Marine Diatoms Grown in Chemostats under Silicate or Ammonium Limitation. II. Transient Response of *Skeletonema costatum* to a Single Addition of the Limiting Nutrient*

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Abstract

Skeletonema costatum was grown at different steady-state growth rates in ammonium or silicate-limited chemostats. The culture was perturbed from its steady-state condition by a single addition of the limiting nutrient, ammonium or silicate. The transient response was followed by measuring nutrient disappearance of the limiting and non-limiting nutrients at frequent time intervals. The results from a typical perturbation experiment indicate that three distinct modes of uptake of the limiting nutrient can be distinguished; surge uptake (V_s), internally controlled uptake (V_i), and externally controlled uptake (V_e). An interpretation of these three modes of uptake is given and their relation to control of uptake of the limiting nutrient is discussed. The uptake rates of the non-limiting nutrients were shown to be depressed during the surge of the uptake of the limiting nutrient. Kinetic uptake parameters, K_s and V_{max}, were obtained from data acquired during the externally controlled uptake segment, V_e. The same V_{max} value of 0.12 h^{-1}, was obtained under either silicate or ammonium limitation. Estimates of K_s were 0.4 µg-at NH_4-N l^{-1} and 0.7 µg-at Si l^{-1}. Short-term ^{15}N uptake-rate measurements conducted on nitrogen-limited cultures appear to be a combination of V_s or V_i, or at lower substrate concentrations V_s and V_e. It is difficult to separate these different uptake modes in batch or tracer experiments, and ensuing problems in interpretation are discussed.

Introduction

The uptake of the limiting nutrient by phytoplankton has been described by a rectangular hyperbola, similar to the Michaelis-Menten equation for enzyme kinetics; $V = V_{max} \cdot S/K_s + S$, where V is the velocity of uptake, V_{max} the maximal velocity, S the concentration of nutrient, and K_s the half-saturation constant, the value of S when $V = V_{max}/2$. In the past, the uptake rates of nitrogen and silicate have been obtained primarily from batch experiments (Eppley and Thomas, 1969; Eppley *et al.*, 1969; Paasche, 1973). In a typical experiment, different amounts of the limiting nutrient were added to a series of culture flasks. Uptake rates were calculated from the difference in the limiting nutrient concentration at the beginning and end of the experimental period. Nitrogen uptake rates, using the stable isotope, ^{15}N, were measured in a similar way (MacIsaac and Dugdale, 1972). The measurement of the uptake rate in this type of experiment, in which only a single end-point measurement is made, gives only an average uptake rate over the experimental time period.

Recently, Caperon and Meyer (1972) introduced an important refinement in the determination of uptake rate through the use of the perturbation experiment. They used chemostats to prepare popula-

tions representing a variety of known conditioning states. At steady state, the culture was perturbed by a spike addition of the limiting nutrient and the rate of decline in its concentration was followed by repeated sequential sampling. Their sampling intervals varied between 4 and 14 min. The use of chemostats to precondition the algal population was also used by Eppley and Renger (1974) in their study of nitrate and ammonium uptake kinetics.

These workers (Caperon and Meyer, 1972; Eppley and Renger, 1974) have shown that the uptake kinetics reflect the preconditioning nutrient experience of the population. However, there is still no general agreement on just how the uptake rate is related to the particulate nitrogen/cell or cell quota, Q. For example, Eppley and Renger (1974) found that V_{max} per unit cell nitrogen was inversely proportional to dilution rate. This agrees with amino acid uptake studies conducted by North and Stephens (1971). However, Caperon and Meyer (1972) observed a direct correlation between V_{max} and the pre-existing dilution rate. Clearly, this relationship between the uptake rate of the limiting nutrient and the pre-existing dilution rate (preconditioning nutrient experience) needs further investigation for nitrogen limitation, and especially for silicate limitation, since no data exist on this relationship.

In the experiments described in this paper, the perturbation technique was used to document the time-series response of the uptake rate of the limiting nutrient and non-limiting nutrients to a single addition of the limiting nutrient, silicate or ammonium. Additional short-term ^{15}N batch experiments on steady-state ammonium-limited cultures were conducted, to determine uptake characteristics for a range of ammonium concentrations, before the degree of nitrogen deficiency was changed drastically by the utilization of large amounts of ammonium.

Materials and Methods

Description of Chemostat System

The chemostat system and the source of the inoculum of *Skeletonema costatum* used in this study have been described previously (Davis *et al.*, 1973). Concentrations of the major nutrients in the ammonium-limited artificial seawater were 4.0 µg-at PO_4 l^{-1}, 20.0 µg-at Si l^{-1}, 0.5 µg-at NO_3-N l^{-1}, and 10.0 µg-at

NH_4-N l^{-1}; concentrations of trace metals and vitamins were approximately f/50 (Guillard and Ryther, 1962).

Cells were grown in ammonium-limited chemostats under continuous light and in silicate-limited chemostats under continuous light or under a 16 h light:8 h dark photoperiod. The light intensity was 0.14 g-cal $cm^{-2}min^{-1}$ and the temperature was 18°C.

Analyses and Sampling

Methods for nutrient analyses and cell measurements have been described elsewhere (Davis *et al.*, 1973). Particulate nitrogen (PN) and particulate silica (PSi) were calculated from inorganic nutrient analyses of inflow minus outflow. Statistical variation of these methods of analyses and ^{15}N uptake rates are given elsewhere (Conway, 1974; Harrison, 1974).

Daily effluent samples were taken and measurements of fluorescence, cell counts, and concentrations of nitrate, ammonium, phosphate and silicate were made. When no trend in these parameters was observed for several days, it was assumed that steady state had been attained and a perturbation experiment was initiated to evaluate the nutrient-uptake response of the population to additions of the limiting nutrient.

Perturbation Experiment

In the perturbation experiment the concentration of the limiting nutrient in the reactor was increased from the steady-state value, usually <0.5 µg-at l^{-1} to a high value, > 7 µg-at l^{-1}, by the spike addition of a known amount of the limiting nutrient. The concentrations of the limiting nutrient and non-limiting nutrients were measured as often as every 6 min, to follow their disappearance from the medium.

Two types of perturbation experiments were used. In the continuous-mode experiment, pumping of the inflow medium into the reactor vessel was continued after addition of the limiting nutrient. In the second type, a batch-mode experiment, pumping of inflow medium was stopped after addition of the limiting nutrient. The latter type of experiment had the advantage of being shorter.

