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CELL QUOTA GROWTH AND UPTAKE MODELS APPLIED TO GROWTH OF SELENASTRUM CAPRICORNUTUM, PRINTZ IN A NON-STEADY STATE ENVIRONMENT

by

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ABSTRACT

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Applied to Growth of Selenastrum Capricornutum,

Printz in a Non-steady State Environment

by

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Recently proposed algal uptake and growth models dependent on the cell quota (Q), the intracellular limiting nutrient to cell population quotient, were analyzed and applied to experimental data.

The data base used for comparing the models consisted of *Selenastrum capricornutum*, PRINTZ, batch cultures maintained under varying degrees of nitrate limitation over a period of 20 days. The cultures were analyzed for extracellular nitrogen as nitrate plus nitrite, intracellular nitrogen, fluorescence, cell dry weights and cell counts with samples taken at intervals as short as 30 minutes after nutrient spikes. During the culture period, lag, logarithmic and senescent growth phases were encountered.

The cell quota, measured as mg N per mg cell dry weight, ranged from 0.017 to 0.046.

The linked growth/uptake models were fitted to the extracellular nitrogen, intracellular nitrogen and cell dry weight data through the use of a computerized nonlinear optimization routine which adjusted the values of coefficients to minimize a specific error function.

The values of the computed error function were used as a basis for comparisons among the different model simulations.

Analysis suggested that cell growth rates could be represented as a linear function of the cell quota during logarithmic and senescent growth phases. The growth lag encountered, apparently induced by a lag in nutrient uptake, could be represented as a function of the preconditioning growth rate.

The minimum cell quota (Q_0) decreased during successive periods of nutrient starvation, a fact not allowed for in the models studied.

(75 pages)

INTRODUCTION

As pointed out by Droop (1974), the earliest suggestion of the link between the rate of growth of algae and the amount of internal limiting nutrient (the cell quota) was made by Eppley and Strickland (1968). Since then, many authors have observed cell growth as a function of internal nutrient levels (for example, Caperon, 1968; Droop, 1968; Fuhs, 1969; Malone, 1976) and have developed mathematical models to predict this observed relationship and to suggest the physiological mechanisms involved.

This study was initiated to compare the ability of these proposed mathematical models to predict growth and assimulation under a wide range of cell nutrient starvation levels in non-steady state environments (batch cultures). Indirectly, the equivalence of different cell population measures was also tested. This study used cell dry weights while applying proposed models which were developed using either cell counts, cell carbon, cell volume or cell dry weight as the predicted measure of cell population.

The models were applied to data consisting of batch cultures of the green alga, *Selenastrum capricornutum*, PRINTZ, grown under nitrogen limiting conditions. *S. capricornutum* has been chosen as a test organism by the EPA for assaying water quality/nutrient potential (USEPA, 1971). Thus, data collected on the growth characteristics of this alga can be compared to previous data and, in addition, any new information gained can have application in the use of this alga as a test organism.

The effects on algal metabolism by nitrogen limitation have been relatively well documented (Fogg, 1959; Syrett, 1962; Richardson, et al., 1969; Fogg, 1971) as have the kinetics of nitrogen limited growth and uptake (for example, Eppley and Coatsworth, 1968; Eppley and Thomas, 1969; Caperon and Meyer, 1972a, 1972b).

Since the growth dynamics were studied in a batch (rather than continuous) culture, it was necessary to attempt to understand transient effects such as lag in growth and/or uptake. Applied to natural populations this lag effect can be significant in determining which algal species dominates in a given situation (Grenney, Bella and Curl, 1973).

Ultimately then, the results provided some insight into how *S. capricornutum* responded to nitrogen limited growth (measured as dry weight) and how this species compared to the observed response of other species (or growth based on other population measures). Specific objectives to achieve this purpose included the following:

1. The collection of data on the growth, as measured by cell nitrogen, cell dry weight, cell counts, and fluorescence (chlorophyll), of nitrogen limited *S. capricornutum* in batch culture.
2. A review of the literature on nutrient limited algal growth with proposed mathematical models.
3. A comparison of the observed response of *S. capricornutum* to the deterministic models in the literature and the selection of a growth and uptake model which had the following characteristics:
   a) The model must have helped in describing the physiology of algal growth in nitrogen limited environments.

b) The model must have employed measurable constants which could, perhaps, be used for comparing the responses of different algal species (for example, K_m, the half-saturation constant in the Monod model).

c) The model must have been able to simulate the transient effects present in the batch culture.

LITERATURE REVIEW

In 1942, Monod defined the growth kinetics of micro-organisms under the influences of a limiting nutrient (Monod, 1949). His data and reasoning suggested that the growth of a micro-organism was dependent on the (external) limiting nutrient in a manner similar to enzymatic reactions described by the Michaelis-Menten (Langmuir isotherm) enzyme kinetic (surface adsorption) equation:

$$V = V_m \frac{S}{K + S} \tag{1}$$

where,

V = rate of reaction

V_m = theoretical maximum when $S \rightarrow \infty$

S = substrate concentration

K = half saturation constant

When used for rate of growth, $V = \mu$ = specific growth rate. Additionally, Monod assumed that the change in cell population would be in constant proportion to the change in substrate concentration, i.e.:

$$\frac{dX}{dS} = -Y \tag{2}$$

where,

X = measure of cell population per culture volume

Y = proportionality constant = yield of organism per substrate removed

With the assumption of time invariant Y, Equation (2) can be rewritten as a time dependent function:

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \tag{3}$$

which says that the growth rate of the organism is directly proportional to the uptake of the external nutrient.

In Monod's experiments with carbon-limited growth of bacteria this relation must have been approximately true for the substrate concentrations studied since the observed decrease in the growth media carbon over time resulted in a constant increase of biomass over time.

Recent work with algae and limiting nutrients other than carbon have shown that:

1. The growth limiting nutrient uptake rates can exceed the utilization rate of that nutrient for growth (Eppley and Thomas, 1969; Toerien, et al., 1971; Daley and Brown, 1973; Droop, 1973b).
2. Growth may continue after the depletion of the external growth limiting nutrient (Eppley and Strickland, 1968; Fuhs, 1969; Rhee, 1973).

These contradictions to Equation (3) suggest Y is not a constant in time. Thus, it is no longer possible to describe the growth of an organism on the basis of external nutrient supply alone; the concentration of the internal supply, its excess (storage) or degree of depletion, must also be considered. A time variable Y implies that the rate of nutrient uptake does not necessarily limit the growth rate (Gerloff and Skoog, 1957; Caperon, 1968; Eppley and Strickland, 1968).

Growth as a Function of Internal Limiting Nutrient

Thomas and Dodson (1972) had defined a variable (Q, the cell quota) which is the amount of limiting nutrient internal to the cells per total cell population. The dimensions of this variable depend on the measure of the cell population (X) used.

The cell quota must necessarily have limits bounded by the physiology of the cell. The lower limit, Q_o, is the cell quota at which the growth rate approaches zero (Eppley and Strickland, 1968). This definition is essentially equivalent to the mathematical definition of Q_o used in the models studied. This value of the cell quota may also have some physiological significance and has been described as the cell subsistance quota (Droop, 1968, 1974), or the minimum value". . . necessary to maintain cell integrity without growth" (Thomas and Dodson, 1972).

An upper bound is also conceivable. There has to be a finite limit to the storage of a substrate dictated in the extreme by cell lysis. More logically an upper limit would be reached when internal feedback prevented further nutrient uptake (Lehman, Botkin and Likens, 1975).

Several models of specific growth rates (μ) as a function of cell quota have recently been proposed and applied. They are summarized in Table 1. The models are similar in that μ approaches zero as Q approaches Q_o. All except Model 4 are non-linear in Q. If $Q - Q_o << K$ in Model 1, it reduces to Model 4. Caperon (Caperon and Meyer, 1972a) also noted a linear relation between μ and $Q - Q_o$ for some ammonium limited species when the population measure was carbon, which was to be expected when $\mu < \frac{1}{2}\mu_m$ (the case when $Q - Q_o < K$). Model 2 had been shown (Rhee, 1973) to be equivalent to Model 1 when $K = Q_o$ or if $Q >> K - Q_o$. Differences among the models are significant only when $\mu/\mu_m > \frac{1}{2}$ (Figure 1).

Table 1. Growth rate as a function of cell quota.

$\mu = \frac{1}{X}\frac{dX}{dt}$	Author	Limiting "Nutrient"	Cell Population Measure
1. $\mu_m \frac{(Q - Q_o)}{K + (Q - Q_o)}$	Caperon, 1968	nitrate	cell count
	Caperon and Meyer, 1972a	nitrogen nitrogen chlorophyll a	cell count carbon carbon
	Paasche, 1973a	silica	cell count
	Rhee, 1973	phosphorus & polyphosphate fractions	cell count cell volume
2. $\mu_m (1 - \frac{Q_o}{Q})$	Droop, 1968	vitamin B_{12}	cell count
	Droop, 1973a	vitamin B_{12}	cell count
	Droop, 1973b	vitamin B_{12}	cell count
	Droop, 1974	vitamin B_{12}	cell count
	Droop, 1975	vitamin B_{12} & phosphorus	cell count
3. $\mu_m [1 - 2^{**}(1 - \frac{Q}{Q_o})]$	Fuhs, 1969	phosphorus	cell count
4. $\mu_m' (Q - Q_o)$	Malone, 1976	nitrate	dry weight

NOTE that as written, μ_m' in Model 4 is a growth constant with units $(\text{days})^{-1}$ $(\text{cell quota measure})^{-1}$. For consistant units Model 4 could be rewritten as $\mu = \mu_m (Q/Q_o - 1)$ where $\mu_m = Q_o \mu_m'$ and would have the units $(\text{days})^{-1}$.

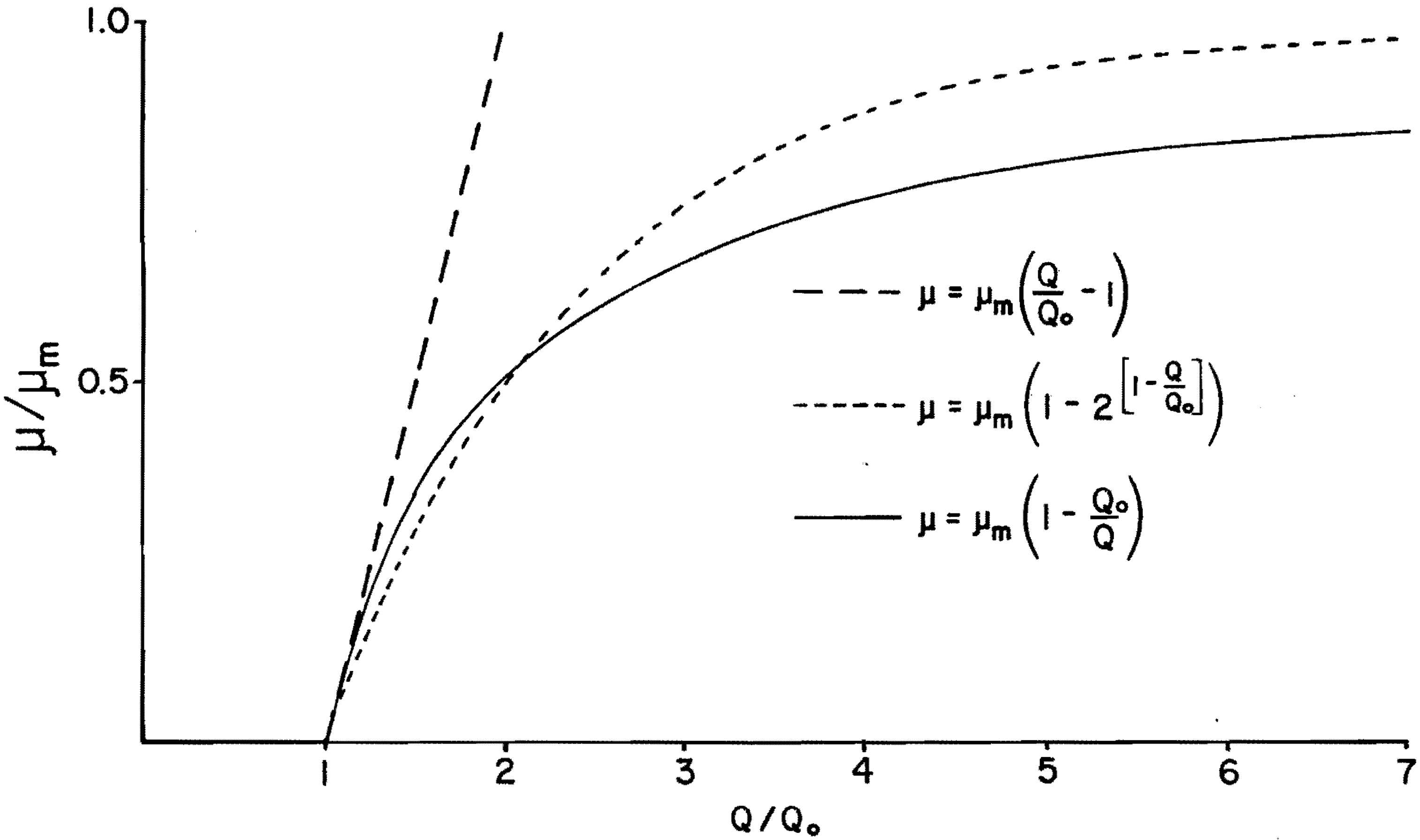

Figure 1. Cell quota model responses, fraction of maximum growth rate as a function of fraction of cell quota above minimum.

Uptake

With Q defined as the mass of growth limiting nutrient internal to the cells per mass of cells (or total cell numbers) and X defined as the mass concentration of cells (or cell concentration), a mass balance equation of the external limiting nutrient concentration (S) can be derived for a constant volume culture:

$$-\frac{dS}{dt} = \frac{d(XQ)}{dt} \tag{4}$$

This relation assumes S changes only with uptake into the cells. Since Q is not constant,

$$\frac{-dS}{dt} = \frac{XdQ}{dt} + \frac{QdX}{dt}$$

Dividing by X and defining

$$u = -\frac{1}{X}\frac{dS}{dt} = \text{relative uptake rate}$$

$$\mu = \frac{1}{X}\frac{dX}{dt} = \text{specific growth rate}$$

$$-\frac{dQ}{dt} = \text{internal nutrient utilization rate}$$

The following relation is established:

$$\mu Q = u - \frac{dQ}{dt} \tag{5}$$

Equation (5) illustrates that the nutrient utilization rate (μQ) is not simply a function of uptake but rather a combination of uptake rate and change in the cell's internal nutrient storage (Figure 2). Paraphrasing Droop (1974), the algal growth potential of a body of water is dependent on both the cell internal nutrient supply as well as the external supply.

Since growth can no longer be considered a constant times the uptake, a separate function for uptake must be described. The most used

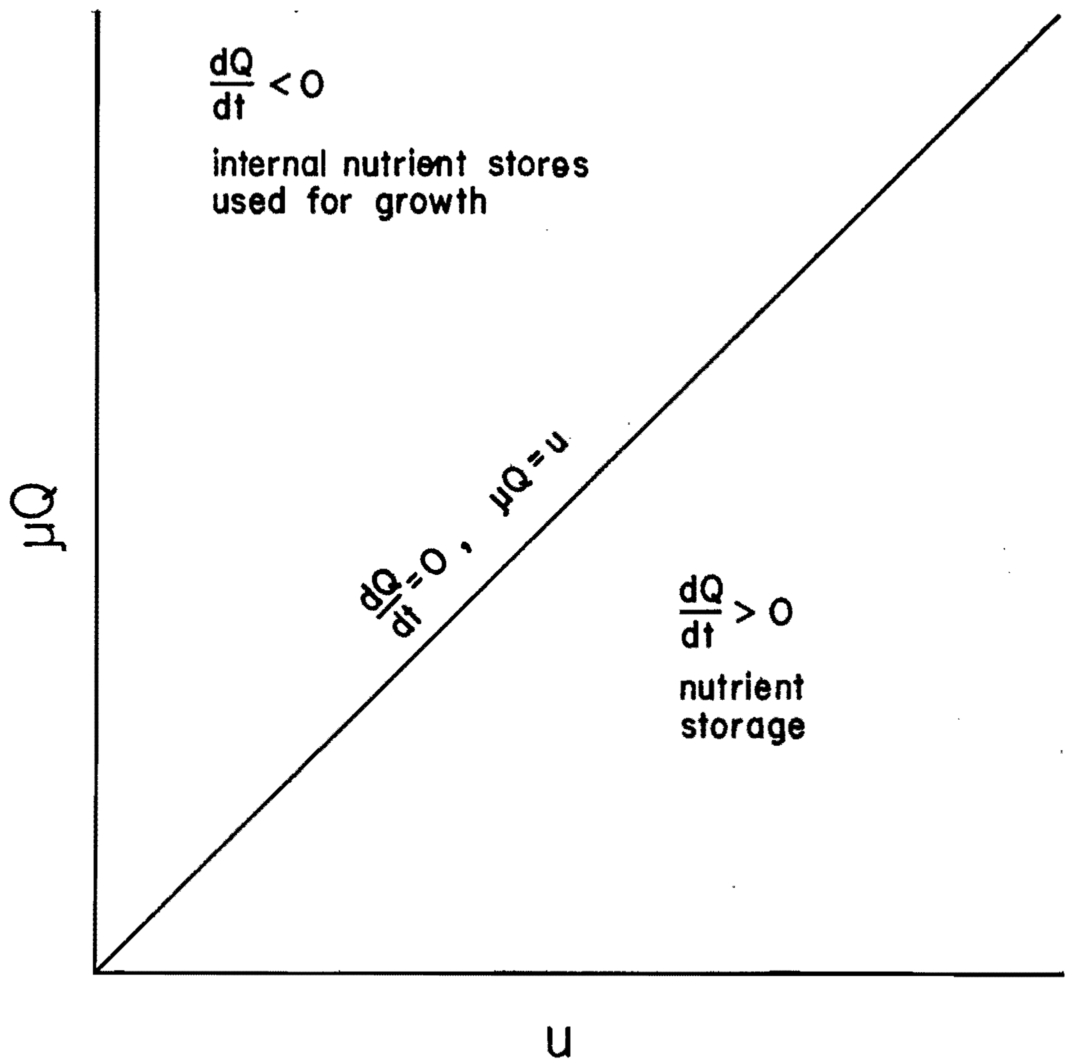

Figure 2. Nutrient utilization (μQ) versus nutrient uptake (u) with internal nutrient storage.

form is the Michaelis-Menten enzyme kinetic equation where the uptake rate is a function of the external growth limiting substrate concentration. Variations of the Michaelis-Menten form have been observed and applied by different authors (Table 2).

