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Adenosine Triphosphate, Chlorophyll *a* & Particulate Oxidisable Carbon in Some Marine Diatoms

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Cellular levels of POC, chl *a* and ATP of 3 marine diatoms have been determined during their exponential stage of growth in culture. A fairly constant ratio has been observed in the levels of these components throughout the growth period irrespective of size and shape of each organism. Increase in levels of these components throughout the exponential growth phase is proportionate in all constituents studied. A relative fall in level of chl *a* and ATP with respect to POC is observed in the late stationary phase. Application of chl *a*:ATP and ATP:POC ratios in the computation of phytoplankton biomass in environmental samples has been discussed.

Adenosine triphosphate is estimated to determine biomass in the oceans[1-3]. Since ATP is a function of only living cells, it may be used a criterion to separate living and dead matter in environmental samples.

The present study has been made to compare intracellular levels of ATP with commonly used parameters for phytoplankton biomass estimation such as particulate oxidisable carbon, cell numbers and chl *a* and to determine if there is any significant variation of the ratios of chl *a*:ATP and ATP:POC during different phases of growth of the organisms in culture. A further objective of this study is to determine whether ATP in conjunction with chl *a*, POC and cell counts can be used to assay phytoplankton biomass in environmental samples.

Three species of Bacillariophyceae, viz. *Rhizoselenia faroensis* (Ostenf), *Nitzschia closterium* (Ehrenberg) W. Smith and *N. bilobata* (Smith) var. *minor* (Grunow) isolated from the marine environment were used. The culture medium employed comprised (mg/l of sea water): soil extract, 50 ml; $NaNO_3$, 200; NaH_2PO_4, 30; Na-EDTA, 0.81; $CuSO_4 \cdot 5H_2O$, 0.0196; $ZnSo_4 \cdot 7H_2O$, 0.44; $CoCl_2 \cdot 6H_2O$, 0.02; $MnCl_2 \cdot 4H_2O$, 0.36; $Na_2MoO_4 \cdot 2H_2O$; 0.000 126 and thiamine HCl, 0.2.

The cultures were maintained in 2 l Erlenmeyer flasks at room temperature (28-29°C) under photoperiod 8:16 using fluorescent illumination with an intensity of 1.35×10^{15} quanta/sec/cm^2.

Aliquots were drawn at intervals of 24 hr from each flask and analysed for chl *a*, ATP, POC and cell counts. Chl *a*—Aliquots (50 ml) of samples were filtered on Whatman GF/C glass fibre filter papers pretreated with 1% suspension of $MgCO_3$ in dist. water. Chl *a* and phaeophytin determinations were carried out by the fluorescence method[4]. Cell counts—Direct enumeration of cells were done with a Sedgwick Rafter. POC—Aliquots (25 ml) of samples were filtered on glass fibre filters precombusted at 450°C. The filter paper was treated with a solution of Na_2SO_4. POC was determined by the wet oxidation method[4]. ATP—Aliquots (20 ml) of phytoplankton culture suspension were filtered through 47 mm HA Millipore filters (pore size 0.45 μm). The filter paper was quickly transferred to a small beaker containing 3-4 ml of boiling tris buffer. The beaker was allowed to stand for about 5 min to facilitate complete extraction of ATP. The supernatant was transferred to a glass vial and the filter paper was rinsed with another 2-3 ml of boiling tris buffer. The extracts were combined and made up to volume (8 ml). They were kept frozen at −20°C till the time of analysis. ATP content was determined by the bioluminescence reaction utilizing firefly luciferin luciferase[1]. ATP measurements were carried out on an ATP photometer model 2000, SAI Technological Co.

ATP when expressed as a percent of other cellular constituents is 0.52 ($\pm$0.15) of POC and 47.84 ($\pm$4.70) of chl *a*. These values are fairly constant for all three organisms irrespective of their morphology. Holm Hansen[3] has found close uniformity of ATP concentration relative to cellular carbon irrespective of the morphology of the organisms studied. However the average value (0.35% ATP of cellular carbon) reported by him is slightly lower than the present value (0.52 ± 0.15%). The value reported here is based on cellular oxidisable carbon while Holm Hansen's value refers to total cellular carbon which explains the discrepancy.

Increase in the levels of ATP, chl *a* and POC is proportionate during exponential growth in the organisms studied (Figs 1 to 3). These results indicate that there is an effective metabolic control in the production of these constituents.