During the perturbation experiment, sampling of a chemostat was carried out through the use of a PDP-11/20 computer (Digital Equipment Corporation), which executed a sequence of programs called

the Laboratory Operating System (LOS)
(Shields and Pollack, 1972). A schematic
diagram of the automated sampling system
is shown in Fig. 1. The sampling proce-
dure began with two rinses of the sample
lines and the sample receptacle. Next, a
40-ml sample from a chemostat was trans-
ferred rapidly across to the sample
receptacle, where it was pumped first
through a fluorometer and then to an
AutoAnalyzer®. The remaining sample was
released through a dump valve, and the
receptacle was washed with deionized
water for 1 min in preparation for the
next sample. Under this sampling scheme,
a chemostat sample could be taken every
6 min.

Fig. 1. Schematic diagram of chemostat and
automated sampling system. NC: Normally closed;
NO: normally open; C: common

*Calculations of Uptake Rate from Nutrient
Disappearance*

Continuous-Mode Perturbation Experiments

The equations describing the chemostat
during transient state were used to cal-
culate the uptake rate during continuous-
mode perturbation experiments. The fol-
lowing equations were adapted from
Herbert *et al.* (1956).

$$\frac{dS}{dt} = DSi - DS - \frac{VX}{Y}, \qquad (1)$$

where X = concentration of organisms in
particulate form (μg-at l^{-1}), t = time,
V = specific uptake rate h^{-1}, D = dilu-
tion rate, i.e., flow rate/volume of
chemostat h^{-1}, S = limiting substrate
concentration in the reactor vessel (μg-
at l^{-1}), S_i = inflowing limiting sub-
strate concentration (μg-at l^{-1}), and

Y = yield "constant"[1] = $\frac{dX}{dS}$. After solv-
ing for V we have:

$$V = \left(D(Si - S) - \frac{dS}{dt} \right) /X .$$

In finite difference form:

$$V = \left(D(Si - \bar{S}) - \frac{\Delta S}{\Delta t} \right) /\bar{X}, \qquad (2)$$

where $\bar{S}$ and $\bar{X}$ are average values during
the time interval Δt.

The changes in particulate nitrogen
(PN) or particulate silica (PSi) can be
described by the equation

$$\frac{dX}{dt} = VX - DX ,$$
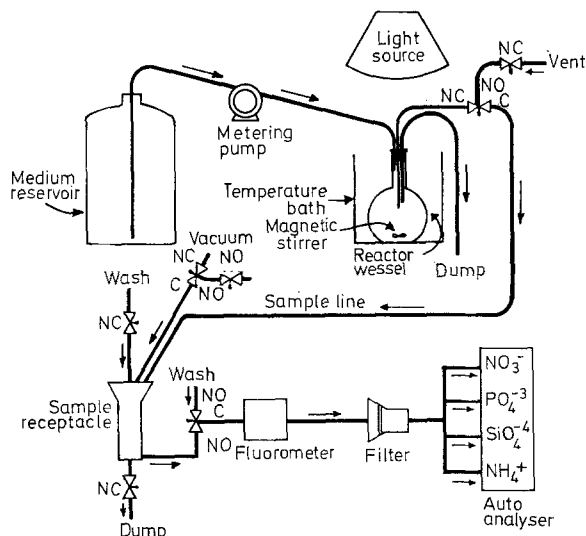
$$\frac{dX}{X} = (V - D) dt,$$

where X = PN or PSi (μg-at l^{-1}). Upon
integration:

$$\ln \frac{X}{X_0} = (V - D) t.$$

Taking the exponents of both sides:

$$X = X_0 e^{(V - D) t} . \qquad (3)$$

The PN or PSi concentration in the reac-
tor during a perturbation experiment is
described by Eq. (3).

Batch-Mode Perturbation Experiments

During a batch-mode perturbation experi-
ment $D = 0$, and since there were no
losses or additions of nitrogen or sili-
ca from the reactor vessel, Eq. (2)
takes the form:

$$V = \frac{\Delta S}{(\Delta t) (X)} .$$

Assuming no excretion of nitrogen or
silica, the increase in PN or PSi should
be equal to the disappearance of in-
organic nitrogen or silicate from the
medium.

^{15}N Studies

The ^{15}N studies consisted of two experi-
ments. Three steady-state chemostat pop-
ulations grown at different dilution
rates were used for each experiment. Sub-
samples of the steady-state populations
were placed in a series of incubation
bottles and appropriate amounts of $^{15}NH_4$

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[1]In short-term studies involving the measurement
of the uptake rate of the limiting substrate,
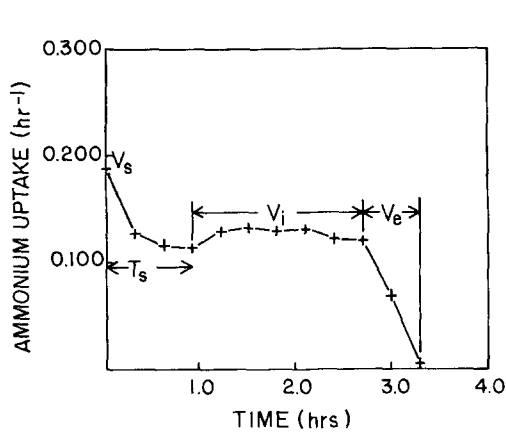Y = 1 if excretion is assumed to be negligible.

Fig. 2. *Skeletonema costatum*. Ammonium-uptake rate during batch-mode ammonium-limited perturbation experiment I-BMP-3 (D = 0.040 h^{-1}). The ammonium-uptake rate has been divided into 3 segments, V_s, V_i, V_e, which are defined in text
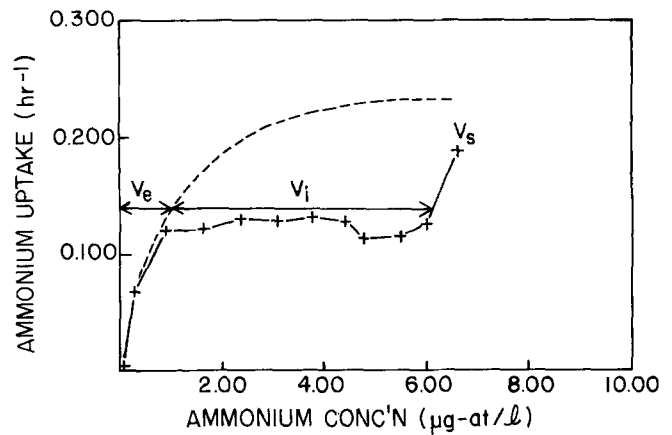
Fig. 3. *Skeletonema costatum*. Ammonium-uptake rate as function of ammonium concentration during batch-mode ammonium-limited perturbation experiment I-BMP-3 (D = 0.040 h^{-1}). The two data points in V_e segment of uptake curve were used to produce dashed hyperbola

were added. Medium f/50 without nitrogen was added to ensure saturation of the other essential micronutrients. Two-hour incubations were carried out under the same conditions of light and temperature that existed in the chemostat. After incubation, the cells were filtered onto glass-fiber filters and dried at 60°C in a vacuum desiccator before analysis. The samples were analyzed for the ^{15}N:^{14}N ratio, and particulate nitrogen content according to Pavlou *et al.* (1974).