All models listed in Table 2 except Model 6 are Michaelis-Menten type; Model 6 being the case where the K of Model 1 is much greater than S. The S_o in Model 4 has been used to allow for finite amounts of substrate remaining in the medium when uptake stops. Two authors have found the maximum uptake rate to vary with the growth rate; Caperon and Meyer (1972b) observed a direct relationship (Model 5), Rhee (1973) observed an inverse relationship which he found to be equivalent to an inverse relation with the cell quota (Model 2). This apparent contradiction will be discussed in a later section. Rhee also discussed previous experiments which showed K to vary directly with the cell quota (Model 3).

Multicompartment Models

The models so far described, except for the Monod model, can be considered two compartment models; the intracellular nutrient is divided into subsistence and growth-producing components. The Monod model is one compartment; all of the nutrient which is taken up is used for growth.

Fuhs (1969) postulated a three compartment model of algal growth with respect to phosphorus supply with:

1. A structural compartment, not affected by nutrient supply, composed of phosphorus compounds required to maintain the integrity and viability of the cell (thus, similar to Q_o).

Table 2. Uptake (u) as a function of external nutrient.

$u = -\frac{1}{X}\frac{dS}{dt}$	Author	Comments
1. $u_m \frac{S}{K + S}$	Droop, 1968, 1973a, 1973b Eppley, Rogers and McCarthy, 1968 Eppley and Thomas, 1969	u_m and K are constants
2. $u_m \frac{S}{K + S}$	Rhee, 1973	$u_m = (K' + \mu)^{-1} = K' (K'' + Q)^{-1}$ K a constant
3. $u_m \frac{S}{K + S}$	Rhee, 1973	$K = (1 + K')(Q - Q_o)$ u_m a constant
4. $u_m \frac{S - S_o}{K + (S - S_o)}$	Droop, 1974, 1975 Paasche, 1973b	u_m and K are constants S_o = finite amount of limiting nutrient remaining in culture when uptake stops
5. $u_m \frac{S - S_o}{K + (S - S_o)}$	Caperon and Meyer, 1972b	$u_m = K'\mu$ K a constant S_o as above
6. u'_m S	Malone, 1976	u_m a growth constant with dimentions $1\ (mg)^{-1}\ (day)^{-1}$

K' and K" are constants.

2. A synthetic (functional) compartment containing phosphorus compounds involved in the cell growth machinery (similar to $Q - Q_o$).

3. A storage compartment which would only become evident when the phosphorus is supplied in excess.

Grenney, Bella and Curl (1973) developed a three compartment model which was applied to the nitrate-limited algal growth data of Caperon (1969). The postulated cell (population) was composed of an inorganic nitrogen compartment (N_1), nitrogenous organic intermediate compartment (N_2), and a cell protein (as nitrogen) compartment (N_3). The cell protein compartment was the cell population measure, with the amount of protein per cell assumed to be constant. This model allowed for a variable cell quota since nitrogen could build up in compartments N_1 and N_2 before being converted to cell protein (N_3). The possibility of protein breaking down to intermediates was also included. Rates between compartments and uptake into N_1 were of the Michaelis-Menten type.

Measures of Cell Population

The model of Grenney, Bella and Curl (1973) used protein as the measure of cell population. Since the amount of protein per cell was assumed constant, the concentration of protein in the reactor (X_p) would be in constant proportion with the concentration of cells (X_n) in the reactor ($X_p = CX_n$, C a constant). With this relation assumed it was possible to compare specific growth rates predicted on the basis of protein (μ_p) with Caperon's (1969) growth data based on cell numbers ($\mu_p = C\mu_n$).

As previously shown, growth kinetics have been based on various measures of the cell population: carbon, cell counts, protein, dry weight, and cell volume. As pointed out by Toerien, et al. (1971), chlorophyll a, ATP, and DNA have also been used. The specific growth rates can be compared for an algal species only if the population measures are the same or are in constant proportions. While in theoretical unrestricted growth (growth with excess of all nutrients), the assumption that two measures of cell population would be in constant proportions may be a good approximation, under stress conditions it is not likely. Fogg (1959), in his discussion of nitrogen-limited growth, described different experiments showing an increase in carbon and dry weight per cell, a decrease in chlorophyll per dry weight, and a variable amount of protein per dry weight. Caperon and Meyer (1972a; see Table 1) have used the variation in chlorophyll a/carbon (a ratio of two different measures of cell population) to predict a carbon based nitrogen-limited growth rate.

Cell population measures are not equivalent. Similarly, what is measured as cell quota (limiting nutrient/unit cell population) for one measure of cell population cannot be assumed equivalent to another (i.e., nitrogen/dry weight $\neq$ nitrogen/carbon). As an example, Caperon and Meyer's (1972b) study of ammonium starved algae showed Q invariant when the population was measured as cell counts but varied when the population was measured as carbon.

MATERIALS AND METHODS

Selenastrum capricornutum, PRINTZ, obtained from a stock culture maintained at the Utah Water Research Laboratory, was grown in a modified version of the synthetic algal nutrient medium (USEPA, 1971) which is shown in Table 3. The medium was so modified to insure that nitrogen would be the limiting nutrient throughout the experiment (Malone, et al, 1975). The modifications included:

1. All distilled water used in dilutions was passed through an ammonium removing ion-exchange column.
2. All concentrations were adjusted to 3.3 times the listed values, except:
   a) $NaNO_3$ concentration was adjusted to provide the degree of limitation desired, and
   b) $NaHCO_3$ concentration was 84.00 mg/1.

Three culture vessels of 3 liter capacity each were used. The cultures were continuously stirred with magnetic stirring bars. Continuous illumination by "cool-white" fluorescent tubes provided an intensity of 6200 lux across the centerline of the base of the growth cabinet.

A mixture of air and carbon dioxide was continuously bubbled through the cultures. The gas mixture was serially bubbled through 1 N H_2SO_4 to remove ammonium (Thomas and Dodson, 1972); a bicarbonate buffer; and distilled water prior to being bubbled through the cultures. The air to carbon dioxide ratio was adjusted to provide a pH of

7.1 ± 0.1 in a separate flask containing 84 mg/l $NaHCO_3$ which was being aerated concurrently with the culture flasks.

Table 3. Algal nutrient medium.

Nutrient	mg/l	μg/l
$NaNO_3$	a	
K_2HPO_4	3.47	
$MgCl_2$ $6H_2O$	40.57	
$MgSO_4$ $7H_2O$	45.00	
$CaCL_2$ $2H_2O$	14.70	
$NaHCO_3$	84.00	
H_3BO_3		618.3
$MuCl_2$ $4H_2O$		1390.
$2nCl_2$		11.0
$CoCl_2$ $6H_2O$		4.7
$CuCl_2$ $2H_2O$		3.7
$FeCl_2$ $6H_2O$		533.3
Na_2EDTA $2H_2O$		1000.
Na_2MoO_4 $2H_2O$		24.33

[a]Varied during experiment.

The experiment was run in a constant temperature and humidity room which maintained the cultures at 25 ± 1°C after an initial temperature instability.

Dry weights were determined with Whatman GF/C filters which had been previously washed, muffled and tared on a Cahn Electrobalance. Algal cell nitrogen fractions were determined with a Coleman Nitrogen Analyzer. It was necessary to store the suspended solids for up to 15 days in a frost-free freezer before final weights and percent nitrogens were determined.

External nitrogen determinations were performed on the filtrate using the cadmium reduction method described in Standard Methods (APHA, 1975). All of these nitrate plus nitrite determinations were made immediately after sampling except those on day 9.92 and day 9.66 which were stored at 4 C for 6 hours and 12 hours, respectively.

Fluorescence was measured on a Turner model 111 Fluorometer equipped with a #110-922 (430 nm) excitation and #110-921 (> 650 nm) emission filters.

Cell counts were determined microscopically with a haemocytometer.

EXPERIMENTAL DESIGN

The experiment consisted of three phases:

Phase I: day 0.0 to day 9.19

Nitrogen enriched cells (cells growing in complete medium where N was not limiting growth) were concentrated by centrifugation, washed three times in 15 mg/1 $NaHCO_3$ (ammonium free) buffer, and suspended in nitrate-free fresh medium. The cells are allowed to grow to senescence for 8.24 days. In preparation for the next two phases and to allow enough volume for future determinations to be made the cultures in the three flasks were mixed and fresh medium (nitrogen-free) added. The nutrient concentrations of the fresh medium was such that the total volume of fresh medium plus Phase I culture would have the nutrient concentrations of Table 3 if all nutrients had been utilized in Phase I. After this dilution the cultures were allowed to stabilize for approximately one day prior to the start of Phase II.

Phase II: day 9.19 to day 11.00

$NaNO_3$ was added to the nitrogen starved cells to give a nitrogen concentration of 1.6 mg/1. Aliquots were taken 5 minutes after the nitrogen addition and every 1/2 hour thereafter for six hours. A less taxing sampling schedule was then assumed until the end of this phase.

Phase III: day 11.00 to day 19.10

The cells having returned to nitrogen starvation were again supplied with $NaNO_3$ to give 1.6 mg N/1. The sampling schedule was the same as described in Phase II.

CURVE FITTING TECHNIQUE

The standard method for computing the specific growth rate (μ) in batch cultures is by the use of the formula (USEPA, 1971):

$$\mu = \frac{\ln (X_2/X_1)}{t_2 - t_1} \tag{6}$$

with

X_2 = biomass at time = t_2

X_1 = biomass at time = t_1

This formula is derived from assuming first order growth and constant μ and solving the differential equation:

$$\frac{dX}{dt} - \mu X = 0 \tag{7}$$

Once μ has been computed by Equation (6) during a small time interval it would be related to the value of the cell quota during the same interval.

The major difficulty with this approach is that at small time intervals the measurement error of the dry weight (especially at low cell densities) can mask the cell density increase. For example, during the lag at the start of Phase II, the computed values of μ ranged from -2.0 to 5.4 days^{-1}.

The computation of uptake rates is also difficult in batch studies. The relative uptake rate is defined by:

$$u = -\frac{1}{X}\frac{dS}{dt}$$

If X and u are assumed to be constant during a small time interval the above relation can be integrated to give:

$$u = \frac{S_1 - S_2}{X\ (t_2 - t_1)}$$

where,

S_2 = external nutrient concentration at time = t_2

S_1 = external nutrient concentration at time = t_1

X = average biomass concentration during interval $t_2 - t_1$

Caperon and Meyer's (1972b) approach was to assume their uptake model (Number 1 in Table 2).

$$u = u_m \frac{S}{K + S} = -\frac{1}{X}\frac{dS}{dt}$$

which integrates to (with the assumption that X is time independent):

$$u_m X\ (t_1 - t_2) = (S_2 - S_1) + K\ \ln\ (S_2/S_1) \qquad (8)$$

A modified form of this solution where a time function of X (Equation 7) is assumed will be used later.

All of the above procedures involve assumptions which are not necessarily based on a nutrient mass balance (Equation 4). An idealistic method would be to assume the model system, uptake plus growth, without any simplifying assumptions; i.e., assume functions for μ and u in the following set of linked differential equations:

$$\frac{dX}{dt} = \mu X \tag{9}$$

$$\frac{dQ}{dt} = u - Q\mu \tag{10}$$

$$\frac{dS}{dt} = - uX \tag{11}$$

For example, assuming the model of Malone (1976)

$$\mu = \mu_m \left(\frac{Q}{Q_o} - 1\right)$$

$$u = u_m S$$

the three coefficients μ_m, Q_o, u_m, would be solved for simultaneously.

The method for solving for coefficients used in this study is shown in flow chart form in Figure 3. The initial estimate of the coefficients ($\overline{\beta}_o$) were used in a forth order Runge-Kutta prediction of the model system state variables (S^p, X^p, Q^p). The values were compared with the observed data (S^o, X^o, Q^o), and an error function (E) computed. The error function chosen in this study was a linear combination of the normalized sums of squares difference between the observed and predicted values:

$$E = \frac{1}{2} \sum_{i=1}^{n} \frac{(S_i^o - S_i^p)^2}{S_i^p + 0.01} + \frac{1}{2} \sum_{i=1}^{n} \frac{\{(XQ)_i^o - (XQ)_i^p\}^2}{(XQ)_i^p} + \sum_{i=1}^{n} \frac{(X^o - X^p)^2}{X^p}$$

where the subscript i represents the value at time = t_i and n is the total number of data points. The number 0.01 was used in the denominator of the external nitrogen error since S_i^p goes to zero.

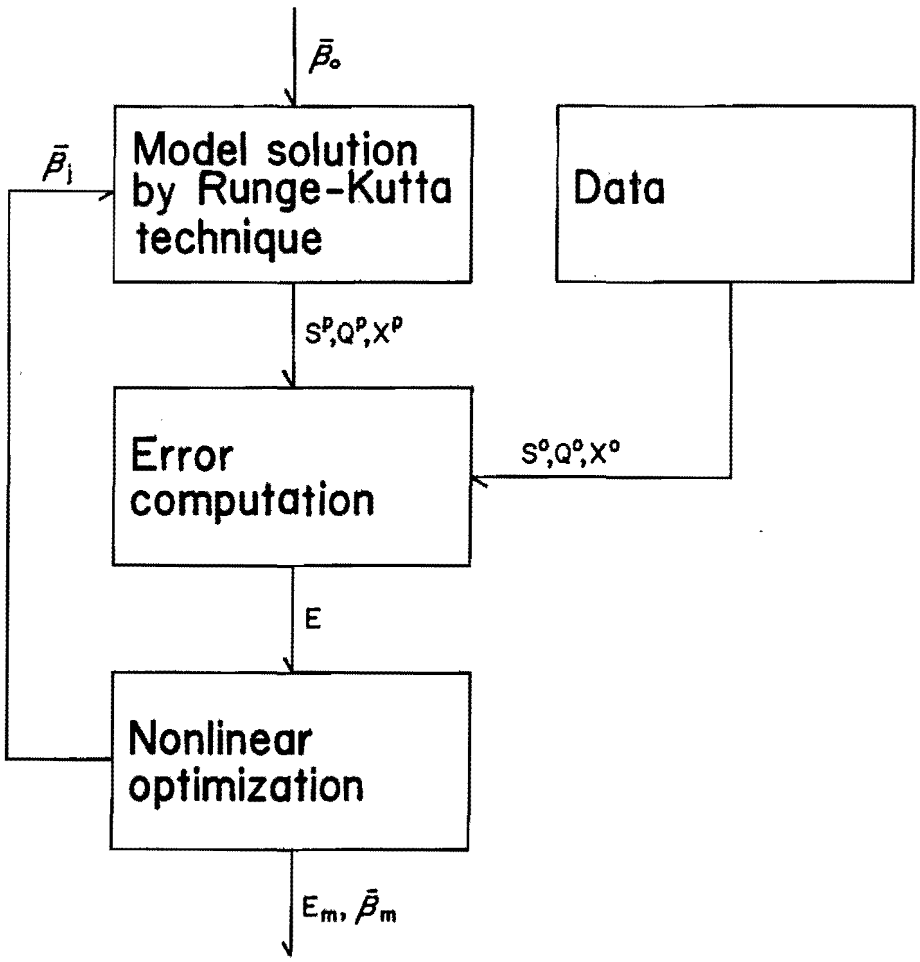

Figure 3. Flow chart of coefficient estimation technique.

This error function was then an input to a nonlinear optimization routine (Grenney, 1975). This can be described as an iterative technique which converges on a minimum of an objective function (E_m in this case) by adjusting the values of the coefficients ($\overline{\beta}_j$) until the set of coefficients giving the minimum error ($\overline{\beta}_m$) is obtained. It was necessary that the technique be used several times with different initial guesses of the coefficients ($\overline{\beta}_o$) to insure that $\overline{\beta}_m$ was a global minimum. The algorithm was based on the Davidon-Fletcher-Powell technique (Hadley, 1964) modified to incorporate upper and lower boundaries on the coefficient being estimated.

The result of this technique then was to arrive at coefficient values for the assumed model which minimizes the observed-predicted error for the entire model system.

The minimized errors can be used for comparing the different model's relative effectiveness in simulating the data.

Computer program listings are given in Appendix B.

RESULTS

Nutrient Budget

The total nitrogen present in a culture should be the sum of what was added plus what was initially present in the algae. Remembering that there was no nitrogen in the medium during Phase I, the total nitrogen present in this phase should have been equal to the nitrogen within the algal cells. Figure 4 shows the concentration of cell nitrogen (deviation about the mean) during Phase I as a function of time.

Similarly, Figure 5 shows the total nitrogen concentration (deviation about the mean) for Phase II. The computed total (2.34 mg/l) is based on an algal nitrogen concentration of 0.74 mg/l (after dilution) plus a computed nitrogen addition of 1.6 mg/l.

Figure 6 shows Phase III total nitrogen concentration; the computed total being equal to the computed total from Phase II plus a computed addition of 1.6 mg/l.

