PHOSPHORUS METABOLISM OF SEVERAL

AQUATIC MICROORGANISMS

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A

THESIS

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ABSTRACT

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AQUATIC MICROPLANKTON

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Several taxonomically diverse aquatic microplankton were described growing at phosphorus (P) concentrations that limit growth in many natural aquatic systems. Because natural aquatic systems are subject to periodic fluctuations in P levels, both steady-state (via continuous culture) and transient (via batch culture) growth were described. Complete growth kinetic descriptions of Synechococcus Nägeli (strain A) and Scenedesmus quadricauda were used to predict the relative competitive abilities of these species when P was the growth-limiting nutrient. These descriptions, coupled to their morphological characteristics, were used to construct partial physiological profiles for each organism. The profiles indicate that S. Nägeli (strain A) (a small

unicellular blue-green alga) is better suited for growth in P-limited oligotrophic niches than is S. quadricauda (a green alga). However, results from kinetic experiments with these and several other microplankton, show that such physiological profiles are not necessarily indicative of profiles for taxonomically related species.

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LIST OF SYMBOLS

Symbol	Meaning	Units
P_t	Total inorganic phosphorus concentation	nMP
P_{sol}	Phosphorus concentration in solution	nMP
Pi	Inorganic (molybdate reactive) phosphorus concentration	nMP
P_{nex}	Molybdate unreactive phosphorus concentration	nMP
P_{cell}	Phosphorus concentration in cells	nMP
D	Dilution rate	day^{-1}
μ	Specific growth rate	day^{-1}
μ_{MAX}	Apparent maximum growth rate	day^{-1}
Q	Cell quota	nMP $(mg\ cell)^{-1}$
Q_o	Minimum (subsistence) cell quota	nMP $(mg\ cell)^{-1}$
Q_{MAXs}	Maximum steady-state cell quota	nMP $(mg\ cell)^{-1}$
Q_{MAXt}	Maximum transient cell quota	nMP $(mg\ cell)^{-1}$
R	Coefficient of luxury consumption	unitless
X	Dry weight	$mg \cdot l^{-1}$
Y	Cell yield	$\mu g\ cell\ (nMP)^{-1}$
V	Instantaneous transport rate	nMP (mg cell-day)-1
V_n	Net transport rate	nMP $(mg\ cell\text{-}day)^{-1}$
V_t	Gross transport rate	nMP $(mg\ cell\text{-}day)^{-1}$
a	affinity	$l\ (mg\ cell\text{-}day)^{-1}$
a_s	Steady-state affinity	$l\ (mg\ cell\text{-}day)^{-1}$

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INTRODUCTION

Although microplankton in natural aquatic systems may be limited at any moment by environmental factors such as temperature, light and growth inhibitors (Nicholls and Dillon, 1978), the single factor most often regulating the microplankton community is the availability of essential nutrients (Tempest and Neijssel, 1978). Traditionally, empirical models relating microplankton growth to either external (Monod, 1942) or internal (cell quota; Droop, 1968) limiting nutrient concentrations have been used to describe the interaction between the microplankton community and essential nutrients. These and other models (Caperon, 1967; Dugdale, 1967) are based primarily on steady-state hyperbolic enzyme theory. The fitting of data to such models has enhanced our understanding of the microplankton community/limiting nutrient relationship.

Many aquatic systems, however, are subject to periodic nutrient perturbations in the levels of essential nutrients. Microplankton responses to nutrient perturbations can not be accurately described with steady-state models (Brown and Harris, 1978). In these systems, microplankton production can best be related to both fluctuating (transient) and

steady-state levels of essential growth-rate-limiting nutrients. For this reason, it is important to describe an organism's response to both steady-state and transient nutrient levels. Properly designed batch culture experiments give good approximations of transient conditions while continuous culture experiments best describe an organism's response to steady-state conditions.