Results

Ammonium Limitation

Perturbation Studies

Three experiments were conducted. Experiment I consisted of batch-mode perturbation (BMP) experiments on the populations from three chemostats, connected in series to form multiple or tandem reactors. Inflow medium was pumped into the first chemostat (BMP-3); the outflow of this reactor flowed into the next (BMP-2), and its outflow entered the last reactor (BMP-1). The result was a series of three populations, each more nitrogen-limited than the preceding one. Experiments II and III were continuous-mode peturbation (CMP) experiments, using chemostats operated as single reactors.

The results from a BMP experiment are shown in Fig. 2. Three distinct modes of uptake of the limiting nutrient can be distinguished:

Surge Uptake, V_s. The time interval, T_s, over which the surge uptake occurs, is

defined as the time from the addition of the limiting nutrient until $\Delta v/\Delta t$ first takes on a positive value, following the high initial uptake rate. The magnitude of V_s was estimated from the highest value of uptake rate obtained during the time interval T_s.

Internally Controlled Uptake, V_i. V_i is defined as the mean uptake rate beginning at the end of time period T_s and continuing until the uptake rate decreases rapidly. V_i can be considered as an apparent substrate-saturated uptake rate.

Externally Controlled Uptake, V_e. V_e begins when the uptake rate appears to be a function of decreasing substrate concentrations.

Grouping of the data into these distinct modes of uptake was necessary since it appeared that different mechanisms were responsible in each case. In Fig. 3, the break in the ammonium-uptake curve signaling the beginning of external control, can be seen at approximately 1 µg-at NH_4-N l^{-1}. It is the uptake rate from this externally-controlled segment that should be used to evaluate the Michaelis constants V_{max} and K_s. The number of useful data points in this experiment was reduced to two or three by this analysis. If a hyperbola was fitted to these points, as shown in Fig. 3, a higher estimate of V_{max} and K_s was obtained than if a hyperbola was fitted to the combined results of V_e and V_i segments of the data, as has been done customarily in the past. For ammonium, the number of data points that can be obtained in this V_e segment are small since the segment generally
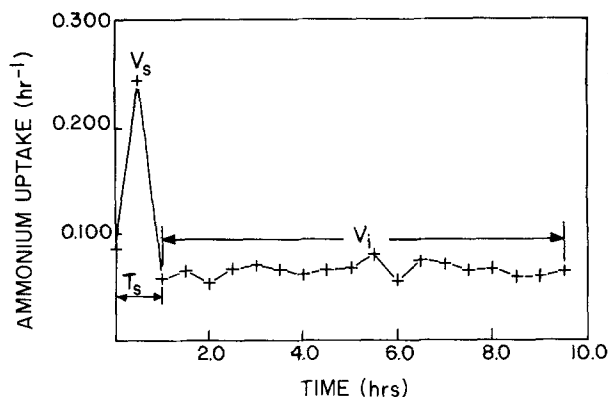
Fig. 4. *Skeletonema costatum*. Ammonium-uptake rate during continuous mode, NH_4-limited perturbation experiment III-CMP-4 ($D = 0.052$ h^{-1})
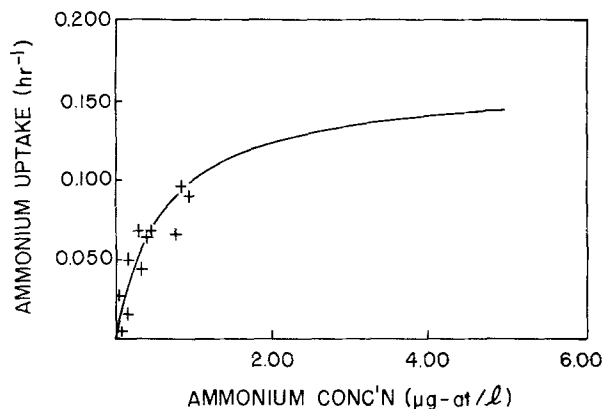

Fig. 5. *Skeletonema costatum*. Ammonium-uptake rate (V_e) as function of ammonium concentration at substrate concentrations less than 1 µg-at l^{-1}. Uptake values and corresponding ammonium concentrations were obtained from Experiments I and II. Values were pooled in order to obtain estimates of V_{max} and K_S, which were 0.125 h^{-1} and 0.44 µg-at l^{-1}, respectively

occurs at concentrations of less than 1 µg-at N l^{-1}. The experimental period can be stretched by using the continuous-mode experiment; however, it may extend for too long a period, as shown in Fig. 4 where values of V_e were not observed within the 10-h duration of the experiment.

The values of V_e and ammonium concentration obtained in Experiments I and II (from 5 perturbation experiments) were pooled, since only 2 or 3 data points from the V_e segment were obtained in each experiment. One hyperbola was fitted to the pooled data (Cleland, 1967), giving a value of $V_{max} = 0.125$ h^{-1} and $K_S = 0.44$ µg-at l^{-1} (Fig. 5). However, V_{max} should be estimated for each experiment if enough data points are available. For example, the estimate of K_S and V_{max} from the pooled data (from 5 experiments) appeared to underestimate the values of the kinetic parameters for one of these experiments (BMP-3, Fig. 3).

Results of the three perturbation experiments (I, II and III) are summarized in Table 1, except for the values of V_e. Within each experiment (I, II and III), the magnitude of V_S was generally inversely proportional to cellular nitrogen content (Q) but there was no apparent correlation with cell surface area or volume. It should be kept in mind that the estimate of V_S was often from a single-point measurement and may not be a good estimate of its absolute magnitude. The values of V_i in Table 1 increase with decreasing nitrogen/cell,

except for BMP-1 and BMP-2; these populations were quite different from the others since they were from the second and third reactors in the tandem arrangement and probably represent cells that were approaching nitrogen starvation.

Phosphate- and silicate-uptake rates were calculated for the three batch-mode perturbation experiments in Experiment I to examine the effect that the surge in ammonium- (the limiting nutrient) uptake had on the utilization of these non-limiting nutrients; the latter were at saturating concentrations throughout the experiment. These data are summarized in Table 2, and one example of the pattern of phosphate and silicate uptake is presented in Fig. 6. These results show that during the surge of ammonium uptake, the uptake rates of phosphate and silicate were depressed, and they have been denoted as V_{lag} in Table 2. The lag time in Table 2 refers to the time from initial exposure to the higher concentration of ammonium until phosphate- and silicate-uptake rates began to continuously increase; this was usually about 0.6 h. The depression of phosphate uptake during the lag period was complete, while silicate utilization was strongly depressed but never to undetectable rates. Under ammonium limitation, the uptake rate of silicate during the depression period was directly proportional to nitrogen/cell, and the uptake rates of phosphate and silicate after the depression were also directly proportional to nitrogen/cell (Table 2).