From Figure 6, and perhaps Figure 4, it is evident that there was an increase in the total nitrogen concentration of the cultures over time which was taken up by the algal cells. This phenomenon could be either real or a result of the analysis technique used. One aspect of the analysis technique could produce such a pattern. It was necessary to store the filtered algae (in the freezer) before performing the nitrogen analyses. For Phase I, the algal nitrogen determinations were made 7 or 8 days after the initial filtering. For Phase II and Phase III, the algal nitrogen determinations were made up to 10 days after

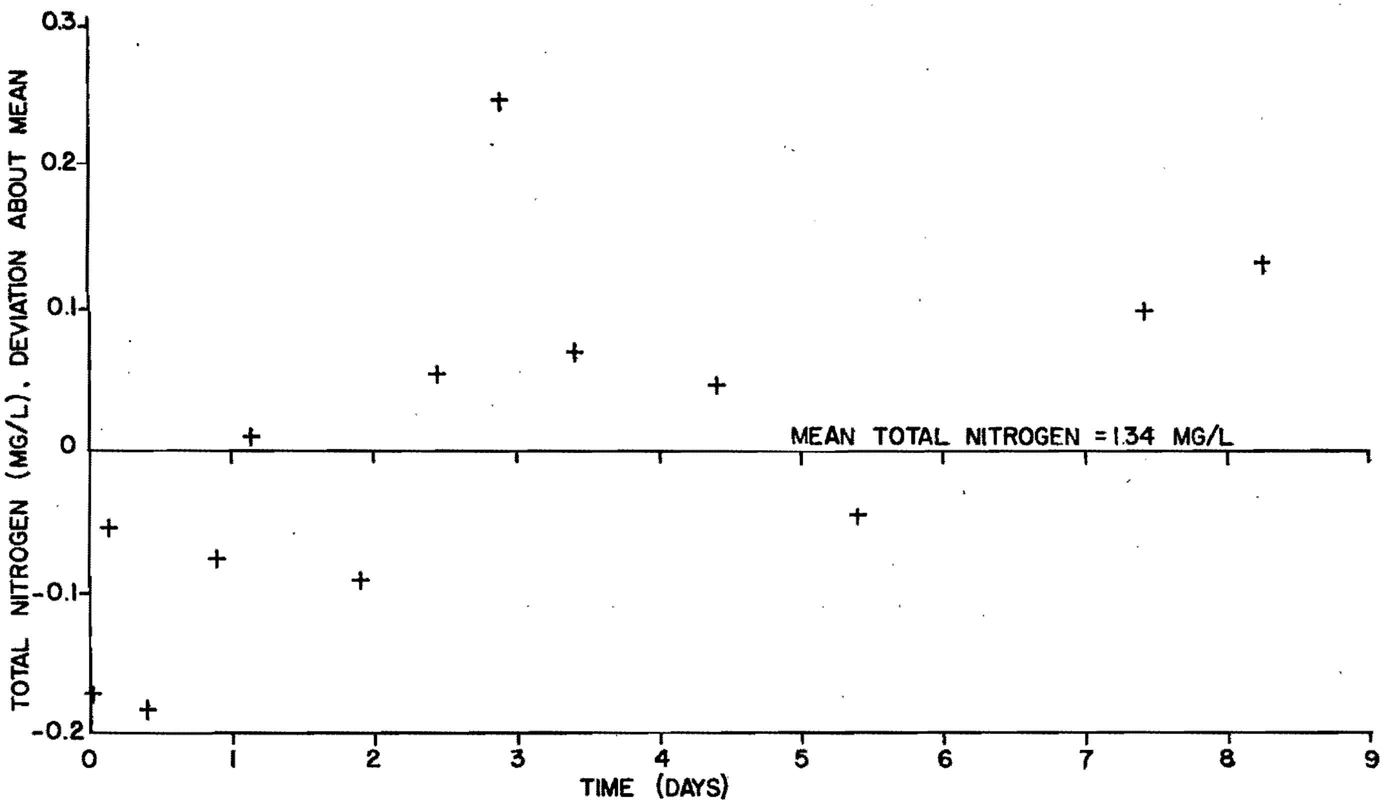

Figure 4. Total nitrogen in culture flask versus time during Phase I.

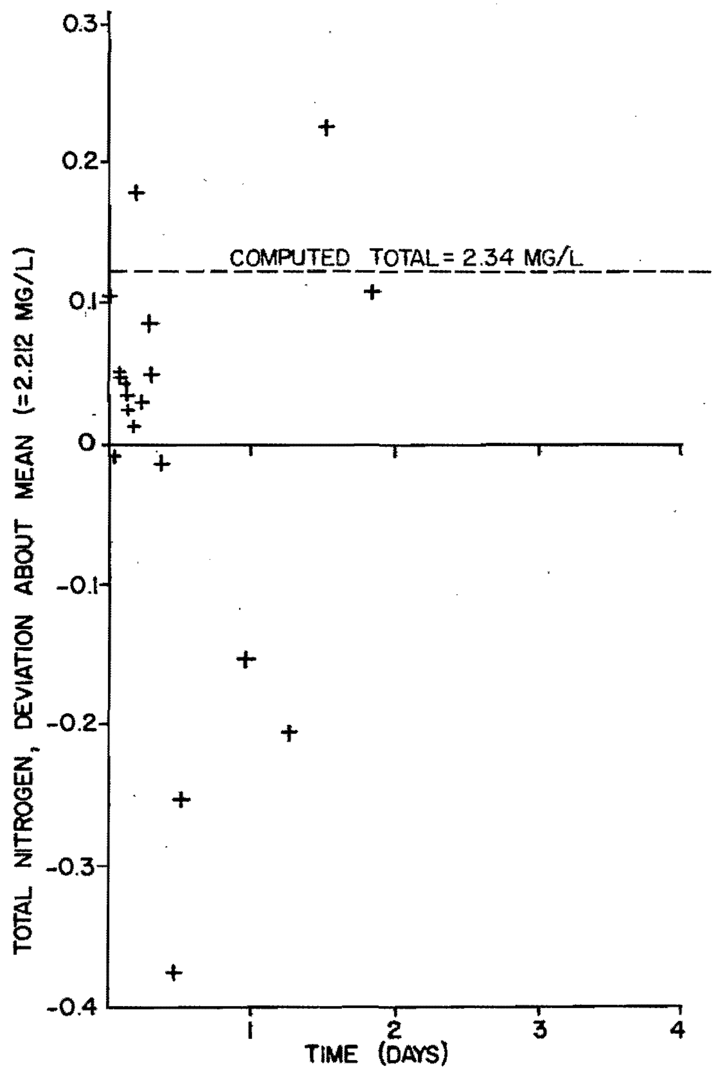

Figure 5. Total nitrogen in culture flask versus time during Phase II.

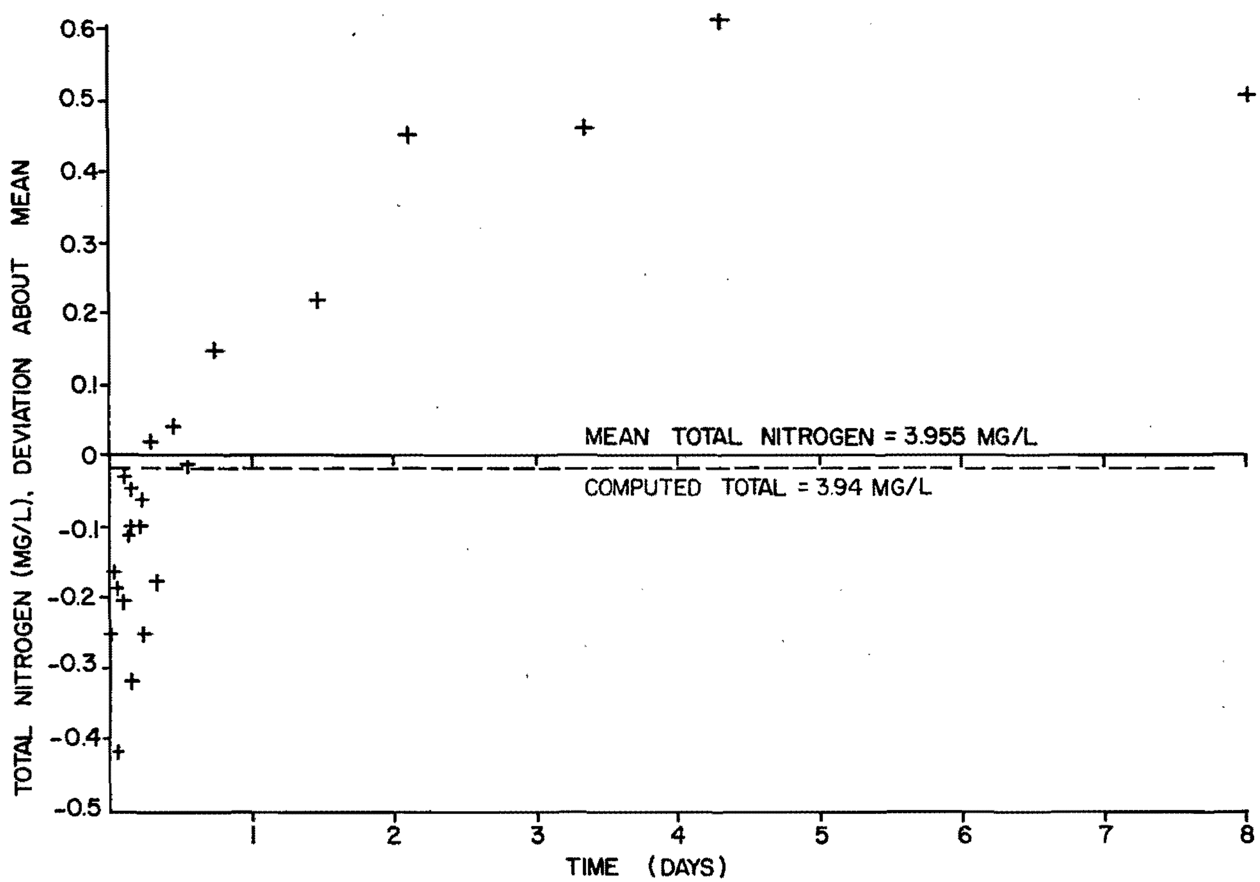

Figure 6. Total nitrogen in culture flask versus time during Phase III.

the initial filtering. It is possible that the nitrogen on the filters (algal nitrogen) was gradually lost while the filters were stored. The longer the filters were stored, the more nitrogen would be lost. Thus, algal nitrogen determinations in the earlier part of the phases would be relatively lower than in the latter part since the determination in the latter part were done sooner after filtering.

The major difficulty with this explanation is that, for it to be true, the computed totals and the external nitrogen determinations would both have to be incorrect. Essentially, two independent measures of nitrogen (what was assumed to be added and what was measured external to the algae) would have had to be lower than the actual total for the above hypothesis to completely explain the lack of nutrient mass balance.

There is another explanation which would be more reasonable; the increase of total nitrogen was real. This could result from a failure to strip the ammonium from the air-CO_2 mixture which was bubbled through the cultures. As discussed in Materials and Methods, a solution of IN H_2SO_4 was used for this purpose. It is possible that this stripping solution became exhausted during the experiment.

During the computer simulations, extra nitrogen inputs were used during Phase III so the total nitrogen would follow the pattern of Figure 6, allowing for the uptake of that nitrogen by the algal cells.

Model Application

An edited list of the data as used for model comparison is given in Table 4. The data was edited to provide measures of all three

Table 4. Mean external nitrogen (S), cell quota (Q), and biomass (X) with coefficients of variation (CV) and number of replicates (N).

Phase	Time (days)	S (mg/l)	CV/N	Q*100 (percent)	CV/N	X (mg/l)	CV/N
Phase I	0	a.	---	3.72	--/1	31.4	--/1
	0.16	a.	---	3.74	1.51/2	34.3	4.54/2
	0.41	a.	---	3.24	2.36/3	35.8	2.01/3
	0.91	a.	---	3.14	2.40/3	40.1	1.75/3
	1.16	a.	---	3.23	7.61/3	41.7	1.68/3
	1.92	a.	---	2.79	8.10/3	44.7	2.07/3
	2.41	a.	---	3.02	5.47/3	46.3	1.95/3
	2.91	a.	---	3.30	16.45/3	48.1	2.13/3
	3.41	a.	---	2.87	12.49/3	49.3	3.10/3
	4.41	a.	---	2.79	17.31/3	49.6	1.85/3
	5.41	a.	---	2.50	6.70/3	51.7	2.57/3
	7.41	a.	---	2.74	15.72/3	52.7	3.53/3
	8.24	a.	---	2.68	15.74/3	53.7	2.79/3
Phase II	9.19	1.555	3.91/2	2.33	3.67/3	32.7	1.41/3
	9.24	1.478	3.02/3	2.21	2.82/2	32.5	4.45/3
	9.26	1.524	5.06/3	2.27	7.39/3	32.5	1.42/3
	9.28	1.489	5.42/3	2.44	11.92/3	31.9	5.02/3
	9.31	1.444	2.76/3	2.49	10.44/3	32.5	1.55/3
	9.33	1.415	4.88/3	2.67	2.96/3	31.2	1.11/3
	9.35	1.435	6.30/3	2.45	10.17/3	32.7	5.48/3
	9.37	1.405	4.82/3	2.59	9.86/3	31.6	2.76/3
	9.39	1.418	5.66/3	2.59	7.70/3	35.4	15.38/3
	9.41	1.409	4.63/3	2.50	11.75/3	33.5	5.35/3
	9.46	1.365	3.83/3	2.88	6.87/3	32.4	2.14/3
	9.50	1.297	1.85/3	2.95	3.82/3	32.8	2.66/3
	9.58	1.221	1.09/3	2.95	13.65/3	33.3	1.51/3
	9.66	0.843	1.01/3	2.92	13.55/3	34.1	2.71/3
	9.91	0.482	3.73/3	3.80	5.61/3	38.9	3.61/3
	10.16	0.042	97.6/3	4.62	1.53/3	44.7	4.01/3
	10.41	a.	---	3.98	0.94/2	50.5	0.84/3
	10.69	a.	---	3.73	9.50/3	65.4	4.43/2
	11.02	a.	---	2.87	13.53/3	81.3	6.31/3

[a] External nitrogen not measured, assumed to be zero in model runs.

Table 4. Continued.

Phase	Time (days)	S (mg/1)	CV/N	Q*100 (percent)	CV/N	X (mg/1)	CV/N
Phase III	11.08	1.328	6.71/3	2.78	8.10/3	85.8	6.28/3
	11.09	1.374	0.00/2	2.91	1.00/3	83.0	6.29/3
	11.11	1.259	9.25/3	2.95	5.63/3	85.0	5.79/3
	11.13	1.142	9.88/3	2.77	16.86/3	86.7	4.37/3
	11.16	0.942	11.31/3	3.24	1.15/3	86.7	4.09/3
	11.18	0.826	3.00/2	3.54	1.20/2	87.5	4.85/2
	11.20	0.669	10.88/3	3.59	9.02/3	88.5	4.27/3
	11.22	0.537	22.91/2	3.85	--/1	86.0	--/1
	11.24	0.427	1.64/3	3.63	7.13/3	88.3	4.09/3
	11.26	0.317	20.68/3	4.07	2.63/3	88.2	5.24/3
	11.28	0.215	22.30/3	3.98	5.12/3	91.5	3.28/3
	11.30	0.090	24.21/2	4.12	2.79/3	92.2	3.61/3
	11.32	0.028	98.88/3	3.95	5.52/3	93.0	5.30/3
	11.36	a.	---	4.11	4.26/3	97.0	4.22/3
	11.41	a.	---	3.79	2.65/3	99.7	4.27/3
	11.53	a.	---	3.56	3.62/3	112	4.53/3
	11.66	a.	---	3.18	3.75/3	124.	5.56/3
	12.00	a.	---	2.73	5.34/3	150.	3.14/3
	12.51	a.	---	2.66	5.11/2	157.	1.80/2
	13.17	a.	---	2.21	9.98/3	199.	3.52/3
	14.43	a.	---	1.98	2.93/2	223.	1.27/2
	15.38	a.	---	1.90	7.43/3	246.	1.72/2
	19.14	a.	---	1.69	8.00/3	264.	3.68/3

[a] External nitrogen not measured, assumed to be zero in model runs.

variables X, Q, S at each time step (for example, if a value of X was not available at a particular time, the values of Q and S determined for that time were edited from the data list). A complete list of the data, including fluorescence and cell counts, is given in Appendix A.

Dry weight and cell quota data are plotted as a function of time over the entire period in Figure 7.

Three observations were made from these figures and will be discussed before any model is applied.

1. Algal growth in the absence of external limiting nutrient was observed during Phase I.
2. A lag in growth and uptake was observed in Phase II.
3. The apparent Q_o at the end of Phase I was different than the apparent Q_o at the end of Phase III.

Growth in the absence of external limiting nutrient

The first observation supports the use of cell quota growth models; that is, growth was a function of the internal stores of limiting nutrient. The Monod model would not predict this.

