.73	1.56
Fourth washing	—	—	0.74	0.79
Fifth washing	—	—	0.62	0.65
Sixth washing	—	—	0.45	0.45
Seventh washing	—	—	—	—
Eighth washing	—	—	—	—
Ninth washing	—	—	—	—
Washed cells	—	0.37	0.15	0.63
	μl $O_2/10^6$ cells/hr		μl $O_2/10^6$ cells/hr	
Respiration	−4.8		−2.9	
Photosynthesis	+7.0		+4.1	

Although the cultures were bacteria-free, there was much suspended matter not recognisable as algal material. Often, *Cylindrotheca* had several particles attached to the outside of the frustule, and these progressively disappeared as washing proceeded. The suspended matter, particularly the particles, might have been hydroxy-carbonate-phosphate complexes with calcium and/or iron.

DISCUSSION

The results presented here show that under the growth conditions used, extra phosphate inhibited rather than increased the magnitude of photosynthesis and respiration. Recently Ebata and Fujita (1971), have shown that photosynthesis by *Phaeodactylum tricornutum* was increased by 40% if cells were grown in five times the normal phosphate; in both normal ($0.9 \mu g$P/ml) and five-fold phosphate photosynthesis was greatest after 60 hours growth, and the decline thereafter was slower with the higher phosphate. The difference between these results and those with *Amphidinium* and *Cylindrotheca* might be explained by the

different species used or by the differing culture conditions e.g. *Phaeodactylum* was grown in the presence of 0.5% CO_2 and at 60 hours was just starting the logarithmic phase of growth.

Previously reported values for the phosphorus content of unicellular marine algae have usually been calculated indirectly by equating the loss of phosphorus from the culture medium to the uptake of phosphorus by the cells. The experiments with *Amphidinium* and *Cylindrotheca* show that this method can be erroneous because phosphorus compounds are precipitated in the alkaline, seawater-based media often used. This precipitation is further increased as the pH is allowed to rise. Previously published studies (Table 3) do not give information on pH or its variation during culture. Values were probably sufficiently high to cause phosphate precipitation unless there was some mixing, especially by bubbling gaseous CO_2.

Table 3 was compiled by recalculating data and measuring graphs designed for purposes other than to give the phosphorus content of cells. Because many assumptions were made in these arithmetic procedures and because experimental conditions differed greatly between the various investigations, no specific conclusions can be made. The general order of magnitude of the phosphorus content per cell and per volume of cell provide a base for further chemical work and for considerations on phosphorus metabolism.

TABLE 3. *Phosphorus Content of Algae*
Time refers to duration of contact with medium

SPECIES	INITIAL μg P /ml medium	TIME	CULTURE METHOD	μg P/10^6 cells	μg P/μl cell	REFERENCE
Phaeodactylum tricornutum	0.04	9hr	Stagnant batch	0.6	0.6	Ketchum (1939*a*)
,,	0.25	6hr	,,	0.2	0.2	Ketchum (1939*b*)
,,	0.9 and 2.5	12hr	,,	2.0	2	Kuenzler and Ketchum (1962)
,,	0.9	160hr	CO_2 mixing	0.2	0.2	Ebata and Fujita (1971)
Asterionella japonica	0.03	some days	Stirred batch	1.6	0.7	Goldberg *et al.* (1951)
Cyclotella nana	0.1	few days	Turbido-Chemo-stats	0.2	3	Fuhs (1969)
Thalassiosira fluviatilis	,,	,,	,,	3.1	5	,,
Amphidinium carteri	2	42–65hr	Stagnant batch	0.9	0.9	Present paper
,,	20	,,	,,	1.2	1.2	,,
Cylindrotheca closterium	2	,,	,,	0.7	0.9	,,
,,	20	,,	,,	0.9	1.2	,,

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