Table 1. *Skeletonema costatum*. Values of surge uptake (V_s), internally controlled uptake (V_i), and time interval (T_s), from 3 sets of ammonium-limited perturbation experiments (designated I, II, and III), with corresponding values of particulate nitrogen/cell, dilution rate, cell surface area, and cell volume. Reactors in Experiment I were connected in series, with result that Populations BMP-1 and -2 may represent populations that were approaching starvation and thus are not directly comparable with the other 5 populations

Experiment identification	D, dilution rate (h^{-1})	Q, PN/cell $(\mu g\text{-at cell}^{-1} \times 10^{-7})$	V_s (h^{-1})	V_i * (h^{-1})	T_s (h)	Cell surface area (μm^2)	Cell volume (μm^3)	Sampling interval (h)
I								
BMP-1	0.040	0.34	0.218	0.098 (n=3)	1.8	213.9	222.0	0.3
BMP-2	0.040	0.36	0.235	0.103 (n=8)	1.2	263.4	300.3	0.3
BMP-3	0.040	0.47	0.188	0.130 (n=6)	0.9	267.0	291.2	0.3
II								
CMP-1	0.040	0.52	0.276	0.096 (n=4)	3.2	146.6	108.9	0.5
CMP-2	0.047	0.67	0.196	0.088 (n=18)	2.7	154.4	128.2	0.5
III								
CMP-3	0.049	0.73	0.284	0.060	1.0	246.4	263.8	0.5
CMP-4	0.052	0.84	0.245	0.064 (n=18)	1.0	234.3	254.3	0.5

*Mean value of n data points are shown in parentheses.

Table 2. *Skeletonema costatum*. Mean uptake rates of non-limiting nutrients, phosphate and silicate, during batch-mode NH_4-limited perturbation experiments on tandem reactors $(D = 0.04 \ h^{-1})$. Numbers of observations in parentheses

Experiment identification	Time lag (PO_4) (h)	Phosphate uptake rate V_{lag} (h^{-1})	V_i (h^{-1})	Time lag (SiO_4) (h)	Silicate uptake rate V_{lag} (h^{-1})	V_i (h^{-1})	Q * $(\mu g\text{-at cell}^{-1} \times 10^{-7})$	$V_i(NH_4)$ * (h^{-1})	T_s * (h)
BMP-1	0.6	ud (3)	0.059 (12)	0.6	0.004 (3)	0.087 (15)	0.34	0.098	1.8
BMP-2	0.6	ud (3)	0.058 (12)	0.6	0.020 (3)	0.067 (15)	0.36	0.103	1.2
BMP-3	0.6	ud (3)	0.075 (12)	1.5	0.020 (6)	0.110 (12)	0.47	0.130	0.9

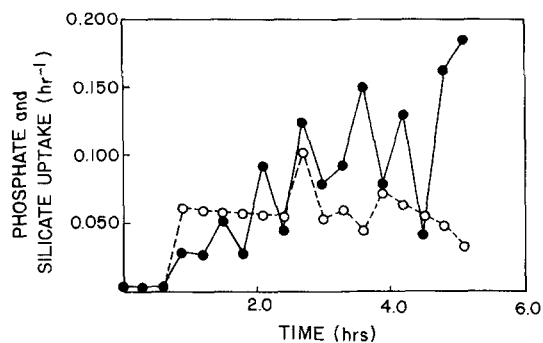ud: Undetectable.
*Data from Table 1.

Fig. 6. *Skeletonema costatum*. Uptake rates of non-limiting nutrients, phosphate (open circles) and silicate (filled circles) during batch-mode, ammonium-limited perturbation experiment I-BMP-1 (D = 0.040 h^{-1}). About 7 μg-at NH_4-N l^{-1} were added at t = 0 h to an ammonium-limited steady-state population
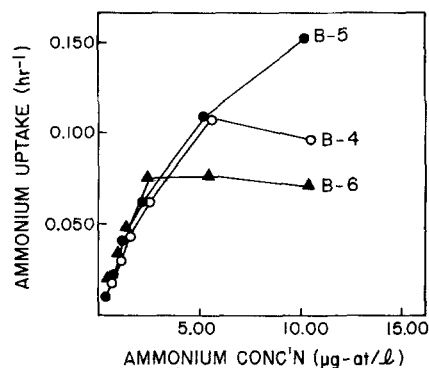

Fig. 7. *Skeletonema costatum*. Ammonium uptake as function of ammonium concentration during ammonium-limited batch ^{15}N experiments. Each uptake-rate measurement represents mean value over 2-h incubation period. Dilution rates were B-5 = 0.044 h^{-1}, B-4 = 0.037 h^{-1}, and B-6 = 0.052 h^{-1}; nitrogen/cell, Q, is given in Table 3; B-5 had lowest value of Q

Table 3. *Skeletonema costatum*. Relationship between V_i and N/cell for both series of batch ^{15}N experiments

Experiment identification	Preconditioning dilution rate (h^{-1})	PN/cell (μg-at cell^{-1} x 10^{-7})	V_i * (h^{-1})	Saturating ammonium concentration (μg-at l^{-1})
B-5	0.044	0.21	0.152[a]	>10.0
B-4	0.037	0.27	0.096	5.0
B-1	0.042	0.27	0.081[a]	>10.0
B-2	0.048	0.46	0.070	5.0
B-6	0.052	0.46	0.066	2.5
B-3	0.058	0.78	0.037	1.5

*Taken as first data point exhibiting substrate saturation in V_{NH_4} versus NH_4 concentration plots.

[a]Not true value of V_i because substrate saturation was not observed; included only for comparison.

^{15}N Studies

Results from the ^{15}N batch experiments (Table 3) on steady-state chemostat populations provided further insight into the nature of the changes in V_i associated with ammonium limitation. The data from one such experiment are plotted in Fig. 7, where the uptake hyperbola is represented by the curve for Population B-5; this population had the lowest nitrogen/cell. Populations B-4 and B-6 followed the same uptake hyperbola, but showed truncation. These truncation levels were interpreted to be the result of internal cellular control and equivalent to V_i of the perturbation experiments as previously defined. V_i values from both series of ^{15}N experiments showed an inverse relation-

ship with corresponding values of PN/cell (Table 3). The ammonium concentration, corresponding to the point at which saturation appears to occur, was inversely proportional to PN/cell. The value of V_i from these ^{15}N experiments were compared to those from the perturbation studies and their relationship to particulate nitrogen/cell (Q) is presented in Fig. 8. The values of V_i showed the same pattern as those values from perturbation experiments, although lying below the latter.