Lag phase

The lag phase at the start of Phase II would not be predicted by any of the growth-uptake models previously presented. To better illustrate the uptake lag it is beneficial to compare the uptake responses of the two nitrogen additions. This was done by first approximating the biomass time responses during the two uptake periods. These exponential growth approximations are shown in Figures 8 (Phase II) and 9 (Phase III). Once the growth curves had been approximated, a Michaelis-Menten uptake function was fit to the uptake data during Phase III (see Figure 10) by a modified form of the exact solution used by Caperon and Meyer

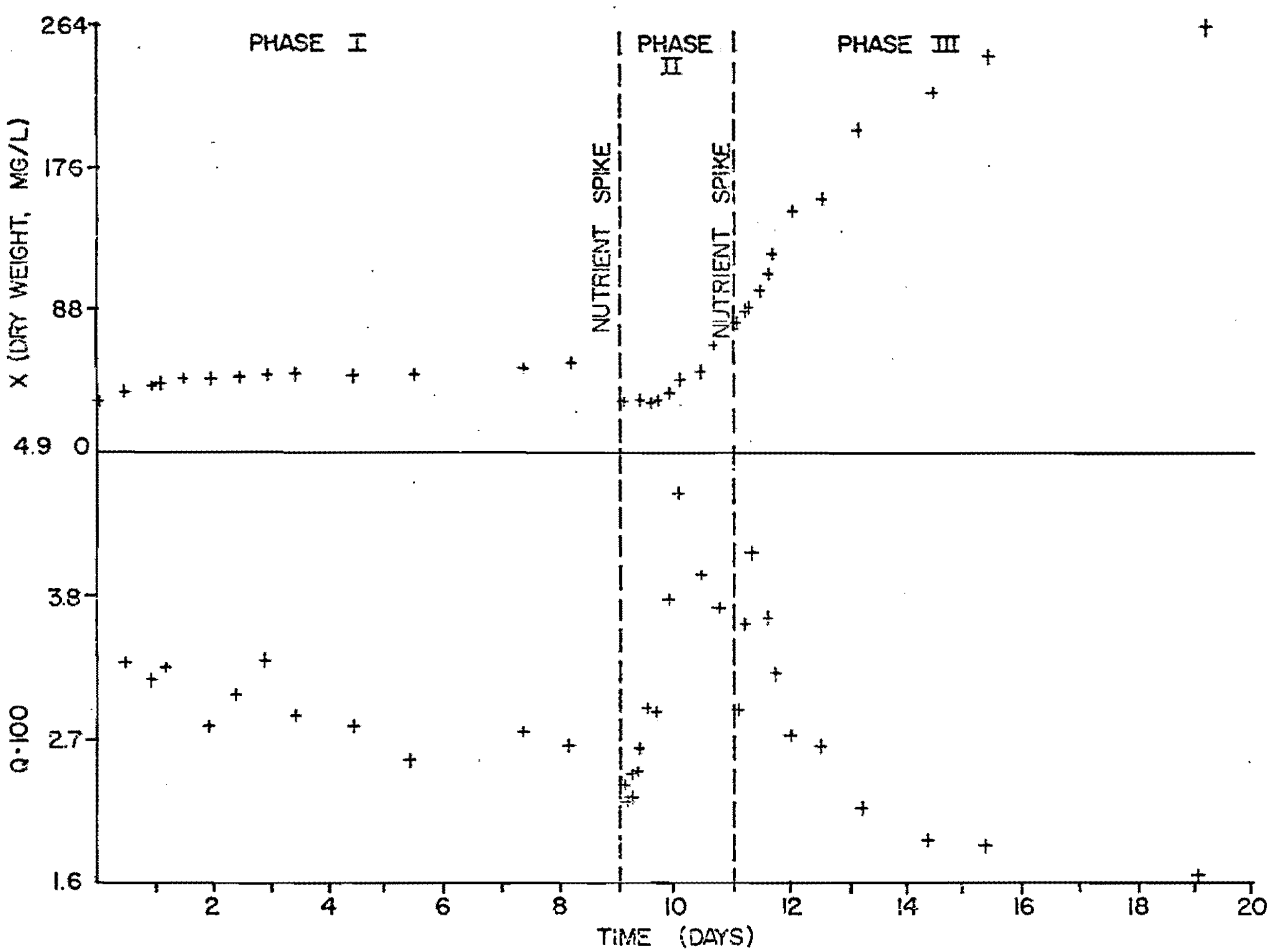

Figure 7. Observed biomass and cell quota response.

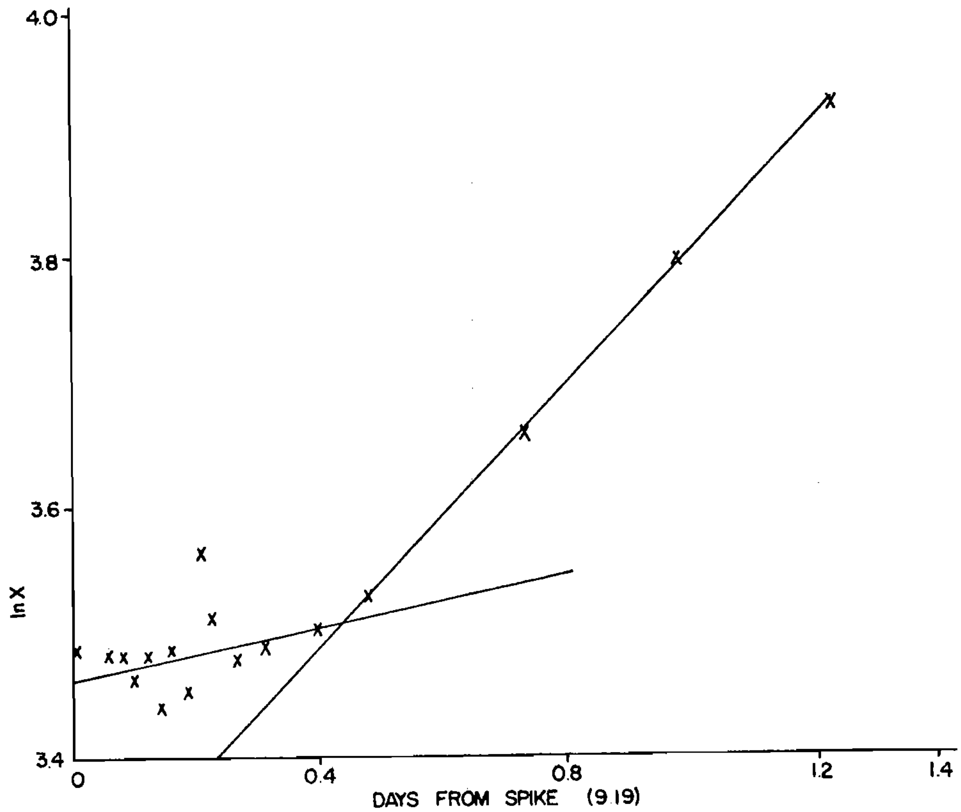

Figure 8. Phase II biomass with growth approximation.

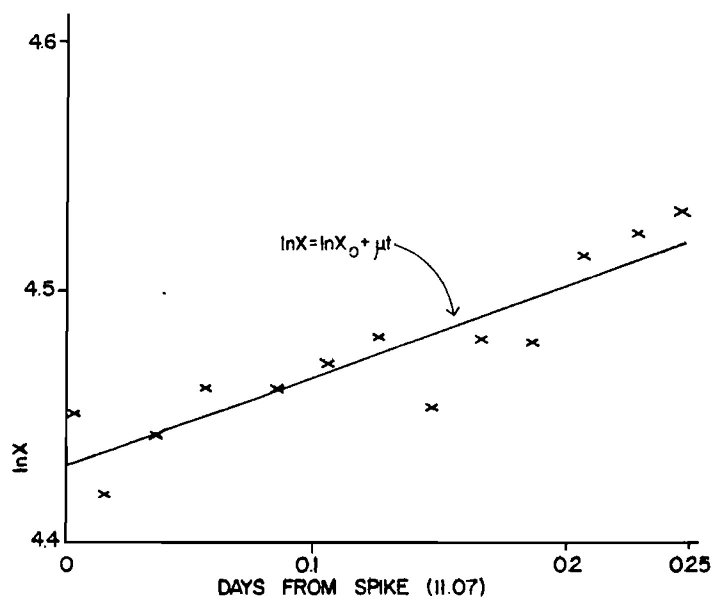

Figure 9. Phase III biomass with growth approximation.

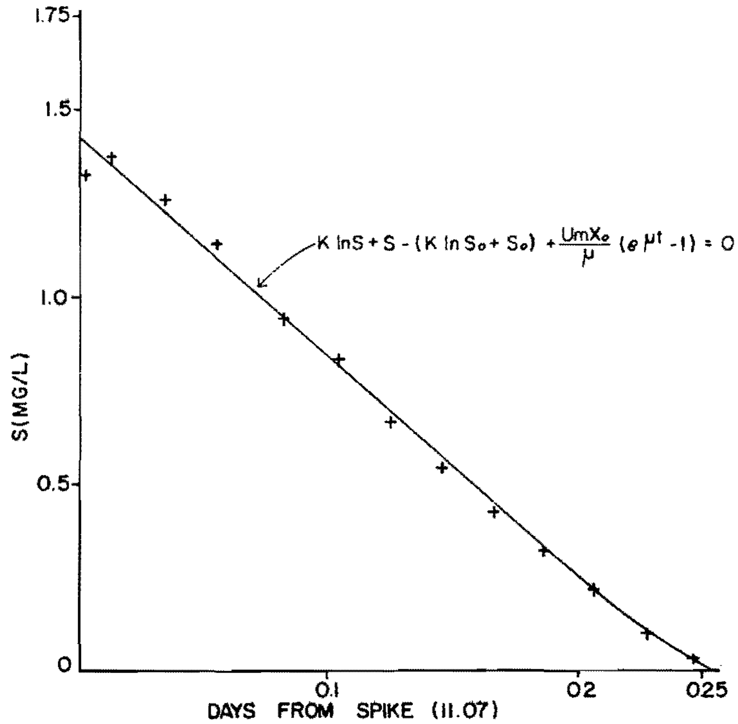

Figure 10. Phase III external nutrient with uptake approximation.

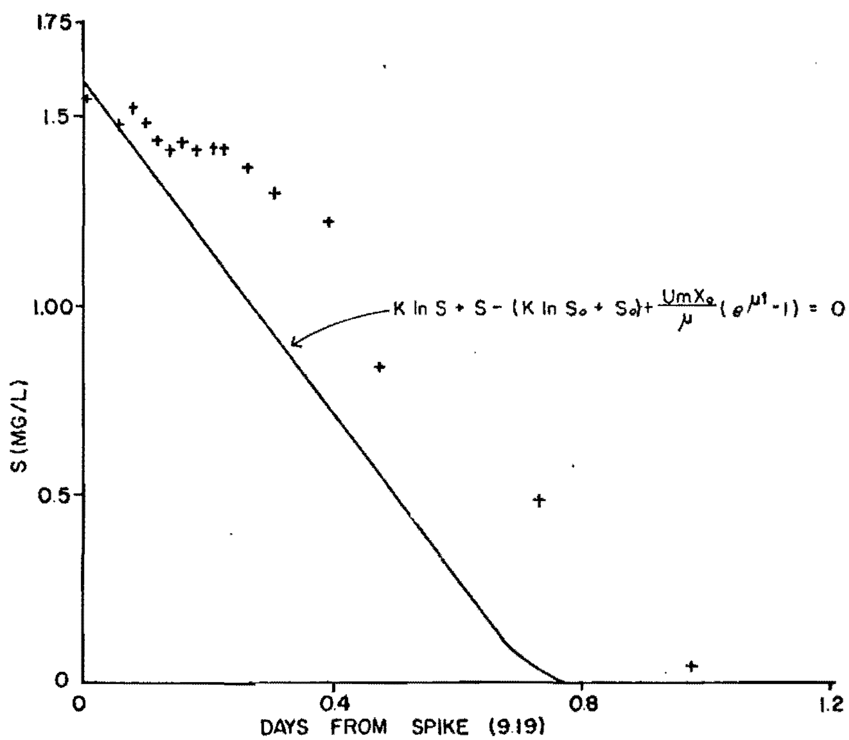

Figure 11. Phase II external nutrient with uptake approximation.

(1972b). This least squares fit gave K, the half saturation constant, and u_m, the maximum uptake rate.

The next step was to assume the same uptake function, with these two constants, for Phase II (Figure 11). The uptake responses for the two spikes were not the same (compare Figure 10 to Figure 11). Phase II exhibited a lag in uptake not simulated by the model.

This type of response, lag in uptake by nitrogen starved batch cultured cells resuplied with nitrogen, has been previously reported. Thomas and Krauss (1954) observed a 2-hour lag in uptake and protein synthesis by nitrogen starved cells. Eppley, Rogers, and McCarthy (1968) observed lag in uptake of N-depleted cells after a nitrate addition but not after an ammonium addition. Eppley and Thomas (1969) found it necessary to preincubate N-starved cells to get a linear NO_3^- uptake response; i.e., to compensate for the uptake lag of N-starved, and presumably, non-growing cells.

A hybrid culturing system has also been used which illustrated this uptake lag. Caperon and Meyer (1972b) cultured N-limited cells in a chemostat to find the steady-state growth rate. They then shut off the nutrient pumps and added ammonium and/or nitrate to the cultures to give, essentially, nutrient uptake in a batch culture. This method enabled them to relate uptake rate to the preconditioning specific growth rate. While the uptake response always seemed to be of the Michaelis-Menden type, they showed the maximum uptake velocity (u_m) to be a linear function of the preconditioning growth rate (see Figure 12), with the half-saturation constant (K) being well behaved.

What linear dependance of u_m on μ does then is to induce an uptake lag when the cells have been nutrient starved or, equivalently, when their

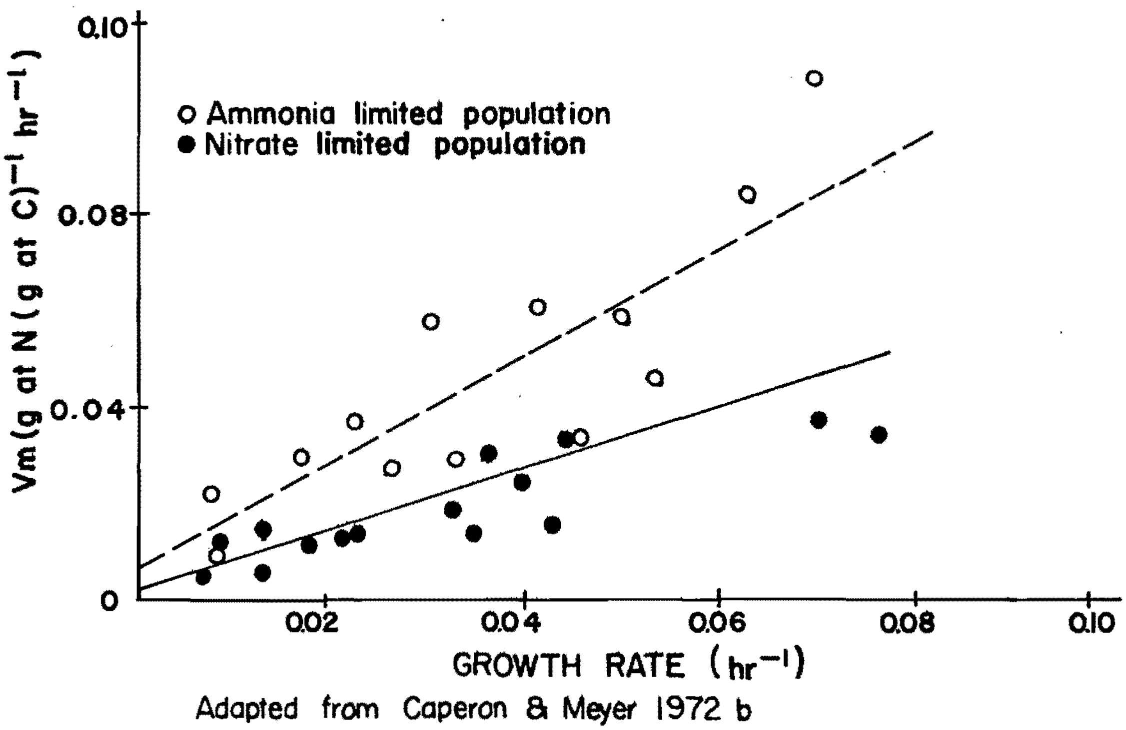

Figure 12. Previously observed relation of maximum uptake rate varying as a function of preconditioning growth rate.

growth rate has previously gone to low values. This function ($u_m = a\mu + b$, a and b constants) will be applied to the data of the present study during the model comparisons.

If one accepts the fact that cell growth is a function of the internal stores of limiting nutrient, it becomes evident that a lag in uptake will induce a lag in cell growth. Referring again to Equations (9), (10), and (11):

$$\frac{dX}{dt} = \mu X \tag{9}$$

$$\frac{dQ}{dt} = u - \mu Q \tag{10}$$

$$\frac{dS}{dt} = -uX \tag{11}$$

If there is a time lag in u, the increase in Q lags (Equation (10)). Since the assumption is that $\mu = f(Q)$, this lag is ultimately passed down to the growth rate. This reasoning suggests that the observed growth lags in this and similar studies may be entirely a result of a lag in uptake. Thus, in Fogg's (1971) definition of the lag pahse as being " . . . a period of restoration of enzyme and substrate concentrations to the levels necessary for rapid growth," the enzyme involved could be a permease and the "substrate concentration" the internal cell quota.

Before going on to a discussion of the third observation, it should be pointed out that several authors have found that u_m increases upon nutrient starvation (for example, uptake Model 2 - Rhee, 1973). It has been argued by Perry (1976) that " . . . it would appear to be sound adaptive strategy for a nutrient-starved cell to increase its potential for nutrient absorption by increasing the machinery for uptake". This does indeed appear to make sense but seems to contradict the observations of Caperon and Meyer (1972b), and the other researchers working with batch cultures. Rhee (1973) and Perry (1976) were working with phosphorus uptake of P-limited cultures and this might be the cause of the difference; different uptake mechanisms exist for phosphorus and nitrogen. Referring again to the work of Eppley and Thomas (1969) with batch nitrogen uptake experiments, they, too, observed an enhanced uptake rate by nutrient starved cells but only after an initial lag period. What Caperon and Meyer (1972b) observed and modeled and what this study was concerned with was the uptake lag. Apparently, after lag the uptake rate will increase as a function of the nutrient prehistory.

Variable Q_o

All of the cell quota growth models previously presented incorporate Q_o, the minimum internal nutrient content, which is assumed to be a physiological constant for an algal species in a constant controlled environment.

Recent work by Perry (1976) has shown Q_o (measured as moles phosphorus per cell) of phosphate-limited chemostat cultures of a diatom to be a variable. He determined the Q_o's in batch studies after culturing the diatoms in a chemostat under known preconditioning growth rates. He found in his data, and in his analysis of Caperon's (1967) nitrate limited batch cultures, that Q_o was some function of the previous maximal growth rate, and therefore, a function of previous nutrient limitation.

What this suggests then, is that some type of population acclimation is occurring. Analysis of Q_o reveals that this supposed constant is the inverse of the maximum or ultimate cell yield, a parameter which must be constant if one is to attempt nutrient biostimulation assays.