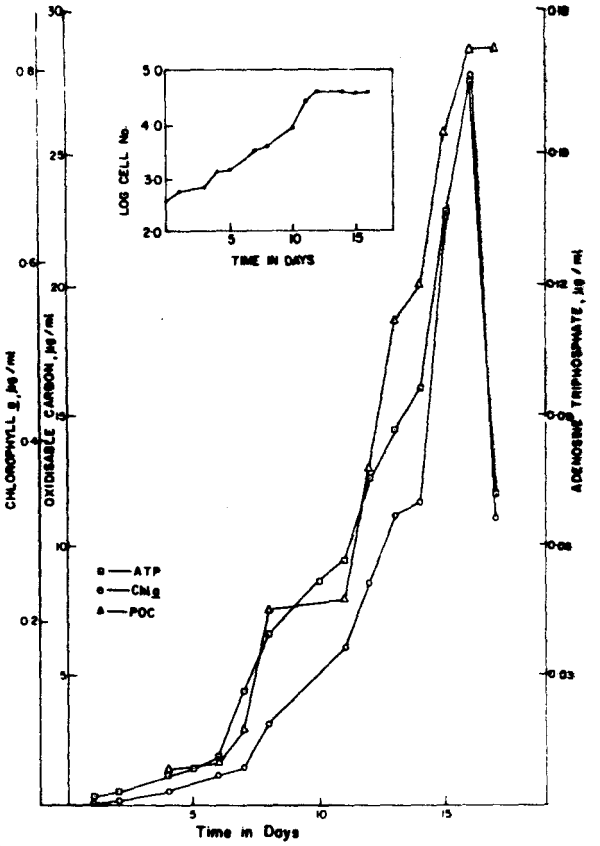

Fig. 1—Concentration of ATP, chl *a* and POC with time in the culture of *N. bilobata* (Inset shows variation in cell number with time)

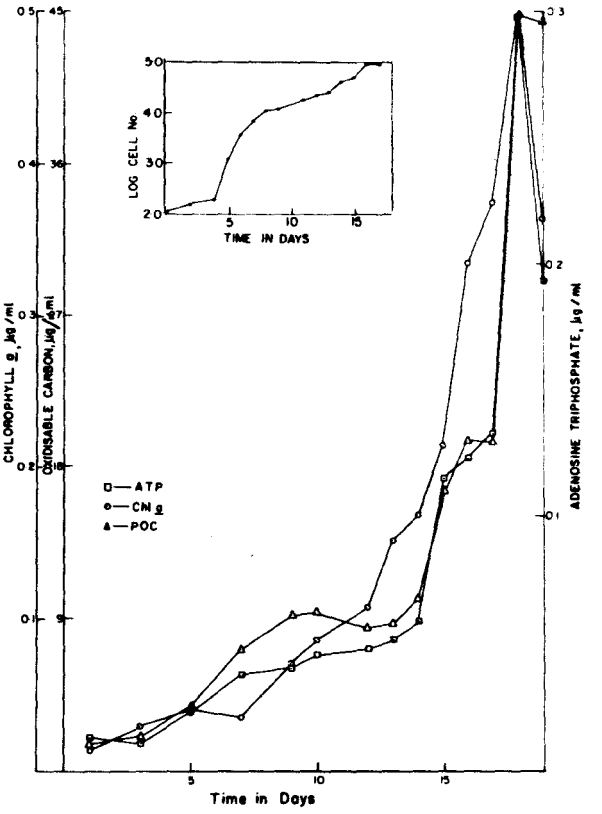

Fig. 2—Concentration of ATP, chl *a* and POC with time in the culture of *N. closterium* (Inset shows variation in cell number with time)