Silicate Limitation

The results of perturbation studies on silicate-limited populations exhibited a time-series pattern of uptake rate similar to that described above for ammonium-limited populations. An example of the uptake rate pattern for one perturbation experiment is shown in Fig. 9, and the data from other perturbation experiments are summarized in Table 4. The silicate concentration where the silicate uptake rate began to decrease rapidly, due to external substrate control, usually occurred at approximately 3 μg-at Si l^{-1}. The value of V_e for three experiments in Table 4 have been pooled and assumed to be described by Michaelis-Menten kinetics (Fig. 10A). Estimates of K_s and V_{max} were 0.7 μg-at Si l^{-1} and 0.112 h^{-1}, respectively. Recent experiments in which the sampling interval in the V_e region was as short as 6 min have yielded better estimates of K_s (1.3 μg-
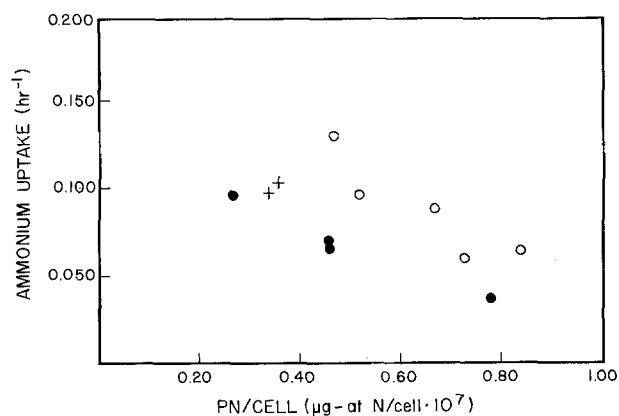
Fig. 8. *Skeletonema costatum*. Ammonium-uptake rate (V_i) as function of PN/cell from [15]N (filled circles) and perturbation experiments (open circles). Uptake rates (crosses) are from second (I-BMP-2) and third (I-BMP-1) reactors of tandem arrangement from Experiment I
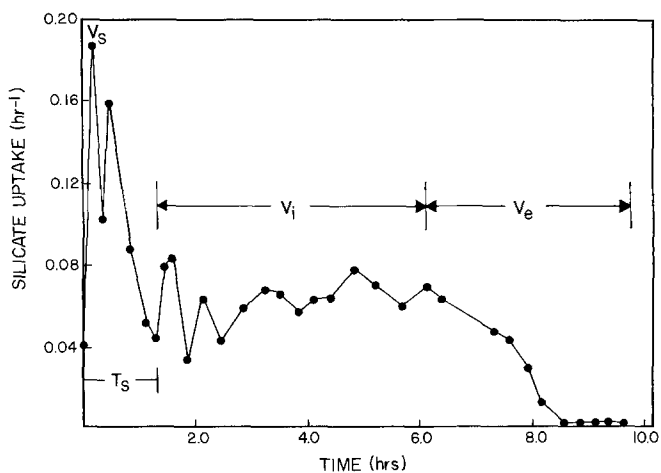

Fig. 9. *Skeletonema costatum*. Silicate-uptake rate after addition of about 10 µg-at Si l^{-1} at $t = 0$ h, during batch-mode, Si-limited perturbation experiment $(D = 0.040 \text{ h}^{-1})$. Symbols are defined in text

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at Si l^{-1}) and V_{max} (0.10 h^{-1}). An example of the data from one such experiment is shown in Fig. 10B. This experiment and others will be described in more detail in a subsequent paper (Breitner and Harrison, in preparation).

The values of V_i and silica/cell (Q) from Table 4 exhibited an approximate inverse relationship (Fig. 11). The values of V_s in Table 4 are higher than the V_{max} values obtained from the data in Fig. 9, in agreement with the results for ammonium.

Uptake rates of the non-limiting nutrients, phosphate and nitrate were calculated for two batch-mode perturbation experiments and are summarized in Table 5. The uptake rates for phosphate and nitrate (denoted as V_{lag}) were depressed for the first hour, coinciding with the surge in the uptake rate of the limiting nutrient, silicate. The time lag in Table 5 refers to the time interval from addition of the limiting nutrient (silicate) until phosphate and nitrate uptake rates begin to increase. The degree of depression of the nitrate uptake rate was slight.

Discussion

Interpretation of Perturbation and [15]N Studies

Perturbation Studies

Ideas and interpretations of the perturbation studies can be presented within a theoretical framework, formulated from

Table 4. *Skeletonema costatum*. Values of V_s, V_i and T_s from Si-limited perturbation experiments, with corresponding values of PSi/cell, dilution rate, and cell volume. Dash: No data

Dilution rate (h^{-1})	Q (µg-at Si cell^{-1} x 10^{-7})	V_s (h^{-1})	V_i* (h^{-1})	T_s (h)	Cell volume (μm^3)
0.031	0.39	0.200	0.095 (8)	1.8	135
0.033[a]	0.38	0.180	0.060 (6)	1.4	150
0.035[a]	0.32	0.195	0.065 (12)	0.9	130
0.036	0.39	0.162	0.065 (11)	2.0	98
0.036	0.45	0.195	0.070 (7)	0.7	199
0.040	0.26	–	0.074 (8)	–	99
0.040	0.33	–	0.088 (8)	0.6	99
0.041	0.33	0.170	0.060 (15)	1.3	–
0.040	0.28	0.180	0.070 (15)	1.3	–
0.050	0.19	0.167	0.133 (4)	0.8	~125
0.050	0.25	0.155	0.104 (8)	1.0	~125
0.054	0.35	0.178	0.110 (6)	0.9	~125

*Mean value of n data points are shown in parentheses.

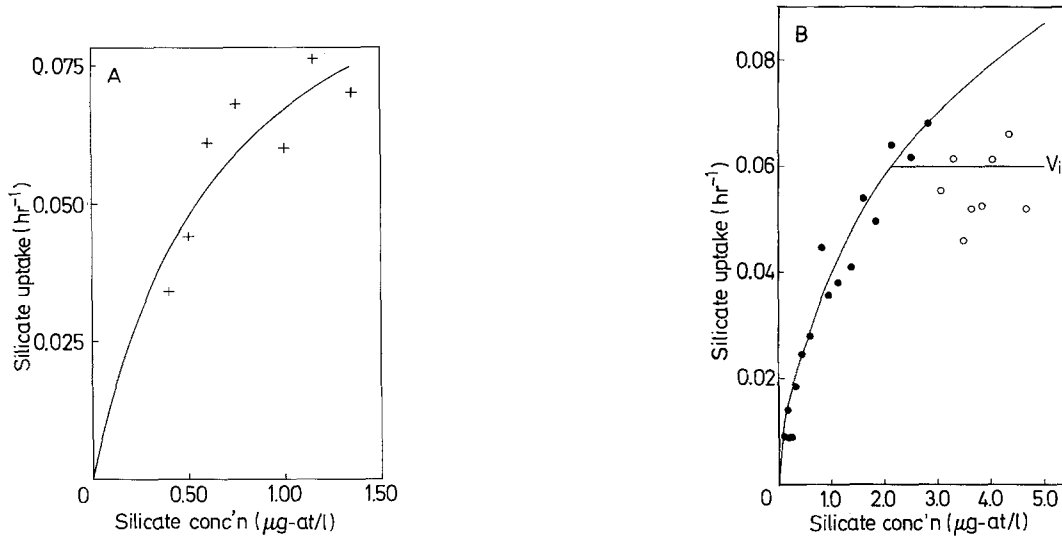[a]V_e values used in Fig. 10A were derived from these experiments.