Model application

Although it is evident that none of the growth models would simulate the observed response throughout the entire time period, initial screening of the models used all the data. The model of Fuhs (1969) was not used because its response as a function of Q is essentially the same as the other nonlinear models. Caperon's (1968) model was also not applied because it is equivalent to Droop's (1968) model when $K = Q_o$ which as Rhee (1973) observed was approximately the case in the studies where this model was applied. Only two models were applied to the data of this study and compared: a non-linear type (Droop's, 1968, model) and a linear type (Malone's, 1976, model).

The biomass response of Droops model shown in Figure 13 and the response of Q as mass percent nitrogen (Figure 14) can be compared to the response of Malone's model illustrated in Figures 15 and 16. Although the general responses are similar and the overall patterns are close to the actual data, lag phenomenon and total biomass data are not approximated by the simulation curves.

The values of the coefficients as optimized and the relative error (E) show that there is little difference between the two models with the linear model simulation being slightly better (Table 5). The similarity between the linear and non-linear models suggests that in this experiment saturating values of Q were never reached, i.e., nitrogen was always limiting, no storage above "functional" (Fuhs, 1969) internal nitrogen supplies occurred (see previous discussion in Multicompartment Models).

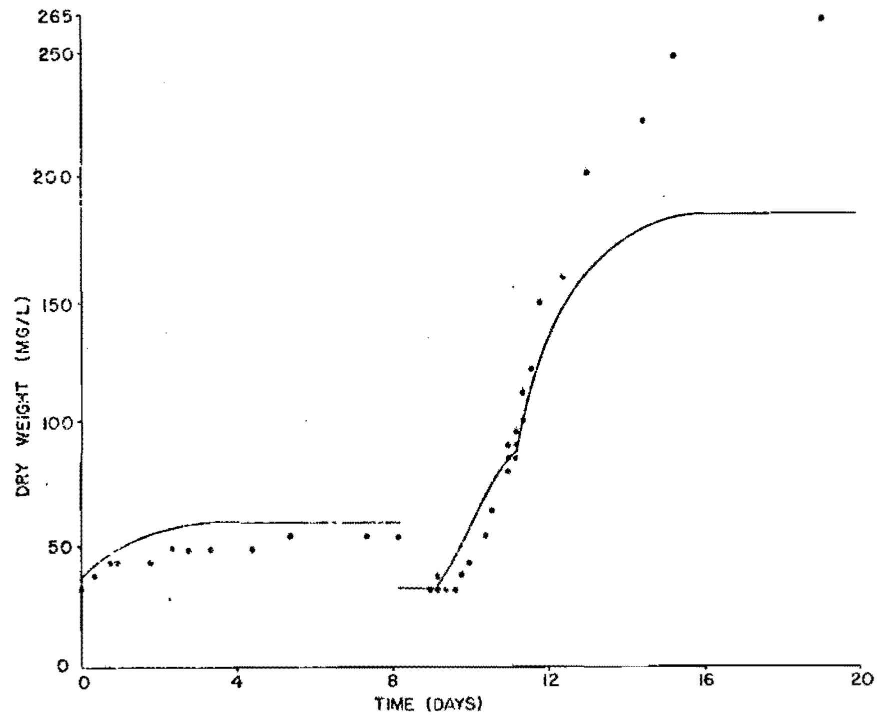

Figure 13. Non-linear growth and uptake (biomass simulation).

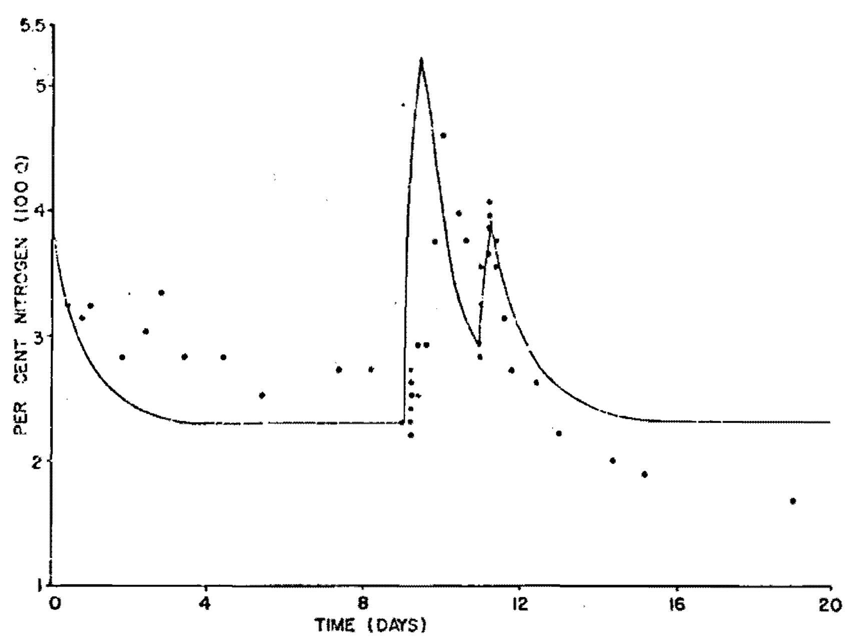

Figure 14. Non-linear growth and uptake (cell quota simulation).

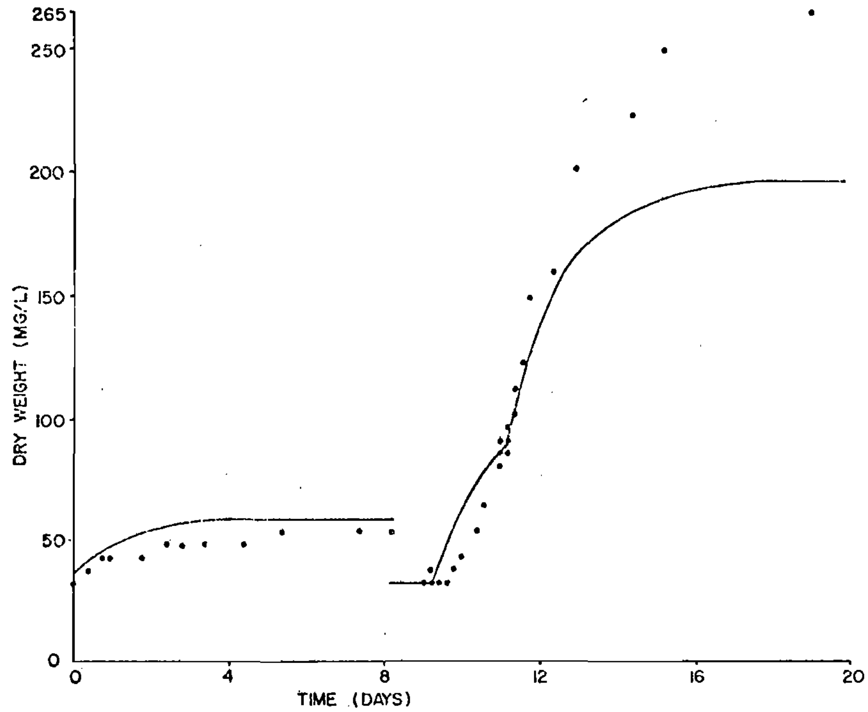

Figure 15. Linear growth and uptake (biomass simulation).

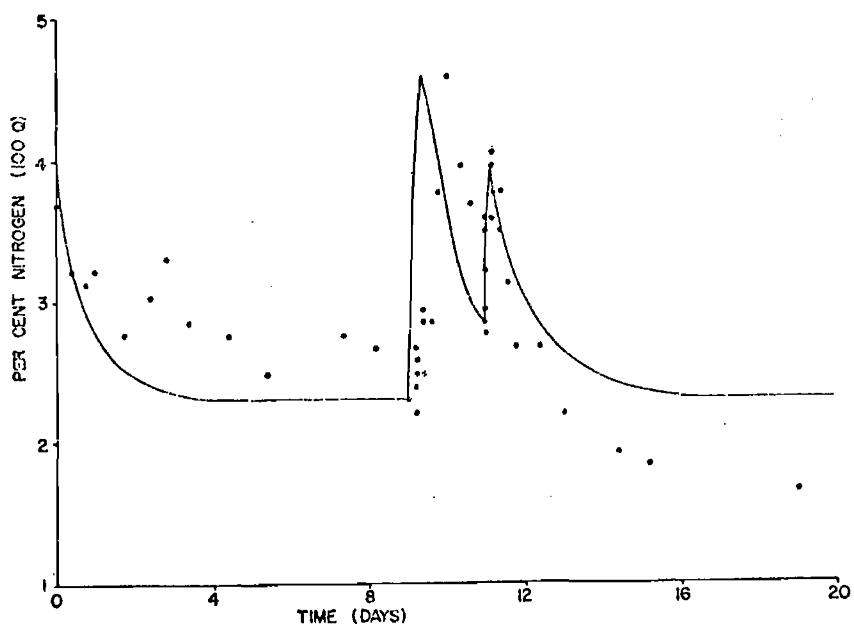

Figure 16. Linear growth and uptake (cell quota simulation).

In an attempt to improve the simulation of uptake lag observed in Phase II, the function suggested by Caperon and Meyer (1972b), $u_m = a\mu + b$, was employed. The growth model used was Malone's (1976) linear model. The percent nitrogen model response (during the period of the two nitrogen additions) when $u = u_m^* S$ (Figure 17) is to be compared to an improved simulation of lag when $u = (a\mu + b)\ S/(K + S)$ (Figure 18). The relative errors of the model responses when different uptake functions were used with the linear growth model can also be compared (Table 5).

When a linear growth model is assumed this uptake lag function makes nutrient uptake a linear function of the internal cell quota. The function $u = (a\mu + b)S/(K + S)$, can just as meaningfully be written $u = (a'Q + b')S/(K + S)$, where a' and b' are constants. The lag is induced by the cells having a low Q resulting from previous nutrient starvation; the uptake rate is dependent" . . . on the previous rate of nitrogen supply" (Caperon and Meyer, 1972b).

The model to this point has the following form:

$$\frac{dX}{dt} = \mu X = \mu_m \left(\frac{Q}{Q_o} - 1\right) X \tag{12}$$

$$\frac{dQ}{dt} = u - \mu Q \tag{13}$$

$$\frac{dS}{dt} = -uX = -\frac{(a\mu + b)\ SX}{K + S} \tag{14}$$

This model's 20 day response is shown in Figure 19, cell biomass simulation, and Figure 20, cell quota simulation. As a result of the variability of Q_o the model does not approximate the total biomass data through the entire 20 day period. What was attempted next was to show that the model could be a good predictive tool if the data is considered

Table 5. Model coefficients and error

Model	a (dimensionless) or a* ($l\ mg^{-1}$)	b (day^{-1}) or b* ($l\ mg^{-1}\ day^{-1}$)	u_m (day^{-1}) or u_m^* ($l\ mg^{-1}\ day^{-1}$)	K (mg/l)	μ_m (day^{-1})	Q_o (in percent)	Error (mg/l)
$u = u_m \frac{S}{K + S}$ $\mu = \mu_m (1 - \frac{Q_o}{Q})$	----	----	5.00	0.0426	1.37	2.33	126.
$u = u_m^* S$ $\mu = \mu_m (\frac{Q}{Q_o} - 1)$	----	----	0.0957	----	0.952	2.34	102.
$u = (a^*\mu + b^*) S$ $\mu = \mu_m (\frac{Q}{Q_o} - 1)$	0.0339	0.072	----	----	0.932	2.29	98.6
$u = (a\mu + b) \frac{S}{K + S}$ $\mu = \mu_m (\frac{Q}{Q_o} - 1)$	0.00784	0.116	----	0.113	0.914	2.25	64.2
Phase I	0.00784	0.116	----	0.113	0.914	2.71	
Phase II	0.00784	0.116	----	0.113	0.914	2.23	37.2
Phase III	0.00784	0.116	----	0.113	0.914	1.53	

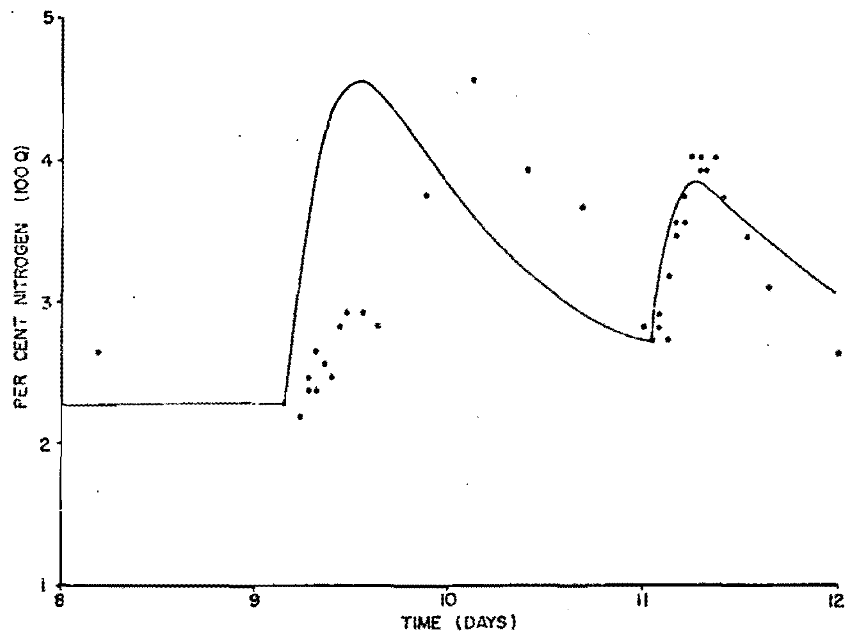

Figure 17. Linear growth, $u = u_m' S$ (cell quota simulation).

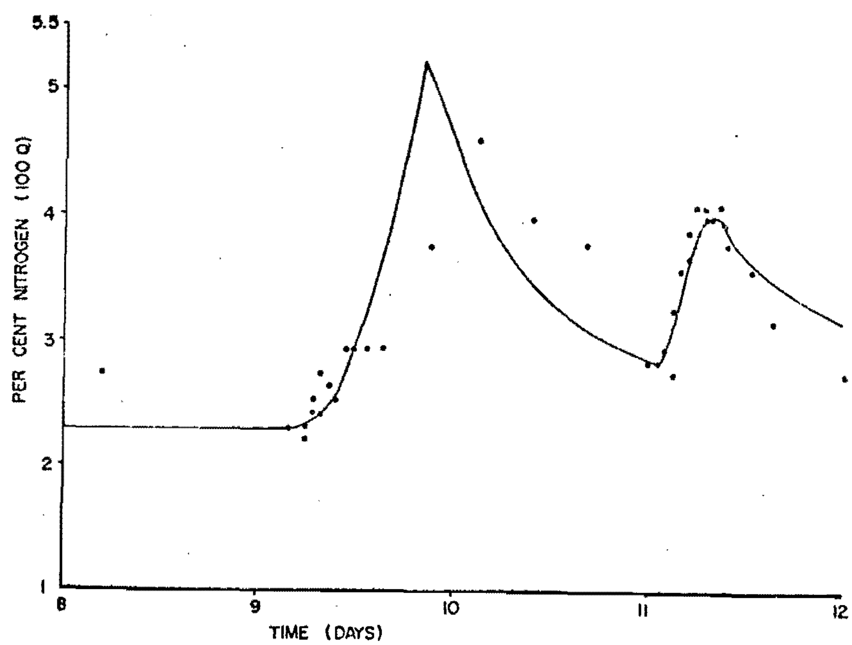

Figure 18. Linear growth, $u = (a\mu + b)S/(K + S)$ (cell quota simulation).

in sections. The three phases were used for dividing up the data into regions approximating different nutritional histories. All coefficients were set at the values previously optimized for the 20-day period with the exception of Q_o which was to be optimized for each phase.

The value of Q_o for Phase I was optimized as 2.71 percent. With this value of Q_o there was no over shoot in the prediction of the biomass (Figure 21) previously exhibited in Figure 19. The value of Q was also more closely approximated (Figure 22) then when the model was applied for the entire 20-day period (Figure 20).

The optimized value of Q_o was lower in Phase II, 2.23 percent, and resulted in an improved simulation of the biomass (Figure 23) and cell quota (Figure 24).

The Phase III Q_o, 1.53 percent, illustrates a continuing decrease in the optimized Q_o. The total biomass values were finally approximated with this value of Q_o (Figure 25) as were the final values of Q (Figure 26).

The relative error accumulated for the 20-day period when Q_o was optimized for each phase individually was almost 50 percent lower than when the same model was applied with and average Q_o (Table 5).

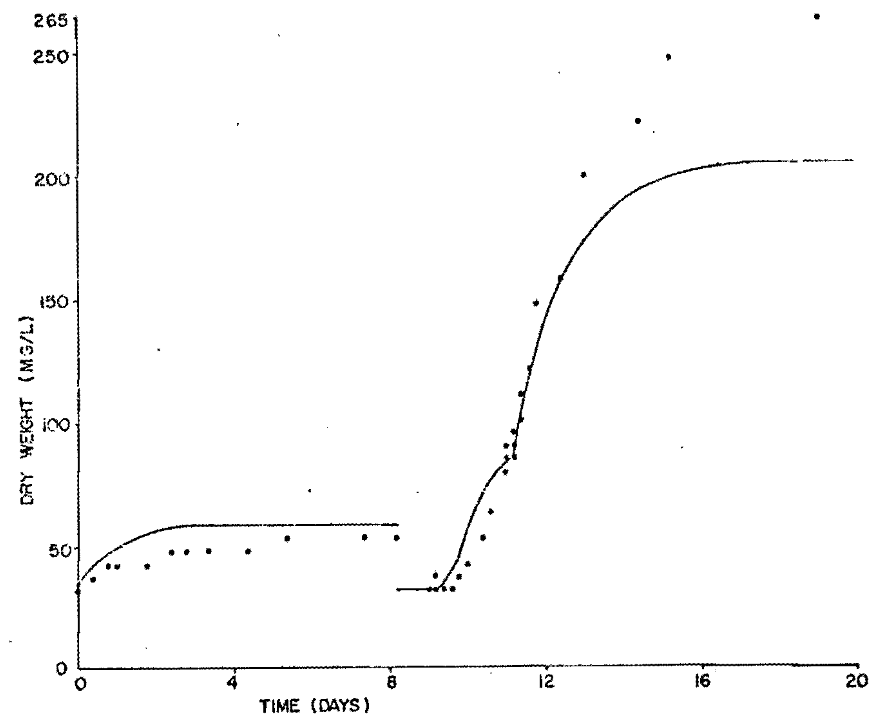

Figure 19. Final model with uptake lag (biomass simulation).