This work describes the phosphorus-limited growth kinetics of seven taxonomically diverse microplankton species. We chose to work with phosphorus as the growth-limiting nutrient since it has been shown to be a nutrient which often limits microplankton biomass in many aquatic systems (Golterman, 1973; Schindler, 1977) and because radiotracer techniques now allow precise measurements of the low phosphorus concentrations that control microplankton growth (Robertson and Rutton, 1979).

LITERATURE REVIEW

PHOSPHORUS AND MICROPLANKTON GROWTH

Phosphorus (P) is an essential nutrient for growth and metabolism in all microbes. However, its biologically available form, orthophosphate (Pi), is often present in the photic zone of natural aquatic systems only in amounts that limit microbial growth and biomass (Sawyer, 1947, 1952; Golterman, 1973; Schindler, 1977). Increased P levels are generally recognized as the key factor in the acceleration of eutrophication in many aquatic systems.

The mechanisms by which P regulates the microbial community, however, are poorly understood. This is due largely to the fact that analytical techniques are not sensitive enough to accurately assess the dilute Pi concentrations that regulate microbial communities in situ. Total P (organic P plus the various forms of inorganic P), however has been related directly to microplankton biomass in situ (Vollenweider, 1976). Unfortunately, even laboratory studies which use conventional chemical analytical techniques to measure the growth of microplankton have not been able to describe microbial growth in dilute Pi environments. However, recent developments in analytical techniques

incorporating the use of radiophosphorus (32-P; Law et al., 1976) do allow precise determinations of dilute Pi concentrations and its control of microplankton growth. These developments have enabled the direct measurement of the external Pi concentrations that control the growth rate of both a green alga (Brown and Button, 1979) and an aquatic heterotrophic yeast (Robertson and Button, 1979).

PHOSPHORUS TRANSPORT AND METABOLISM

Microbes actively transport P, principally in the form of Pi. The transport of Pi has been linked to specific binding proteins present in the periplasmic space and transport proteins present in the cell membrane (Argast and Boos, 1980; Rosenburg et al., 1977; Burns and Beever, 1979). These authors have also shown evidence that Pi transport in certain microbes consists of two mechanisms, a constitutive mechanism with a high half saturation constant (called low affinity) and a repressible mechanism with a low half saturation constant (called high affinity). It is hypothesized that the amount and type of transport is regulated by the presence and concentration of Pi transporter proteins, which in turn are regulated by cellular levels of P via genetic feedback control. Some microbes, under conditions of P limitation also excrete extracellular

alkaline phosphatases which enzymatically catalyze the breakdown of organic P molecules to Pi, which is then available for transport (Patni et al., 1977; Hassan and Pratt, 1977). There is now evidence that alkaline phosphatase production and Pi transport are genetically linked (Aragast and Boos, 1980) in at least some microorganisms.

Once transported, the cell channels P into a variety of metabolic pathway whose endproducts can be grouped into three general catagories ; structural, functional and storage components (Fuhs, 1972).

Structural components. Phosphorus is required for the maintainence and synthesis of certain structural components of the cell, including both the genetic (DNA) and membrane (phospholipids) fractions of the cell. The requirements for (but not necessarily synthesis of) these components are often discontinuous and potentially cyclic, since the major demand for phosphorus for these components occurs immediately prior to cell division.

Functional components. This category includes those P-compounds required for cell function including the low molecular weight phosphorolated metabolic intermediates such as ribonucleic acids (RNA) and

nucleotides (such as ATP and NADH). In addition, intracellular membrane structures such as ribosomes , mitochrondia and chloroplasts, which provide energy charge for cell function, are included in this category.

Storage components. Many microorganisms accumulate P above basal physiological requirements into cellular pools (Fuhs, 1972; Rhee, 1973; Brown and Button, 1979; Robertson and Button, 1979). Microbial growth and nutrient uptake have been related, through hyperbolic relationships, to such intracellular P pools (Rhee, 1973). At least two major P pools have been identified. The first, an "acid (TCA) soluble fraction", consists of polyphosphate granules which are composed of high molecular weight linear chain polyphosphates. This pool is characterized by a relatively long turnover time. The second type, an "acid (TCA) insoluble fraction is composed of polymers of phosphoric acid. This pool is charaterized by a relatively rapid turnover time.