Fig. 10. *Skeletonema costatum*. Silicate-uptake rate (V_e) as function of silicate concentration at substrate concentrations less than 5 µg-at Si l^{-1}. In (A), the values of V_e and silicate concentration were obtained from 3 experiments in Table 4, and have been pooled. Estimates of V_{max} and K_s were 0.112 h^{-1} and 0.7 µg-at Si l^{-1}, respectively. In (B), values of V_e (filled circles) and V_i (open circles) were obtained from one experiment; estimates of V_{max} and K_s were 0.10 h^{-1} and 1.3 µg-at Si l^{-1}, obtained from a hyperbola fitted to V_e values only. V_i values have been included to demonstrate truncation effect

Table 5. *Skeletonema costatum*. Mean uptake rates of non-limiting nutrients, phosphate and nitrate, during two batch-mode Si-limited perturbation experiments. Number of observations in parentheses

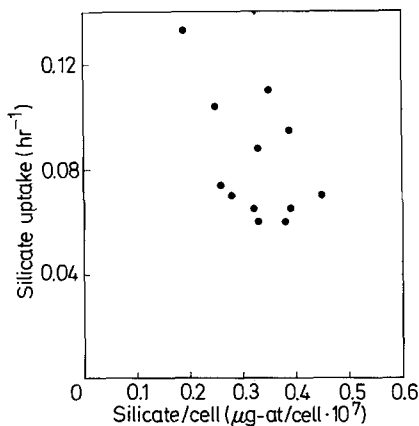| Dilution rate (h^{-1})	Time lag PO_4 (h)	Phosphate uptake rate		Time lag NO_3 (h)	Nitrate uptake rate		V_i (SiO_4) (h^{-1})	T_s (h)
		V_{lag} (h^{-1})	V_i (h^{-1})		V_{lag} (h^{-1})	V_i (h^{-1})		
0.040	0.6	0.016 (10)	0.040 (15)	0.9	0.035 (10)	0.040 (15)	0.070	1.3
0.041	0.8	0.030 (5)	0.038 (15)	1.2	0.035 (5)	0.040 (15)	0.060	1.3

Fig. 11. *Skeletonema costatum*. Relationship between cell quota, Si/cell, and internally controlled uptake rate, V_i, for Si-limited perturbation experiments in Table 4

the results of these studies (Fig. 12). The observed uptake rate (V_e) is represented as a function of external substrate concentration until this concentration increases to a level sufficient to saturate the assimilatory enzyme system. The external substrate concentration at this point is designated S_t. At substrate concentrations greater than S_t, the uptake rate (V_i) is approximately constant until the cellular nitrogen or silica content is increased to a non-deficient level. When this occurs the uptake rate and growth rate are coupled and the cell can effectively maintain a non-deficient chemical composition. The capacity of the permease system, at increasing substrate concentration, is represented as a hyperbolic curve from which V_i branches. The maximum capacity
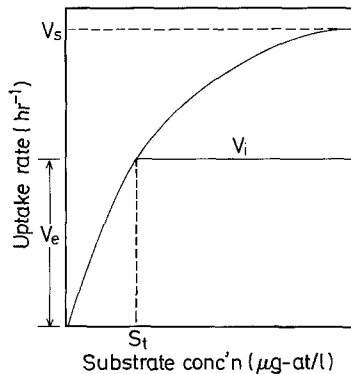
Fig. 12. *Skeletonema costatum*. Theoretical interpretation of V_s, V_i, and V_e formulated from results of ammonium- and silicate-perturbation experiments
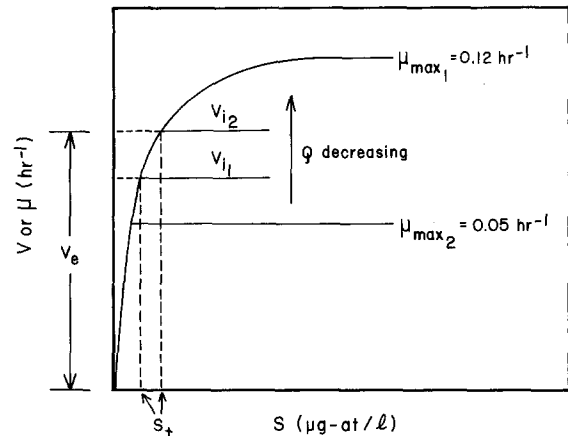

Fig. 13. *Skeletonema costatum*. Relationship between uptake rate measured during perturbation studies and maximal growth rate measured at steady state

of the permease system would be proportional to the surge of uptake V_s. Since this surge in uptake appears to last up to 1 h, it appears that we are not measuring the absorption phenomenon that Droop (1973) has observed.

Our initial experiments indicated that the optimum procedure for obtaining estimates of the V_e segment is to add a large enough spike of the limiting nutrient to allow the V_s and V_i segments to be separated from V_e. Other experiments (Harrison and Conway, in preparation) indicate that two small additions of the limiting nutrient produce estimates of the uptake kinetics similar to those produced by a single large addition; however, a third small addition resulted in a much lower estimate of V_{max}. Therefore, kinetic estimates may be sensitive to the duration of exposure to the saturating nutrient concentration (limiting nutrient). The experimental design should also be such that the maximum number of data points are obtained in the V_e segment by increasing the number of measurements taken during this segment.

The estimates of K_s and V_{max} obtained for silicate-limited populations are more precise than for ammonium limitation because the hyperbola is truncated at a higher substrate concentration, thereby allowing more measurements of V_e to be taken. The estimates of V_{max} and K_s for *Skeletonema costatum* of 0.11 h^{-1}

and 0.7 µg-at Si l^{-1}, respectively, agree with literature values (Davis, 1973; Paasche, 1973).

The V_i segment of the uptake curve (see Fig. 3) is represented as a truncation or branching from the permease uptake hyperbola (Fig. 12). The data in Tables 1 and 4 indicate that the magnitude of V_i is approximately inversely proportional to Q, the cell quota; this is shown in Fig. 13 as a series of truncation levels, accompanied by a series of S_t values. The range of V_i's was approximately 0.05 to 0.12 h^{-1}, which is approximately the same as the two maximal growth rates established for steady-state populations of *Skeletonema costatum* under ammonium and silicate limitation (Harrison *et al.*, 1976).