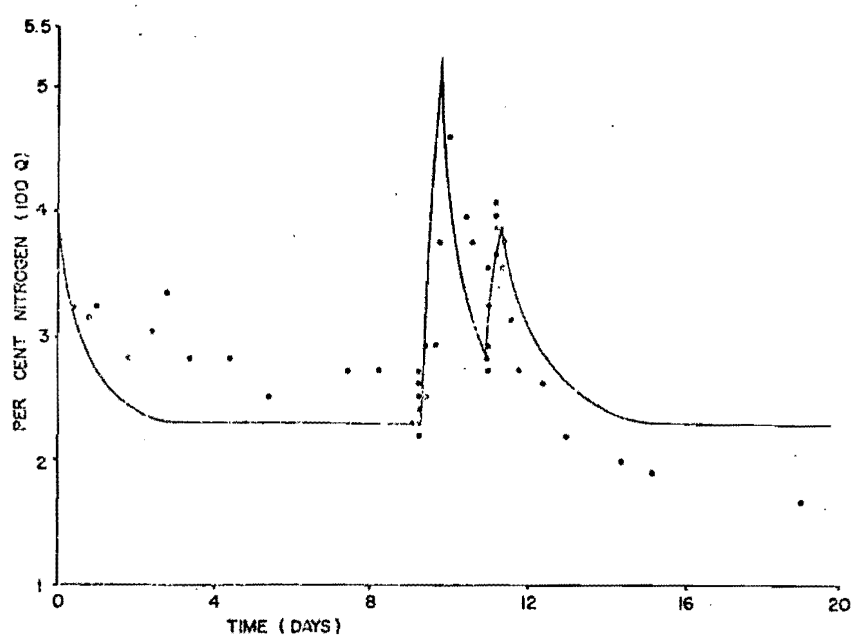

Figure 20. Final model with uptake lag (cell quota simulation).

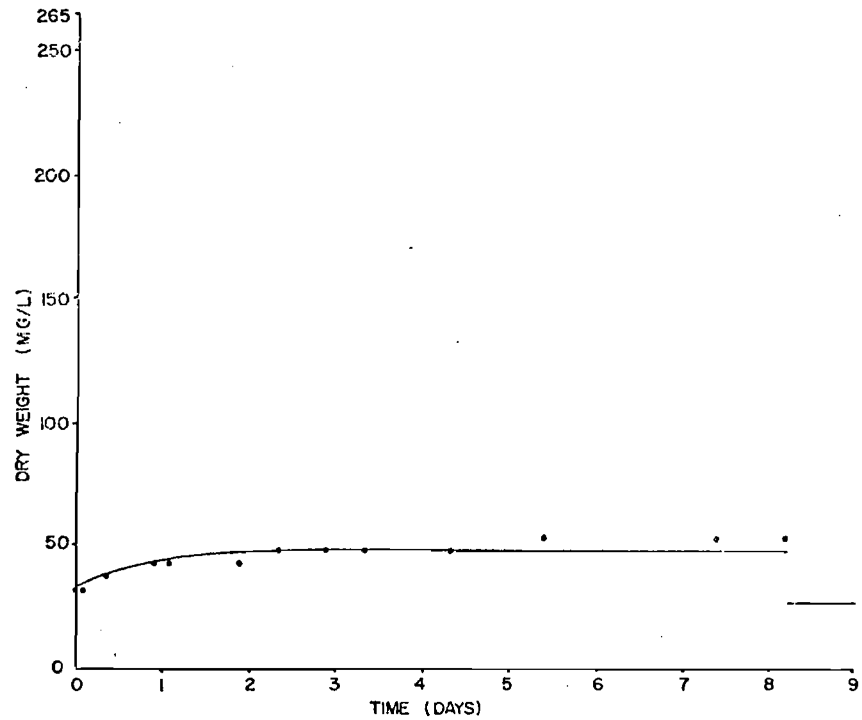

Figure 21. Phase I biomass simulation, Q_o = 2.71 percent.

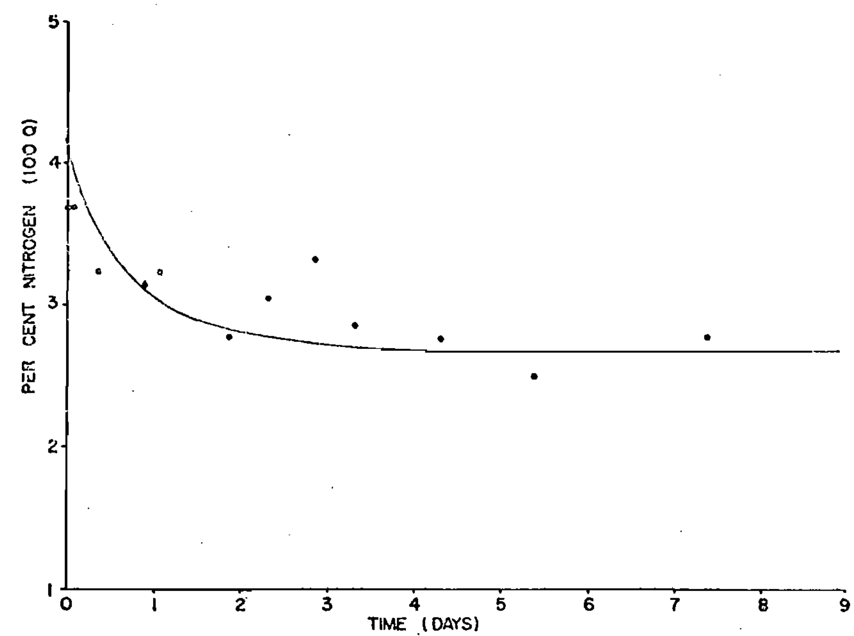

Figure 22. Phase I cell quota simulation, Q_o = 2.71 percent.

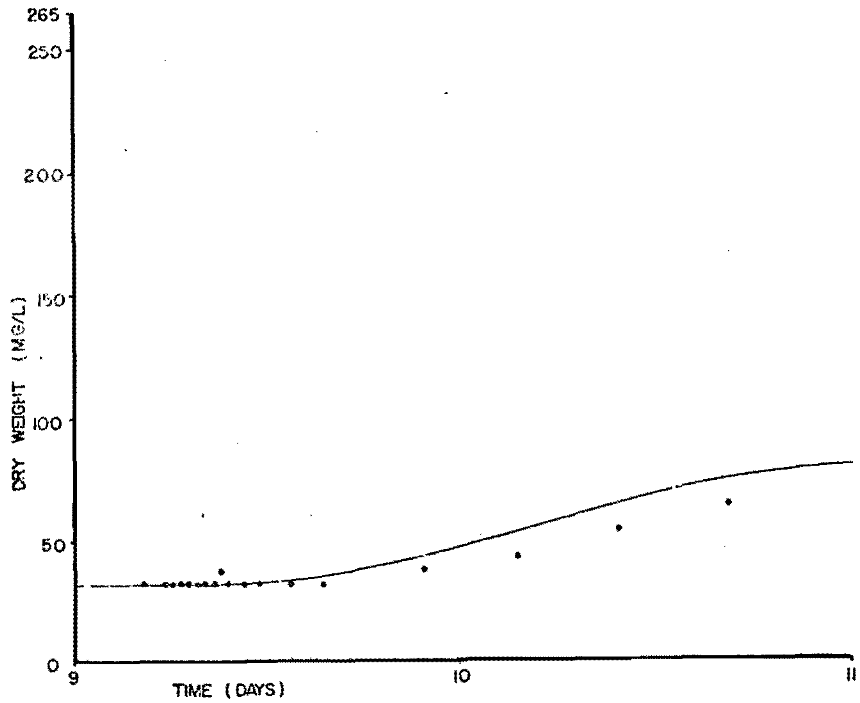

Figure 23. Phase II biomass simulation, Q_o = 2.23 percent.

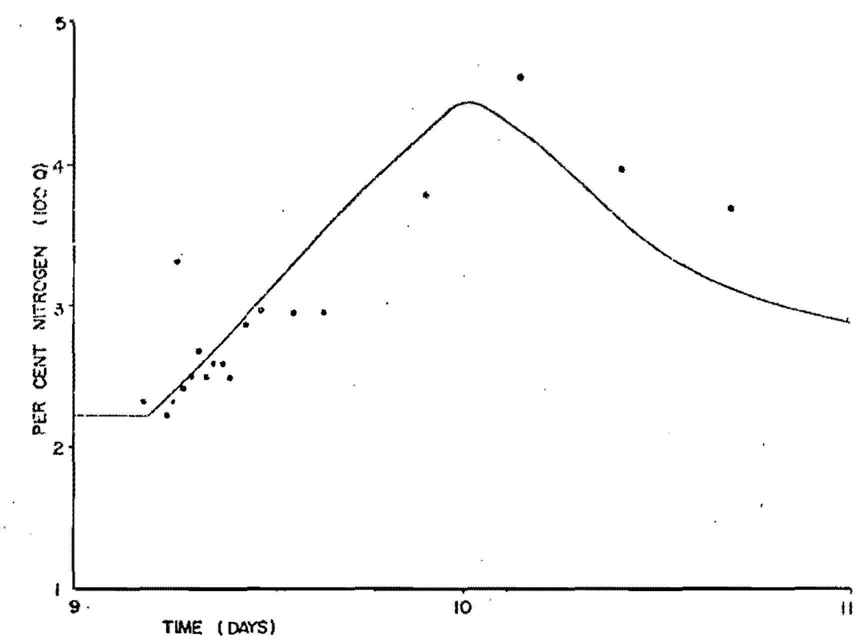

Figure 24. Phase II cell quota simulation, Q_o = 2.23 percent.

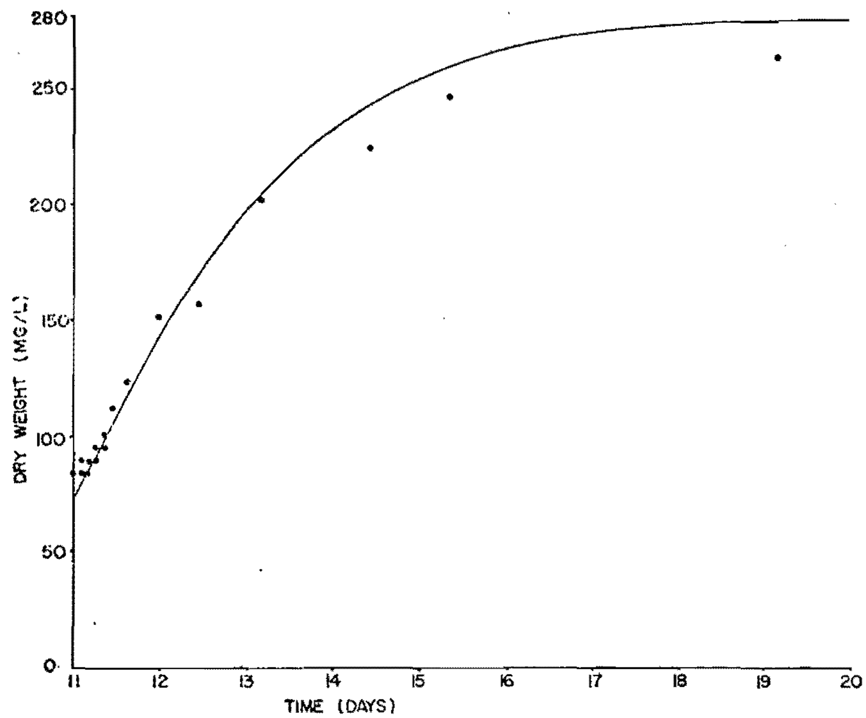

Figure 25. Phase III biomass simulation, Q_o = 1.53 percent.

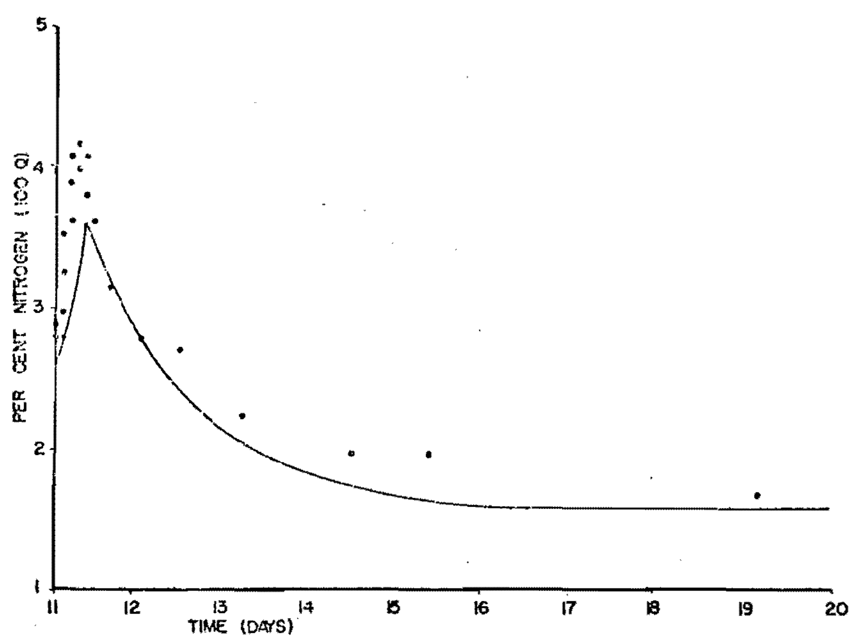

Figure 26. Phase III cell quota simulation, Q_o = 1.53 percent.

DISCUSSION

Two types of algal growth models were compared in this study. The model of Malone (1976) had a linear dependence on Q, the cell quota, and the model of Droop (1968) had a non-linear dependence on Q where the maximum rate of growth (μ_m) was approached asymptotically. These two types of models were combined with the respective author's uptake models and compared in simulations of data of nitrogen starved cells in batch culture. Droop's (1968) uptake-growth model produced simulations of the data which were similar to Malone's (1976) uptake-growth model.

The observed similarity between these two models can be explained by the fact that the non-linear model is approximately linear at low values of Q and only low values of Q were observed in this study (Q/Q_o was at most 2.4, see Figure 1). Thus, it is possible that the non-linear model is more representative of nutrient limited algal growth but the high, saturating values of Q which would have demonstrated its superiority in predicting growth were not observed.

It has been suggested, however, that high values of Q may be an indication of the onset of storage of the supposed limiting nutrient and growth limitation by another factor (Fuhs, 1969). If high values of Q did indeed represent nutrient storage, the linear growth model of Malone (1976) would be a more realistic model of single nutrient limitation.

It is also possible that high values of Q would only be evident when cell population measures other than cell dry weights are used in defining X and Q (see previous discussion in Cell Population Measures). This speculation would be supported by the data of Malone (1976) who also worked with cell dry weight.

Still, Droop's (1968) non-linear model has two factors which might make it superior even if only low values of Q are observed. The maximum growth rate (μ_m) is this equation could be a constant which represents the physiological maximum that could be obtained by the particular algae under the controlled environmental conditions of the study. If this were true, the μ_m of one species could be compared to the μ_m of another species giving insight as to how these two species would compare in thier growth responses in a given situation. Since Q/Q_o was at most equal to 2.4 in this study, μ_m was extrapolated from values of μ which would not have exceeded 58% of μ_m (see Figure 1).

Second, Droop's (1968) model can be rearranged to a familiar form:

$$\frac{dX}{dt} = \mu_m \left(\frac{R - X}{R}\right) X = \mu_m X - \frac{\mu_m}{R} X^2$$

where,

$R = \frac{XQ}{Q_o}$ = limiting nutrient concentration contained within the algae/Q_o = (self crowding) carrying capacity

This equation has been called the logistic equation (Odum, 1971) with R, the species carrying capacity, being the concentration of the biomass that is asymptotically approached as the species approaches the environment's capacity for supporting further growth. This variable could be used for predicting the maximum (single species) biomass a given environment would support.

Both of the cell quota models compared were a considerable improvement over the Monod model, as evidenced by the growth without uptake observed in Phase I. The cell quota models consider the growth potential of the external nutrient concentration (as does Monod's model) and, in addition, consider the growth potential of the nutrients already within the cell.

Unfortunately, the cell quota models do not predict the adaptation of the cells reflected in the variable Q_o. This adaptation may be a result of the changes in tha algae's environment; all nutrients, limiting and non-limiting, plus the change in light intensity resulting from the biomass dependent self-shading; or only a result of the previous (single) nutrient starvation. This study can not distinguish between the two. This is the major hazard of working with a batch study. The use of a chemostat would have been beneficial by minimizing the effects of the cell's nutrient pre-history and by providing a constant cell-external environment.

On the other hand, a chemostate would not have provided the in-depth look at what was going on with the algae cells; there would have been no gradual decay of Q to the Q_o (observable in batch culture). To find Q_o in a chemostat study, it is necessary to first assume a cell quota model and then to extrapolate the steady-state values to zero growth.

By the nature of their use, chemostats often conceal pertinent data, for example, growth and/or uptake lags (transient effects in general). The use of a batch culture in this study permitted the observation of growth lag and provided some insight into the possibility of the growth lag being induced by a lag in limiting nutrient uptake.

CONCLUSIONS

1. Algal growth was a function of both cell-internal limiting nutrient and external limiting nutrient concentrations.
2. Lag in growth was apparently a result of lag in nutrient uptake.
3. Uptake lag was a function of the level of nutrient starvation of the algal cells, or equivalently, a function of the cells preconditioning growth rate.
4. The minimum cell nutrient quota (Q_o) varied over the study period. this resulted from:
   a) population adaptation to nitrogen starvation and/or
   b) population adaptation to the changing environment of the batch culture.
5. None of the proposed cell quota growth methods allowed for the observed variation in Q_o.
6. Droop's (1968) model (growth rate a hyperbolic function of cell quota) and Malone's (1976) model (growth rate a linear function of cell quota) gave similar fits to the cell mass, external nutrient and cell quota data.
7. When applied to sections of the data which represented different nutritional histories, the linear model simulated the data after adjusting the value of Q_o (μ_m remaining constant).