TRANSPORT AND STORAGE

The ability to transport and store nutrients, such as

P, above basal requirements has been termed luxury uptake. Luxury uptake has been demonstrated for both P (Rhee, 1973; Brown and Button, 1979; Fuhs, 1972) and nitrogen (Caperon and Meyer, 1972; Rhee, 1974) in microplankton species. Nutrient transport can be operationally divided into at least two very distinctive types, each of which are best observed under different environmental and growth conditions. The first type is that transport which is required to sustain basal growth and metabolism and is called subsistence transport. It is best measured in steady-state nutrient-limited continuous cultures where it is characterized as the rate of transport required to sustain a cellular nutrient level (Q) which is needed to maintain steady-state growth and metabolism at each specific growth rate (μ). Many studies have shown that nutrient transport rates and Q vary with μ, each reaching a maximum at the maximum growth rate (μ_{MAX}). At μ_{MAX}, Q maintains a maximum value (Q_{MAXs}) characteristic for every organism. The second type of nutrient transport known as overplus (Fuhs, 1972) is best observed when nutrient starved cells are perturbed with a large excess of that nutrient. Under such conditions, transport rates greatly exceed maximum steady-state transport rates. Such rapid transport rates result in a temporary Q which is much higher than Q_{MAXs}. This value, Q_{MAXt}, is also variable among and characteristic for each

organism. Under certain conditions cells can maintain a Q value between Q_{MAXs} and Q_{MAXt}. This can occur when nutrient starved cells receive a perturbation (but not a large excess) of that nutrient, or under steady-state conditions when the cells are not growth-rate-limited by the nutrient in question, but by another factor which does not affect transport of this nutrient. These types of transport are conceptualized in Figure 1.

The coefficient of luxury uptake (R) has been used as an index of luxury uptake. It was first defined by Droop (1974, 1975) and later by Tilman and Kilham (1976) as a measure of the nutrient storage capacity of an organism. It is the ratio of maximum cellular nutrient content to minimum cellular nutrient content (Q_{MAX}/Q_o), and is thus dimensionless. It is important to note that the maximum value for R is Q_{MAXt}/Q_o, and not Q_{MAXs}/Q_o.

NUTRIENT TRANSPORT KINETICS

Most models attempting to describe regulation of microplankton communities by essential nutrients assume that the kinetics of nutrient transport by microplankton can be described by curves analogous to Michaelis-Menten enzyme hyperbolas. Michaelis and Menten (1913) described an enzyme reaction rate (equation 1) for a single substrate-enzyme