Values of S_t, obtained from perturbation experiments, were approximately 1 µg-at NH$_4$-N l^{-1} and 3 µg-at SiO$_4$-Si l^{-1}. The slope of the hyperbola is sufficiently steep in the region where V_i branches that S_t appears to be a constant value for each limitation (ammonium or silicate). In reality, analytical measurements are not sensitive enough to distinguish differences in S_t. Sampling intervals also restrict the effectiveness of separating S_t's. Because of the rapid change in substrate with time, an infinitely small Δt would have to be approached before differences in S_t could be distinguished.

In a previous paper (Harrison *et al.*, 1976), the growth kinetics of *Skeletonema costatum* were described. Two and possibly three physiological states were identified, each state having a different maximal growth rate. The theoretical interpretation presented in Fig. 13 is based on populations that have a common maximal growth rate of 0.05 h^{-1} (i.e., from Region 2 as defined by Harrison *et al.*, 1976). For these populations, uptake and growth in terms of cell N or cell Si, are uncoupled during a perturbation experiment. The uptake rate, represented by V_i, is higher than the maximal growth rate (μ_{max}). When the cell quota increases and approaches a maximum ($Q \longrightarrow Q_{max}$) during a long perturbation experiment, V_i would be expected to shift to a maximal growth rate of 0.12 h^{-1} as previously suggested (Harrison *et al.*, 1976). During the course of a typical perturbation experiment, the coupling of V_i and μ_{max} has not been observed because of the short duration of the experiment (4 to 12 h).

The elevated rate of ammonium uptake by N-depleted cells is well known, i.e., uncoupling between growth and uptake (Eppley and Thomas, 1969; Caperon and Meyer, 1972; Eppley and Renger, 1974). The above workers have measured values of V_{max} which we interpret to be equivalent to V_i values. The observation in our study that V_i was inversely proportioned to the degree of nitrogen deficiency of the cell or pre-existing dilution rate agrees with observations made by North and Stephens (1971) and Eppley and Renger (1974).

There is no published data on the relationship between V_i and the pre-existing dilution rate or silica deficiency. However, the increased values of V_i at lower dilution rates may simply be due to the decreasing values of Q, since it is used in the normalization of the specific uptake rate (h^{-1}). In a subsequent paper of this series, Dugdale (in preparation) examines the data in this paper and others, using cellular transport of the limiting nutrient as a more reliable indicator to evaluate shift-up (incremental increase) in the nutrient uptake rate.

The V_s segment of the uptake curve was observed in all perturbation experiments on silicate-limited or ammonium-limited *Skeletonema costatum* populations. The V_s segment implies a permease capacity that exceeds (initially) the internal utilization rate. Recent studies in this laboratory, on ammonium-limited *S. costatum* populations, have shown an increase in the intercellular pool of ammonium during the V_s segment (Quay

Dortch, personal communication) lending credence to the idea of an enhanced permease capacity during the initial stages of a perturbation experiment. This type of phenomenon has also been observed in bacteria (Fisher *et al.*, 1973; Bremer and Dennis, 1975) and marine algae (Caperon and Meyer, 1972; Droop, 1973), although in the two studies on marine algae the surge in uptake was either not mentioned or attributed to adsorption.

^{15}N Studies

The same features observed in the perturbation studies can be seen in the results of the ^{15}N experiments. V_e was evident until a saturating ammonium concentration was reached, S_t. At this point the apparent substrate-saturated uptake rate V_i was observed, with the exception of two of the more nitrogen-deficient populations (B-1 and B-5). The uptake curves of these two populations (B-1 and B-5) may be proportional to the capacity of the permease system at increasing substrate levels (see Fig. 12), since neither population exhibited a substrate saturation uptake rate (V_i). All V_i values fell within the growth rate range of 0.05 to 0.12 h^{-1}, as observed in the perturbation studies, except for the population exhibiting the largest value of Q (B-3).

The biggest difference between the results of the ^{15}N and perturbation studies was in values of S_t. S_t values for the ^{15}N studies ranged from 1.5 to 5 µg-at NH_4 1^{-1}, compared to values of approximately 1 µg-at NH_4-N 1^{-1} from the perturbation studies. However, there was a basic difference in the two types of experiments. The duration of the ^{15}N experiments was ≤ 2 h. In perturbation experiments, S_t values were determined after the populations had been exposed to high levels of ammonium for approximately 2.5 to 7.5 h. Another complication is that ^{15}N uptake-rate measurements from N-limited populations are usually a combination of V_s and V_i, or at lower substrate concentrations V_s and V_e.

The most immediate problem for batch and tracer studies on nutrient-limited populations lies in separating V_s from V_e or V_i measurements. It would appear that modifications in the experimental design should be made for some experiments. For example, pre-incubation of N-limited cells under saturating nitrogen conditions for 1 to 2 h, would allow a measurement of V_i to be made after that time.

Proposed Mechanism for Control of Uptake and Assimilation of Ammonium and Silicate

Sims *et al.* (1968), working with yeast, found that the level of total amino acids inside the cell appeared to regulate the maximal rate of ammonium uptake by feedback control. In the present study, the uptake and assimilation of ammonium was assumed to be influenced by the level of total amino acids in the cell through feedback control of the permease system. The uptake of ammonium and subsequent rise in the total amino acid pool could theoretically result in a decrease of the effective permease concentration. Variations in the effective permease concentration may be the result of: (1) an inhibitor affecting the site of enzyme synthesis; or (2) an inhibitor combining with the enzyme or enzyme-substrate complex, thereby preventing the enzyme from functioning. This inhibition would result from a rise in the total amino acid level during ammonium uptake. The total amino acid level would, in turn, be determined by the nutritional state of the cell. Cells grown at high dilution rates (>0.060 h^{-1}) have a higher nitrogen content (Harrison *et al.*, 1976). In these cells, the protein content is assumed high and all amino acid pools are theoretically filled. Since the cell has no large nitrogen quota to fill, a reduction in the catalytic capacity of the permease system occurs. Restraint is placed on the permease system by the high level of total amino acids found in the non-limited cell. As the dilution rate decreases, the cells shift into a nitrogen-depleted condition and the amino acids inside the cell are reduced. The subsequent reduction in the total amino acid pool would increase the effective permease concentration, thereby increasing the rate of ammonium utilization.

Evidence for increasing protein levels (and probably amino acid levels) with increasing dilution rate has recently been documented and will be described further in a mathematical model of nitrogen uptake and assimilation (Conway *et al.*, in preparation). The proposed mechanism for the control of the uptake and assimilation of ammonium is analogous to that proposed for phosphorus-limited *Scenedesmus* sp. (Rhee, 1973).