RECOMMENDATION

To eliminate the disadvantages of batch culturing while providing a good view of transient growth and uptake, it is suggested that a hybrid culturing system be used (Caperon and Meyer, 1972b) where the cell's previous history is known and can be related to the transient responses.

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APPENDICES

Appendix A

COMPLETE DATA LISTING

Table A-1. Complete data listing with coefficients of variation (CV) and number of replicates (N).

Time (days)	External Nitrogen (mg/l)	CV/N	Cell Quota (percent)	CV/N	Cell Dry Weight (mg/l)	CV/N	Fluorescence (relative units)	CV/N	Cell Counts (10^{-6} cells/ml)	CV/N
0.0	a.	----	3.72	--/1	31.4	--/1	----	----	0.77	0.0/3
0.03	a.	----	3.90	1.94/3	30.1	3.35/3	----	----	----	----
0.09	a.	----	----	----	----	----	12.33	1.15/3	----	----
0.16	a.	----	3.74	1.51/2	34.3	4.54/2	11.61	0.85/3	0.85	--/1
0.41	a.	----	3.24	2.36/3	35.8	2.01/3	11.00	4.00/3	0.82	23.3/3
0.64	a.	----	----	----	----	----	11.78	4.54/3	1.20	8.33/3
0.66	a.	----	3.27	6.29/3	36.9	1.37/3	----	----	----	----
0.91	a.	----	3.14	2.40/3	40.1	1.75/3	11.52	2.35/3	1.46	7.29/2
1.16	a.	----	3.23	7.61/3	41.7	1.68/3	11.17	2.59/3	1.61	8.26/3
1.41	a.	----	3.21	3.19/3	43.5	3.39/3	13.70	6.98/3	1.99	1.07.2
1.52	a.	----	----	----	----	----	10.50	2.75/3	----	----
1.92	a.	----	2.79	8.10/3	44.7	2.07/3	10.83	0.0/3	2.18	4.88/2
2.41	a.	----	3.02	5.47/3	46.3	1.95/3	9.95	2.56/3	2.27	9.05/2
2.41	a.	----	3.30	16.5/3	48.1	2.13/3	8.56	2.29/3	2.21	4.72/3
3.41	a.	----	2.87	12.5/3	49.3	3.10/3	7.33	2.25/3	2.03	8.03/2
4.41	a.	----	2.79	17.3/3	49.6	1.85/3	6.50	4.44/3	2.12	2.67/2
5.41	a.	---	2.50	6.70/3	51.7	2.57/3	3.88	6.35/3	2.31	11.5/3
7.41	a.	----	2.74	15.7/3	52.7	3.53/3	2.38	8.48/3	2.34	4.32/3
8.24	a.	----	2.68	15.7/3	53.7	2.79/3	1.78	9.25/3	----	----
9.19	1.56	3.91/2	2.33	3.67/3	32.7	1.41/3	0.86	5.99/3	1.53	23.6/2
9.20	1.60	8.84/3	2.44	6.54/3	31.9	2.90/3	----	----	----	----
9.21	----	----	----	----	----	----	0.88	5.35/3	1.49	--/1
9.22	1.56	2.83/3	2.75	--/1	32.4	--/1	----	----	----	----
9.24	1.48	3.02/3	2.21	2.82/2	32.5	4.45/3	0.93	2.72/3	1.27	1.11/2
9.26	1.52	5.06/3	2.27	7.39/3	32.5	1.42/3	0.91	3.96/3	1.52	9.80/2
9.28	1.49	5.42/3	2.44	11.9/3	31.9	5.02/3	0.91	1.27/3	1.27	2.23/2
9.31	1.44	2.76/3	2.49	10.4/3	32.5	1.55/3	0.85	5.46/3	1.38	8.74/2

[a] External nitrogen not measured, assumed to be zero in model runs.

Table A-1. Continued.

Time (days)	External Nitrogen (mg/l)	CV/N	Cell Quota (percent)	CV/N	Cell Dry Weight (mg/l)	CV/N	Fluorescence (relative units)	CV/N	Cell Counts (10^{-6} cells/ml)	CV/N
9.33	1.42	4.88/3	2.67	2.96/3	31.2	1.11/3	0.91	3.55/3	1.36	23.9/2
9.35	1.44	6.30/3	2.45	10.2/3	32.7	5.48/3	0.97	5.971/3	----	----
9.37	1.40	4.82/3	2.59	9.86/3	31.6	2.76/3	0.91	5.62/3	1.30	19.1/2
9.39	1.42	5.66/3	2.59	7.70/3	35.4	15.4/3	0.92	4.88/3	1.09	16.3/2
9.41	1.41	4.63/3	2.50	11.8/3	33.5	5.35/3	1.03	7.00/3	1.43	4.94/2
9.46	1.36	3.83/3	2.88	6.87/3	32.4	2.14/3	1.08	6.97/3	----	----
9.50	1.30	1.85/3	2.95	3.82/3	32.8	2.66/3	1.09	8.30/3	1.82	19.1/2
9.58	1.22	1.09/3	2.95	13.6/3	33.3	1.51/3	1.57	10.8/3	1.24	1.72/2
9.66	0.84	1.01/3	2.92	13.6/3	34.1	2.71/3	1.91	11.1/3	1.59	22.8/2
9.91	0.48	3.73/3	3.80	5.61/3	38.9	3.61/3	3.07	12.4/3	1.36	6.32/3
10.16	0.042	97.6/3	4.62	1.53/3	44.7	4.01/3	3.90	13.0/3	1.28	5.85/3
10.28	0.001	86.6/3	4.38	5.78/3	48.5	6.24/3	----	----	----	----
10.41	a.	----	3.98	0.94/2	50.5	0.84/2	4.58	15.5/3	1.17	12.8/3
10.69	a.	----	3.73	9.50/3	65.4	4.43/2	5.30	13.2/3	3.72	12.1/3
11.02	a.	----	2.87	13.5/3	81.3	6.31/3	5.38	10.8/3	5.99	1.77/3
11.08	1.33	6.71/3	2.78	8.10/3	85.8	6.23/3	5.30	16.4/3	4.27	21.7/3
11.09	1.37	0.0/2	2.91	1.00/3	83.0	6.29/3	5.42	16.6/3	3.73	12.6/3
11.11	1.26	9.25/3	2.95	5.63/3	85.0	5.79/3	5.73	4.39/3	5.04	7.20/3
11.13	1.14	9.88/3	2.77	16.9/3	86.7	4.3713	6.80	14.0/3	5.15	4.90/3
11.16	0.94	11.3/3	3.24	1.15/3	86.7	4.09/3	7.10	9.86/3	4.46	11.3/3
11.18	0.83	3.00/2	3.54	1.20/2	87.5	4.85/2	6.07	12.4/3	4.50	6.06/3
11.20	0.67	10.9/3	3.59	9.02/3	88.5	4.27/3	5.45	10.3/3	4.81	5.94/3
11.21	0.54	22.9/2	3.85	--/1	86.0	--/1	5.52	13/9/3	5.40	19.5/3
11.24	0.43	1.64/3	3.63	7.13/3	88.3	4.09/3	6.27	3.94/3	4.37	8.92/3
11.26	0.32	20.7/3	4.07	2.63/3	88.2	5.24/3	6.30	12.4/3	4.65	27.2/3
11.28	0.22	22.3/3	3.98	5.12/3	91.5	3.28/3	6.63	11.3/3	3.96	16.1/3
11.30	0.090	24.2/2	4.12	2.79/3	92.2	3.61/3	7.53	9.49/3	4.40	5.94/3

[a]External nitrogen not measured, assumed to be zero in model runs.

Table A-1. Continued.

Time (days)	External Nitrogen (mg/l)	CV/N	Cell Quota (percent)	CV/N	Cell Dry Weight (mg/l)	CV/N	Fluorescence (relative units)	CV/N	Cell Counts (10^{-6} cells/ml)	CV/N
11.32	0.028	98.9/3	3.95	5.52/3	93.0	5.30/3	7.72	12.5/3	3.94	9.79/3
11.36	a.	----	4.11	4.26/3	97.0	4.22/3	11.8	16.2/3	4.96	12.9/3
11.41	a.	----	3.79	2.65/3	99.7	4.27/3	13.6	11.6/3	----	----
11.53	a.	----	3.56	3.62/3	112.	4.53/3	18.8	17.1/3	5.13	3.88/3
11.66	a.	----	3.18	3.75/3	124.	5.56/3	22.2	9.24/3	9.37	80.2/3
12.00	a.	----	2.73	5.34/3	150.	3.14/3	25.7	8.21/3	6.33	11.7/2
12.51	a.	----	2.66	5.11/2	157.	1.80/2	21.7	9.54/3	7.97	7.57/3
13.17	a.	----	2.21	9.98/3	199.	3.52/3	16.0	4.77/3	17.3	13.3/3
14.08	a.	----	----	----	----	----	12.1	12.0/3	----	----
14.43	a.	----	1.98	2.93/2	223.	1.27/2	11.7	2.87/3	8.69	152/3
15.38	a.	----	1.90	7.43/3	246.	1.72/2	11.1	4.58/3	5.57	39.1/3
17.12	a.	----	----	----	----	----	5.97	4.62/3	----	----
18.12	a.	----	----	----	----	----	4.92	8.22/3	----	----
19.14	a.	----	1.69	8.00/3	264.	3.68/3	3.70	6.19/3	---	----

[a] External nitrogen not measured, assumed to be zero in model runs.

Appendix B

PROGRAM LISTING

Table B-1. Main program.

```
C******** ************************************************************* ********C
C*****                                                                     *****C
C*****   MAIN PROGRAM FOR ALGAE MODEL                                      *****
C*****   18 SEPTEMBER 1976                                                 *****
C*****                                                                     *****C
C******** ************************************************************* ********C

      COMMON /SPIKE / SPIKTM(11),SSPIKE(11),DILTM,DILFAC                25MAY77
      COMMON /DAPLOT/ MAXPAR,IPARAM(6),MXPLOT
      COMMON /DIFFEQ/ COEF(10)
      COMMON /START/  ISTART,ISPIKE,TSTART,TSTOP
      COMMON /DTIME/ NTIME,DASTEP,IPSTEP,LSKIP
      COMMON /COMPUT/ S(10),NEQN                                        31MAY77
      COMMON /OBSERV/ DATIME(56),DATA(56,6),N(56,6),CV(56,6),
     $                XMAX(6),NPOINT
      COMMON /ZERO  / SZERO(6)
      DIMENSION T(3)
      DATA XMAX /1.555,4.568,4.62,25.72,0.3949,264.0/
      DATA NPOINT /55/
      DATA DILTM,DILFAC/8.3      ,.550429/
C     DATA SPIKTM,SSPIKE/9.186111,11.08333,200.0,1.87,1.87,0.0/         24MAY77
C     DATA SPIKTM,SSPIKE/9.186111,11.08333,200.0,1.6,1.6,0.0/
      DATA SPIKTM /9.186,11.083,11.531,11.656,12.000,12.510,            25MAY77
     $             13.167,14.427,15.385,19.135,200.0/                   25MAY77
      DATA SSPIKE /1.6,1.6,0.171,0.086,0.144,0.107,0.070,0.064,0.025,   25MAY77
     $             0.043,0.0/                                           25MAY77
      DATA NEQN /6/                                                     31MAY77
C
      READ( 5, 5)NEQN

    5 FORMAT(I2)
      READ( 5,10)SZERO
   10 FORMAT(10F5.3)
C*****   ARRAY SZERO  SERVES AS INITIAL VALUES                              *
      WRITE( 6,15)SZERO
   15 FORMAT(1X,10F10.5)
C
      READ(5,10)COEF
      WRITE( 6,15)COEF
C
      READ(5,20)T
   20 FORMAT(3F2.0)
      TSTART=T(1)-7.0+(T(2)-8.0)/24.0+(T(3)-15.0)/1440.0
C*****   T IS A DUMMY ARRAY FOR READING IN DATE (T(1)),                *****C
C*****                                                                 *****C
C*****      HOUR (T(2)), AND MINUTE (T(3))                             *****C
C*****   TSTART = STARTING TIME OF SIMULATION                          *****C
C
      READ(5,20)T
      TSTOP =T(1)-7.0+(T(2)-8.0)/24.0+(T(3)-15.0)/1440.0
      TOTIME=TSTOP-TSTART
C*****   TSTOP = TIME OF END OF SIMULATION                             *****C
C*****   TOTIME = TOTAL TIME OF RUN (DAYS)                             *****
C
      READ(5,20)T
      DASTEP=T(1)+T(2)/24.0+T(3)/1440.0
      NTIME=IFIX(TOTIME/DASTEP+0.5)+1
      IPSTEP=IFIX(1.0/(10.0*DASTEP)+0.5)
C*****   DASTEP = TIME STEP IN DAYS                                    *****C
C*****   IPSTEP = TIME STEP FOR PLOTTING (10* A DAY)                   *****C
C
```

Table B-1. Continued.

```
      READ(5,30)MXPLOT,MAXPAR,IPARAM
   30 FORMAT(8I1)
C*****   MXPLOT = THE NUMBER OF PLOTS THE TOTAL TIME WILL BE DIVIDED        *****C
C*****   MAXPAR = THE NUMBER OF PARAMETERS TO BE PLOTTED                    *****C
C*****      INTO                                                            *****C
C*****   IPARAM = AN ARRAY OF THE PARAMETERS TO BE PLOTTED                  *****C
C
      CALL DATRED
C
      DO 44 I=1,NPOINT
      IF(TSTART-DATIME(I))53,53,44
   44 CONTINUE
   53 ISTART=I
      DATIME(NPOINT+1)=1000.0
C*****   ISTART IS THE STARTING INDEX FOR THE DATA                          *****C
      LSKIP=1
C
C     IF(TSTART .GT. DILTM    )LSKIP=2
C     IF(TSTART .GT. SPIKTM(1))LSKIP=3
C     IF(TSTART .GT. SPIKTM(2))LSKIP=4
      IF(TSTART .GT. DILTM)      LSKIP= 2
      IF(TSTART .GT. SPIKTM( 1))LSKIP= 3
      IF(TSTART .GT. SPIKTM( 2))LSKIP= 4
      IF(TSTART .GT. SPIKTM( 3))LSKIP= 5
      IF(TSTART .GT. SPIKTM( 4))LSKIP= 6
      IF(TSTART .GT. SPIKTM( 5))LSKIP= 7
      IF(TSTART .GT. SPIKTM( 6))LSKIP= 8
      IF(TSTART .GT. SPIKTM( 7))LSKIP= 9
      IF(TSTART .GT. SPIKTM( 8))LSKIP=10
      IF(TSTART .GT. SPIKTM( 9))LSKIP=11
      IF(TSTART .GT. SPIKTM(10))LSKIP=12
C*****   SPIKTM = AN ARRAY OF THE TIMES THE CULTURES WERE SPIKED            *****C
C*****   DILFAC = THE DILUTION FACTOR AT DILTM                              *****C
C*****   DILTM = THE TIME THE CULTURES WERE DILUTED                         *****C
C*****   SSPIKE = THE VALUE OF THE EXTERNAL NITROGEN AT SPIKTM              *****C
      IF(MXPLOT .EQ. 0)GO TO 66                                            5JUN77
      CALL MODEL                                                           5JUN77
      CALL PLOT                                                            5JUN77
      GO TO 68                                                             5JUN77
   66 CONTINUE                                                             5JUN77
      CALL NONLIN                                                          5JUN77
   68 CONTINUE                                                             5JUN77
      END
```

Table B-2. Model subroutine.

```
C******* ************************************************************* *******C
C*****                                                                   *****C
C*****   SUBROUTINE MODEL TIME STEPS FROM TSTART TO TSTOP, WHILE         *****C
C*****      INSURING THAT THE SIMULATION HITS THS DATA POINTS,           *****C
C*****      DILUTIONS, AND SPIKES                                        *****C
C*****                                                                   *****C
C******* ************************************************************* *******C
C                                                                     MOD00000

      SUBROUTINE MODEL                                                MOD00100
C     COMMON /SPIKE/ SPIKTM(3),SSPIKE(3),DILTM,DILFAC                 MOD00200
      COMMON /SPIKE / SPIKTM(11),SSPIKE(11),DILTM,DILFAC              25MAY77
      COMMON /START/  ISTART,ISPIKE,TSTART,TSTOP                      MOD00300
      COMMON /OBSERV/ DATIME(56),DATA(56,6),N(56,6),CV(56,6),         MOD00400
     $                XMAX(6),NPOINT                                  MOD00500
      COMMON /DPLOT/ TPLOT(650),SPLOT(650,6),NSPNT                    MOD00600
      COMMON /DTIME/ NTIME,DASTEP,IPSTEP,ISKIP                        MOD00700
      COMMON /COMPUT/ S(10),NEQN                                      31MAY77
      COMMON /DAPLOT/ MAXPAR,IPARAM(6),MXPLOT                         MOD00900
      COMMON /ERROR / SUMSQ1,SUMSQ2,SUMSQ3                            MOD01000
      COMMON /ZERO  / SZERO(6)
      COMMON /DIFFEQ/ COEF(10)                                        31MAY77
      SUMSQ1=0.0
      SUMSQ2=0.0
      SUMSQ3=0.0
C
C        ESTABLISH INITIAL CONDITIONS
      S(1)=SZERO(1)                                                   6JUN77
      S(4)=SZERO(3)                                                   11JUN77
      S(2)=SZERO(2)*COEF(7)                                           11-UN77
      S(3)=SZERO(2)*(1.0-COEF(7))                                     11JUN77
      LSKIP=ISKIP                                                     MOD01100
      IERR=0                                                          MOD01200
      NSPNT=0                                                         MOD01300
      KPLOT=IPSTEP                                                    MOD01400
      JDATA=ISTART                                                    MOD01500
      TDATA=DATIME(JDATA)                                             MOD01600
      SUMDLT=0.0                                                      MOD01700
      ASSIGN 222 TO IPLT                                              5JUNE77
      IF(MXPLOT .NE. 0)ASSIGN 215 TO IPLT                             5JUNE77
  201 CONTINUE                                                        MOD01800
      DO 266 I=1,NTIME                                                MOD01900
      TIME=SUMDLT+TSTART                                              MOD02000
      DELT=DASTEP                                                     MOD02100
C
C****** ************************************************************* *******C
C        IPLT USED FOR SKIPPING AROUND PLOT ROUTINE
      GO TO IPLT                                                      5JUNE77
  215 CONTINUE
C*****   DEFINING VALUES FOR PLOT                                       *****C
      IF(KPLOT-IPSTEP)222,218,222
  218 CONTINUE
      KPLOT=0
      NSPNT=NSPNT+1
      TPLOT(NSPNT)=TIME
      WRITE( 6,220)TIME,S                                             1JUN77

  220 FORMAT(' T=',F6.3,' S=',10F8.4)                                 1JUN77
      DO 222 J=1,MAXPAR
      IP=IPARAM(J)
      SPLOT(NSPNT,IP)=EQPLOT(IP)
```