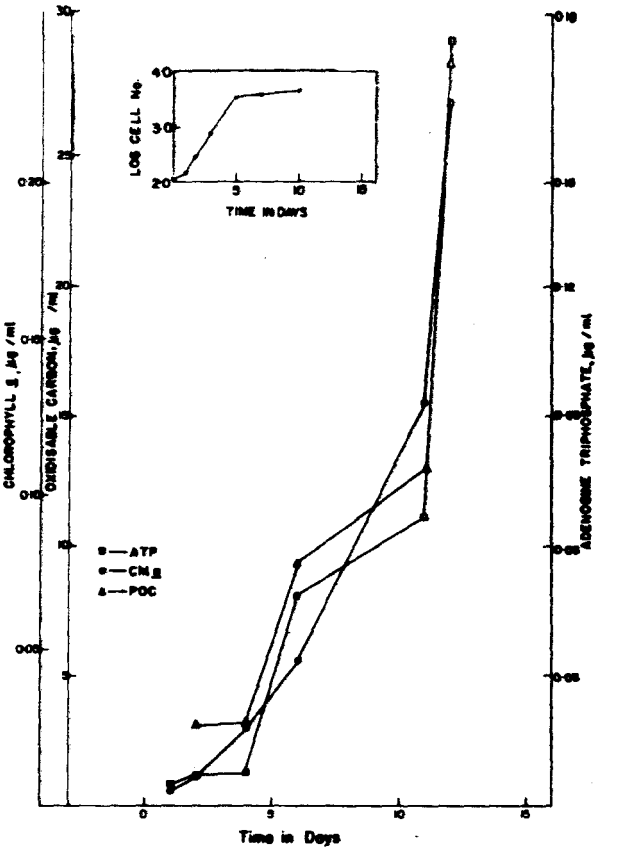

Fig. 3—Concentration of ATP, chl *a* and POC with time in the culture of *R. faroensis* (Inset at top shows variation in cell number with time)

Ratios of chl *a*:ATP and ATP:POC in the three diatoms harvested during the exponential phase are presented in Table 1. The organisms maintain a fairly constant ratio between levels of these cellular constituents throughout the exponential phase of growth. A two-way analysis of variance was used to test the hypothesis that there is no difference in ratios either during the exponential phase or between organisms. Variance ratios are not significant, therefore the null hypothesis was accepted.

Table 2 shows that there is a wider range in correlation coefficients of ATP-POC for each of the organisms. Chl *a*:ATP correlations on the other hand show a narrower range in values and chl *a* is a better indicator of the physiological state of the organisms than POC. A decrease of about 20% in the ATP levels relative to cellular carbon in bacterial as well as algal cells in the late stationary phase has been attributed to extreme nutrient deficiencies and death of the organisms[3,5]. The present results suggest that chl *a*:ATP ratios may be validly used to calculate phytoplankton biomass in environmental samples provided diatoms are the dominant organisms.

Although the measurement of ATP offers a rapid and accurate method for the estimation of cell biomass, it does not differentiate between the

Table 1—Day to Day Variation in Ratios of Chl *a*:ATP and ATP:POC in the Cultures during Exponential Growth Phase

Day	ATP-chl *a*			POC-ATP		
	N. bilobata	*N. closterium*	*R. faroensis*	*N. bilobata*	*N. closterium*	*R. faroensis*
1	0.49	0.48	1.11	208.52	197.88	—
2	0.80	0.47	0.58	204.39	195.79	415.04
3	0.37	0.28	—	172.66	169.11	—
4	0.57	0.45	0.24	221.40	118.89	—
5	0.58	0.51	—	195.59	188.49	—
6	0.46	0.51	0.99	169.23	169.03	192.28
7	0.34	0.54	—	162.50	142.07	—
8	0.34	0.56	0.46	172.22	156.52	192.18
9	0.49	0.51	—	151.86	195.60	—
10	0.32	0.53	—	155.90	192.39	—
11	0.17	0.39	0.72	143.29	186.31	161.80
12	0.60	0.37	0.56	151.07	168.73	240.78

Table 2—Biomass Correlations for Algal Cultures

Organism	ATP-chl *a*		ATP-POC		Chl *a*-POC	
	Correlation coefficient r-value	95% Confidence interval	r-value	95% Confidence interval	r-value	95% Confidence interval
N. bilobata	0.95	0.84-0.98	0.82	0.51-0.94	0.75	0.36-0.90
N. closterium	0.98	0.92-0.99	0.77	0.34-0.93	0.81	0.44-0.94
R. fraoensis	0.93	0.26-0.99	0.84	−0.16-0.99	0.88	−0.10-0.99

contribution of bacteria, phytoplankton and zooplankton. One method of separation is to relate cellular carbon computed on basis of volume of cells to ATP. Sinclair *et al.*[6] have reported a positive correlation between these parameters in St Lawrence estuary. Although in certain areas within the euphotic zone phytoplankton counts are replicable, chl *a* is a better indicator of phytoplankton biomass than cell counts. Thus chl *a*:ATP appears to be more useful for calculating the contribution of phytoplankton to the total biomass.

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