A similar mechanism has been proposed for silicate utilization. For silicate-limited populations, it is assumed that there is one internal pool containing approximately 3% of the total cellular silica (Werner, 1966; Azam *et al.*, 1974). The level of this pool controls the uptake of silicate by feedback control and, when the pool is full, the rate-limiting step becomes the incorporation of silicate from the pool into the shell. These relationships will be described further in a mathematical model of silicate uptake and growth of diatoms (Davis *et al.*, in preparation).

Conclusions

The consequences of this view of nutrient-limited uptake and growth affect not only the interpretation of the results of chemostat-culture experiments, but also the interpretation of ^{29}Si and ^{15}N tracer experiments. The values of V_{max} in the literature, obtained from short-term batch experiments would contain the same problems of interpretation as those for ^{15}N measurements. Perturbation experiments, in which V_i and V_s values were not isolated from V_e values, would not yield true estimates of V_{max} and K_s. Kinetic data obtained and analyzed properly should yield values of V_{max} and K_s which are more reliable, based on data corresponding more closely to Michaelis-Menten kinetics.

In the first paper of this series (Harrison *et al.*, 1976), a shift-up in the maximal growth rate was documented for populations that were grown at high dilution rates. Bacteriological studies indicate that the shift-up in the growth rate is the last of a sequence of events to occur (Schaechter, 1968). In this study we have looked at the initial step in the shift-up, the response in uptake rate to an addition of the limiting nutrient, which would be somewhat analogous to a large increase in the dilution rate. The uncoupling between uptake and growth and cellular control over uptake, by increasing the uptake rate of the limiting nutrient and depressing the uptake rate of the non-limiting nutrients, are some of the important features of this early phase of shift-up. Other intermediate events, such as an increase in cellular amino acids and protein, are now under study. Knowledge of this sequence of events should prove to be important in understanding the mechanisms by which the cell controls uptake and growth and in understanding the outcome of species competition experiments.

Literature Cited

Azam, F., B.B. Hemmingsen and B.E. Volcani: Role of silicon in diatom metabolism. Arch. Mikrobiol. 97, 103-114 (1974)

Bremer, H. and P.P. Dennis: Transition period following a nutritional shift-up in the bacterium Escherichia coli B/r: stable RNA and protein synthesis. J. theor. Biol. 52, 365-382 (1975)

Caperon, J. and J. Meyer: Nitrogen-limited growth of marine phytoplankton. Part II. Uptake kinetics and their role in nutrient limited growth of phytoplankton. Deep-Sea Res. 19, 619-632 (1972)

Cleland, W.: The statistical analysis of enzyme kinetic data. Adv. Enzymol. 29, 1-32 (1967)

Conway, H.L.: The uptake and assimilation of inorganic nitrogen by Skeletonema costatum (Grev.) Cleve, Ph.D. Dissertation, 125 pp. University of Washington, Seattle 1974

Davis, C.O.: Effects of changes in light intensity and photoperiod in the silicate-limited continuous culture of the marine diatom Skeletonema costatum (Grev.) Cleve, Ph.D. Dissertation, 122 pp. University of Washington, Seattle 1973

-, P.J. Harrison and R.C. Dugdale: Continuous culture of marine diatoms under silicate limitation. I. Synchronized life cycle of Skeletonema costatum. J. Phycol. 9, 175-180 (1973)

Droop, M.R.: Some thoughts on nutrient limitation in algae. J. Phycol. 9, 264-272 (1973)

Eppley, R.W. and E.H. Renger: Nitrogen assimilation of an oceanic diatom in nitrogen-limited continuous culture. J. Phycol. 10, 15-23 (1974)

-, J.N. Rogers and J.L. McCarthy: Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. 14, 912-920 (1969)

- and W.H. Thomas: Comparison of half-saturation constants for growth and nitrate uptake of marine phytoplankton. J. Phycol. 5, 375-379 (1969)

Fisher, S., L. Buxbaum, K. Lath, E. Eisenstadt and S. Silver: Regulation of manganese accumulation and exchange in Bacillus subtilis W23. J. Bact. 113, 1373-1380 (1973)

Guillard, R.R.L. and J.H. Ryther: Studies of marine plankton diatoms. I. Cyclotella nana (Hustedt) and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8, 229-239 (1962)

Harrison, P.J.: Continuous culture of the marine diatom Skeletonema costatum (Grev.) Cleve, under silicate limitation, Ph.D. Dissertation, 140 pp. University of Washington, Seattle 1974

-, H.L. Conway and R.C. Dugdale: Marine diatoms grown in chemostats under silicate or ammonium limitation. I. Cellular chemical composition and steady-state growth kinetics of Skeletonema costatum. Mar. Biol. 35, 177-186 (1976)

Herbert, D., R. Elsworth and R.E. Telling: The continuous culture of bacteria: a theoretical and experimental study. J. gen. Microbiol. 14, 601-622 (1956)

MacIsaac, J.J. and R.C. Dugdale: Interactions of light and inorganic nitrogen in controlling nitrogen uptake in the sea. Deep-Sea Res. 19, 209-232 (1972)

North, B.B. and G.C. Stephens: Uptake and assimilation of amino acids by Platymonas. II. Increased uptake in nitrogen deficient cells. Biol. Bull. mar. biol. Lab., Woods-Hole 140, 242-254 (1971)

Paasche, E.: Silicon and the ecology of marine plankton diatoms. II. Silicate-uptake kinetics in five diatom species. Mar. Biol. 19, 262-269 (1973)

Pavlou, S.P., G. Friederich and J.J. MacIsaac: Quantitative determination of total organic nitrogen and isotope enrichment in marine phytoplankton. Analyt. Biochem. 61, 16-24 (1974)

Rhee, G-Y.: A continuous culture study of phosphate uptake, growth rate and polyphosphate in Scenedesmus sp. J. Phycol. 9, 495-506 (1973)

Schaechter, M.: Growth: cells and populations. In: Biochemistry of bacterial cell growth, pp 136-162. Ed. by J. Mandelstom and K. McQuillen. New York: Wiley Press 1968

Shields, D.B. and J.E. Pollack: Chemostat laboratory operating system. Decoscope 12, 1-2 (1972)

Sims, A.P., B.F. Folkes and A.H. Bussey (in part): Mechanisms involved in the regulation of nitrogen assimilation in microorganisms and plants. In: Recent aspects of nitrogen metabolism in plants, pp 91-114. Ed. by E.J. Hewitt and C.V. Cutting. New York, Academic Press 1968

Werner, D.: Die Kieselsäure im Stoffwechsel von Cyclotella cryptica Reiman, Lewis and Guillard. Arch. Mikrobiol. 55, 278-308 (1966)

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