Table B-2. Continued.

```
  222 CONTINUE
      KPLOT=KPLOT+1
C****** *************************************************************** *******C
C
C     GO TO (231,235,239,243),LSKIP                                     MOD02200
      GO TO (231,235,235,235,235,235,235,235,235,235,235,235,           25MAY77
     $         243,243),LSKIP                                           25MAY77
  231 CONTINUE                                                          MOD02300
C****** *************************************************************** *******C
C*****    DILUTION ROUTINE                                                *****C
      TDIFF=DILTM -TIME                                                 MOD02400
      IF(TDIFF-DASTEP)232,232,243                                       MOD02500
  232 CONTINUE                                                          MOD02600
      S(1)=S(1)*DILFAC                                                  MOD02700
      S(2)=S(2)*DILFAC                                                  MOD02800
      S(3)=S(3)*DILFAC                                                  MOD02900
      S(4)=S(4)*DILFAC                                                  MOD03000
      DELT=TDIFF                                                        MOD03100
      LSKIP=2                                                           MOD03200
      KPLOT=IPSTEP                                                      MOD03300
      GO TO 243                                                         MOD03400
C****** *************************************************************** *******C
C*****    SPIKE ROUTINE                                                   *****C
  235 CONTINUE                                                          MOD03500
C     TDIFF=SPIKTM(1)-TIME                                              MOD03600
      LTEMP=LSKIP-1                                                     25MAY77
      TDIFF=SPIKTM(LTEMP)-TIME                                          25MAY77
      IF(TDIFF-DASTEP)237,237,243                                       MOD03700
  237 CONTINUE                                                          MOD03800
C     S(1)=SSPIKE(1)                                                    MOD03900
      S(1)=SSPIKE(LTEMP)                                                25MAY77
      DELT=TDIFF                                                        MOD04000
C     LSKIP=3                                                           MOD04100
      LSKIP=LSKIP+1                                                     25MAY77
      KPLOT=IPSTEP                                                      MOD04200
C     GO TO 243                                                         MOD04300
C 239 CONTINUE                                                          MOD04400
C     TDIFF=SPIKTM(2)-TIME                                              MOD04500
C     IF(TDIFF-DASTEP)241,241,243                                       MOD04600
C 241 CONTINUE                                                          MOD04700
C     S(1)=SSPIKE(2)                                                    MOD04800
C     DELT=TDIFF                                                        MOD04900
C     LSKIP=4                                                           MOD05000
C     KPLOT=IPSTEP                                                      MOD05100
  243 CONTINUE                                                          MOD05200
C****** *************************************************************** *******C
C*****    DATA ROUTINE                                                    *****C
      TDIFF=TDATA -TIME                                                 MOD05300
      IF(TDIFF-DELT  )245,245,255                                       MOD05400
  245 CONTINUE                                                          MOD05500
      DELT=TDIFF                                                        MOD05800
      KPLOT=IPSTEP                                                      MOD05900
      IERR=1                                                            MOD06000
C****** *************************************************************** *******C
C*****    CALL RUNGE-KUTTA                                                *****C
  255 CONTINUE                                                          MOD06100
      SUMDLT=SUMDLT+DELT                                                MOD06200
      CALL RK4(DELT)                                                    MOD06300
C****** *************************************************************** *******C
C
C         ERROR ROUTINE
```

Table B-2. Continued.

```
      IF(IERR-1)266,261,266                                          ERR00100
  261 CONTINUE                                                       ERR00200
      IERR=2                                                         ERR00300
      D1=DATA(JDATA,1)
      D2=DATA(JDATA,2)
      D3=DATA(JDATA,6)
      SUMSQ1=SUMSQ1+(S(1)-D1)**2/(D1+0.01)
      SDM2=S(2)+S(3)                                                 11JUN77
      SUMSQ2=SUMSQ2+(D2-SDM2)**2/D2                                  11JUN77
      SUMSQ3=SUMSQ3+(D3-S(4))**2/D3                                  11JUN77
      JDATA=JDATA+1                                                  MOD05600
      TDATA=DATIME(JDATA)                                            MOD05700
  266 CONTINUE                                                       ERR00700
      JDATA=JDATA-1                                                  DEBUG
      WRITE(6,270)SUMSQ1,S(1),D1,                                    DEBUG
     5            SUMSQ2,SDM2,D2,SUMSQ3,S(4),D3                      11JUN77
  270 FORMAT(5X,3(3F10.3,'*'))                                       DEBUG
      IF(MXPLOT .EQ. 0)GO TO 276                                     5JUNE77
      I=0                                                            PLOT
      NTIME=IFIX((TSTOP-TIME)/DASTEP+0.5)                            PLOT
      IF(NTIME .GT. 3)GO TO 201                                      PLOT
  276 CONTINUE                                                       5JUNE77
      RETURN                                                         MOD06400
      END                                                            MOD06500
```

Table B-3. Data reading subroutine.

```
C******* ****************************************************************** *******C
C*****                                                                       *****C
C*****   DATRED READS IN THE OBSERVED MEAN DATA                              *****C
C*****                                                                       *****C
C****** ****************************************************************** *******C

      SUBROUTINE DATRED
      COMMON /OBSERV/ DATIME(56),DATA(56,6),N(56,6),CV(56,6),
     $                XMAX(6),NPOINT
C
C****** ****************************************************************** *******C
C*****                                                                       *****C
C*****   DATA(I,1) = EXTERNAL NITROGEN, (MG/L)                               *****C
C*****   DATA(I,2) = INTERNAL NITROGEN, (MG/L)                               *****C
C*****   DATA(I,3) = % NITROGEN                                              *****C
C*****   DATA(I,4) = FLUORESENCE, (RFU)                                      *****C
C*****   DATA(I,5) = NORMALIZED FLUORESENCE, (RFU/MG/L)                      *****C
C*****   DATA(I,6) = DRY WEIGHT, (MG/L)                                      *****C
C*****                                                                       *****C
C*****   CV(I,J) ARE THE CV'S OF THE DATA, J=1,6                             *****C
C*****   N(I,J) ARE THE NUMBER OF REPLICATES THE CV'S ARE BASED ON           *****C
C*****                                                                       *****C
C****** ****************************************************************** *******C
C
      D=0.03
      NPOINT=55
      DO 888 I=1,NPOINT
C     READ(11,800)DATE,HOUR,AMINIT,(DATA(I,L),N(I,L),CV(I,L),L=1,6)       24MAY77
      READ(10,800)DATE,HOUR,AMINIT,(DATA(I,L),N(I,L),CV(I,L),L=1,6)

  800 FORMAT(3F2.0,2(F4.3,I1,F4.2),2(F4.2,I1,F4.2),F4.0,I1,F4.2,
     $       F4.1,I1,F4.2)
      DATIME(I)=DATE-7.0+(HOUR-8.0)/24.0+(AMINIT-15.0)/1440.0
      DATA(I,5)=DATA(I,5)/10000.0
C     DATA(I,3)=DATA(I,3)/100.0   CONVRTS %N TO W
  888 CONTINUE
      END
```

Table B-4. Runge-Kutta subroutine.

```
C****** *************************************************************** *******C
C*****                                                                    *****C
C*****   RUNGE-KUTTA ROUTINE                                              *****C
C*****                                                                    *****C
C****** *************************************************************** *******C

      SUBROUTINE RK4(DELT)
      COMMON /COMPUT/ S(10),NEQN                                         31MAY77
      DIMENSION F(4,10),SINTAL(10)
C
C****** *************************************************************** *******C
C*****                                                                    *****C
C*****   S(NEQN) IS AN ARRAY OF TIME DEPENDANT VARIABLES                  *****C
C*****   DELT IS THE SIZE OF THE TIME STEP                                *****C
C*****   F IS AN ARRAY OF RUNGE-KUTTA APPROXIMATIONS                      *****C
C*****   NEQN IS THE NUMBER OF TIME DEPENDANT VARIABLES                   *****C
C*****   SINTAL IS AN ARRAY OF INITIAL VALUES                             *****C
C*****                                                                    *****C
C****** *************************************************************** *******C
C
      DELTB2=0.5*DELT
      DELTB6=DELT/6.0
C
      DO 311 I=1,NEQN
      SINTAL(I)=S(I)
  311 F(1,I)=DSDT(I)
      DO 322 I=1,NEQN
  322 S(I)=SINTAL(I)+DELTB2*F(1,I)
C
      DO 333 I=1,NEQN
  333 F(2,I)=DSDT(I)
C
      DO 344 I=1,NEQN
  344 S(I)=SINTAL(I)+DELTB2*F(2,I)
C
      DO 355 I=1,NEQN
  355 F(3,I)=DSDT(I)
C
      DO 366 I=1,NEQN
  366 S(I)=SINTAL(I)+DELT  *F(3,I)
C
      DO 377 I=1,NEQN
  377 F(4,I)=DSDT(I)
C
      DO 388 I=1,NEQN
  388 S(I)=SINTAL(I)+DELTB6*(F(1,I)+2.0*F(2,I)+2.0*F(3,I)+F(4,I))
      RETURN
      END
```

Table B-5. Example of linked differential equation set.

```
C******** *************************************************************** *******C
C*****                                                                      *****C
C*****   FUNCTION OF THE DIFFERNTIAL EQUATIONS                              *****C
C*****                                                                      *****C
C******* *************************************************************** *******C

      FUNCTION DSDT(INTGER)
      COMMON /DIFFEQ/ COEF(10)
      COMMON /COMPUT/ S(10),NEWN                                          31MAY77
      GO TO (401,402,403,404,405,406),INTGER
  401 S1=S(1)
      S4=S(4)
      S5=COEF(6)*S(3)/S4
      S(5)=S5
      S6=(COEF(2)*S5+COEF(1))*S1*S4/(S1+COEF(3))
      S(6)=S6
      DSDT=-S6
      GO TO 411
  402 S7=COEF(4)*(S(2)-COEF(5)*S(4))
      S(7)=S7
      DSDT=S(6)-S7
      GO TO 411
  403 DSDT=S(7)
      GO TO 411
  404 DSDT=S(5)*S(4)
      GO TO 411
  405 CONTINUE
      GO TO 411
  406 CONTINUE
  411 CONTINUE
C*****                                                                      *****C
      RETURN
      END
```

Table B-6. Function for setting plotted variables.

```
      FUNCTION EQPLOT(INTGER)
      COMMON /COMPUT/ S(10),NEQN                                       31MAY77
      COMMON /DIFFEQ/ COEF(10)
      GO TO(601,602,603,604,605,606,607,608,609,610),INTGER           31MAY77
  601 EQPLOT=S(1)
C         EQPLOT = EXTERNAL NITROGEN
      GO TO 611
  602 EQPLOT=S(2)+S(3)                                                 11JUN77
C         EQPLOT = INTERNAL NITROGEN
      GO TO 611
  603 EQPLOT=(S(2)+S(3))*100.0/S(4)                                    11JUN77
C         EQPLOT = PERCENT NITROGEN
      GO TO 611
  604 EQPLOT=S(4)
C         EQPLOT = FLUORESENCE
      GO TO 611
  605 EQPLOT=S(4)/S(3)
C         EQPLOT = FLUORESENCE/DRY WEIGHT
      GO TO 611
  606 EQPLOT=S(4)                                                      11JUN77
C         EQPLOT = DRY WEIGHT
      GO TO 611
  607 CONTINUE
  608 CONTINUE
  609 CONTINUE
  610 CONTINUE
  611 CONTINUE
      RETURN
      END
```

Table B-7. Subroutine EQUA, the link between the algae model and NONLIN (Grenney, 1975).

```
      SUBROUTINE EQUA(B,E)
      COMMON /DIFFEQ/ COEF(10)
      COMMON /ERROR / SUMSQ1,SUMSQ2,SUMSQ3
      DIMENSION B(15)
      WRITE( 6,10)B(1),B(2),B(3),B(4),B(5),B(6),B(7),B(8),B(9),B(10)
   10 FORMAT(' BETAS=',10F10.6)
      E=0.0                                                             00006340
      COEF( 1)=B( 1)
      COEF( 2)=B( 2)
      COEF( 3)=B( 3)
      COEF( 4)=B( 4)
      COEF( 5)=B( 5)
      COEF( 6)=B( 6)
      COEF( 7)=B( 7)
      COEF( 8)=B( 8)
      COEF( 9)=B( 9)
      COEF(10)=B(10)
      SUMSQ1=0.0
      SUMSQ2=0.0
      SUMSQ3=0.0
      CALL MODEL
      E=0.5*SUMSQ1+0.5*SUMSQ2+SUMSQ3
C     E=          0.5*SUMSQ2+SUMSQ3                                     7JUN77
      RETURN
      END
```

Table B-8. Plot routine.

```
      SUBROUTINE PLOT
      COMMON /OBSERV/ DATIME(56),DATA(56,6),N(56,6),CV(56,6),
     $                XMAX(6),NPOINT
      COMMON /DPLOT/ TPLOT(650),SPLOT(650,6),NSPNT
      COMMON /DAPLOT/ MAXPAR,IPARAM(6),MXPLOT
      COMMON /START/  ISTART,ISPIKE,TSTART,TSTOP
      DIMENSION A(1045),H(12),YH(9),TH(12)
      DIMENSION TS(650),YS(650)
      DATA A/1045*'      '/
      DATA H/12*'      '/
      DATA YH/9*'      '/
      DATA TH/5*'      ',' TIME ','(DAYS)',5*'      '/
      DAYS=(TSTOP-TSTART)/FLOAT(MXPLOT)
      DO 699 I=1,MAXPAR
      IP=IPARAM(I)
      GO TO(601,602,603,604,605,606),IP
  601 CONTINUE
      H(5)='      '
      H(6)='EXTERN'
      H(7)='AL NIT'
      H(8)='ROGEN '
      YH(3)='      '
      YH(4)='MG PER'
      YH(5)=' LITER'
      YH(6)='      '
      GO TO 607
  602 CONTINUE
      H(5)='      '
      H(6)='INTERN'
      H(7)='AL NIT'
      H(8)='ROGEN '
      YH(3)='      '
      YH(4)='MG PER'
      YH(5)=' LITER'
      YH(6)='      '
      GO TO 607
  603 CONTINUE
      H(5)='      '
      H(6)='PERCEN'
      H(7)='T NITR'
      H(8)='OGEN  '
      YH(3)='      '
      YH(4)='   PER'
      YH(5)='CENT  '
      YH(6)='      '
      GO TO 607
  604 CONTINUE
      H(5)='      '
      H(6)='FLUORE'
      H(7)='SENCE '
      H(8)='      '
      YH(3)='      '
      YH(4)='      '
      YH(5)='RFU   '
      YH(6)='      '
      GO TO 607
  605 CONTINUE
      H(5)='  FLUO'
      H(6)='RESENC'
      H(7)='E/DRY '
```

Table B-8. Continued.

```
      H(8)='WEIGHT'
      YH(3)='     RF'
      YH(4)='U PER '
      YH(5)='MG PER'
      YH(6)=' LITER'
      GO TO 607
  606 CONTINUE
      H(5)='      '
      H(6)='  DRY '
      H(7)='WEIGHT'
      H(8)='      '
      YH(3)='      '
      YH(4)='MG PER'
      YH(5)=' LITER'
      YH(6)='      '
  607 CONTINUE
      YSMX=0.0
      YSMN=0.0
      TMX=ISTART
      K1=1
      K2=ISTART
      DO 688 J=1,MXPLOT
      NS=0
      ND=0
      TMN=TMX
      TMX=DAYS+TMN
      DO 611 K=K1,NSPNT
      TIME=TPLOT(K)
      IF(TIME-TMN)611,608,608
  608 CONTINUE
      IF(TIME-TMX)609,609,613
  609 CONTINUE
      NS=NS+1
      TS(NS)=TIME
      Y=SPLOT(K,IP)
      YS(NS)=Y
      IF(YSMX .LT. Y)YSMX=Y
      IF(YSMN .GT. Y)YSMN=Y
  611 CONTINUE
  613 CONTINUE
      K1=K
      YMX=AMAX1(YSMX,XMAX(IP))
      YMN=AMIN1(0.0,YSMN)
      CALL PL360(NS,A,TS,TMN,TMX,TH,YS,YMN,YMX,YH,H,-78)
      DO 622 K=K2,NPOINT
      TIME=DATIME(K)
      IF(TIME-TMN)622,615,615
  615 CONTINUE
      IF(TIME-TMX)616,616,623
  616 CONTINUE
      ND=ND+1
      TS(ND)=TIME
      YS(ND)=DATA(K,IP)
  622 CONTINUE
  623 CONTINUE
      K2=K
      CALL PL360(ND,A,TS,TMN,TMX,TH,YS,YMN,YMX,YH,H,231)
      TMX=DAYS+TMN
  688 CONTINUE
  699 CONTINUE
      RETURN
```