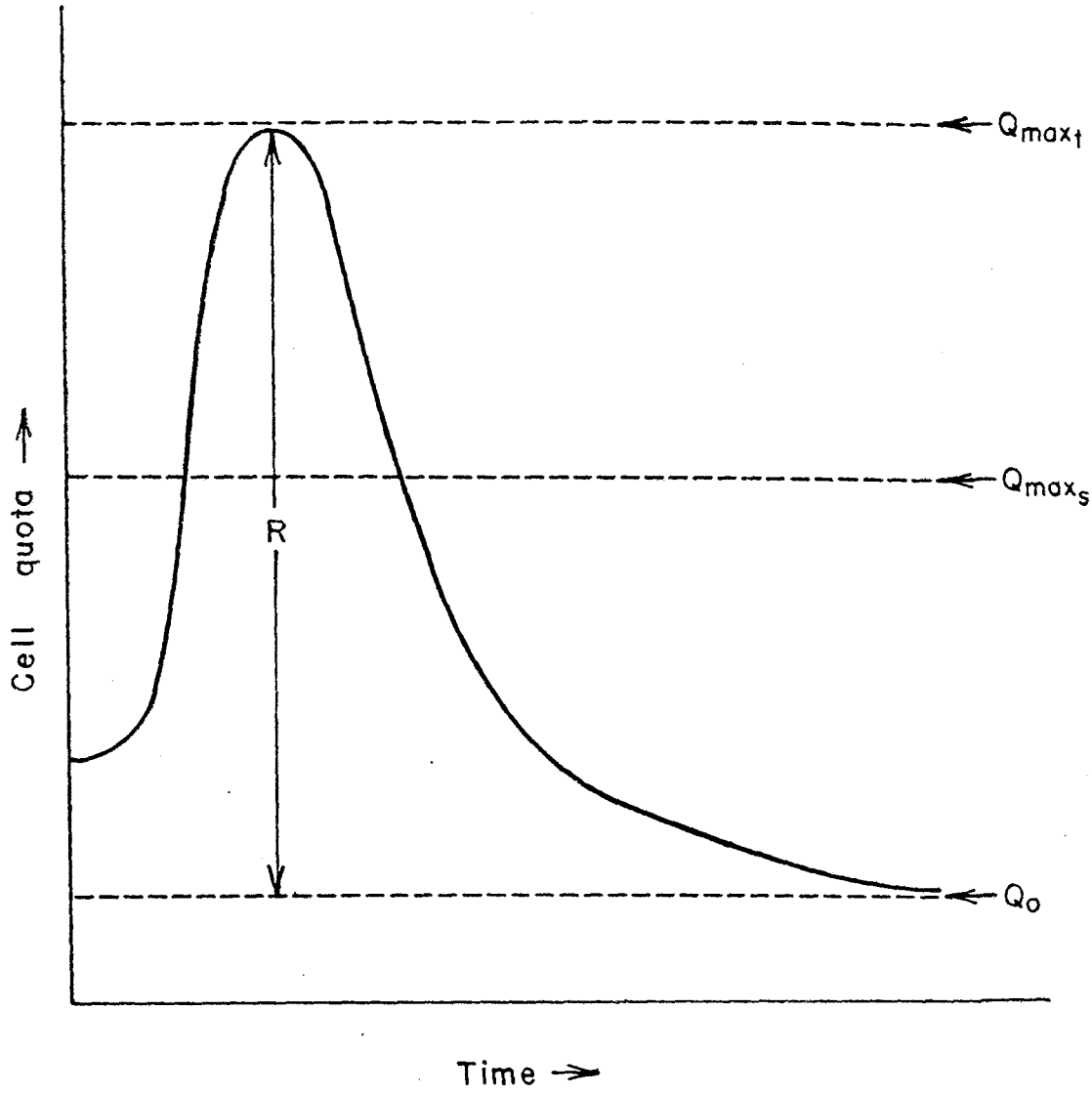

Figure 1. Conceptual diagram representing nutrient storage as a function of time for an individual cell within a population which experiences first an excess (perturbation) and then a growth-limiting supply of the nutrient. Symbols are defined in text.

catalyzed reaction as:

$$v_o = \frac{V_{MAX}\ S_o}{K_s + S_o} \qquad (1)$$

where v_o is the initial reaction velocity, V_{MAX} is the maximum reaction velocity, S_o is the initial substrate concentration and K_s is the Michaelis-Menten half saturation rate constant. K_s is equal to the substrate concentration at which the initial reaction velocity is one half V_{MAX}. Michaelis-Menten half saturation constants have been used as an index of an enzymes' affinity for its substrate. A low K_s represents a high affinity and vice versa. This is only valid, however when comparing enzymes of equal maximum velocity. K_s is more useful as an indication of the range over which an enzyme is responsive to changes in substrate concentrations.

The Michaelis-Menten rate equation describes a rectangular hyperbola (Figure 2) when initial reaction velocity is plotted against initial substrate concentration. A useful linearization of the Michaelis-Menten expression is the Lineweaver-Burke plot (Figure 2) in which $1/S_o$ is plotted versus $1/v_o$. Graphically this plot yields $-1/K_s$ and $1/V_{MAX}$ as the X and Y intercepts respectively and a slope of K_s/V_{MAX}.

It is useful to list the underlying assumptions inherent in the derivation of the Michaelis-Menten expression,

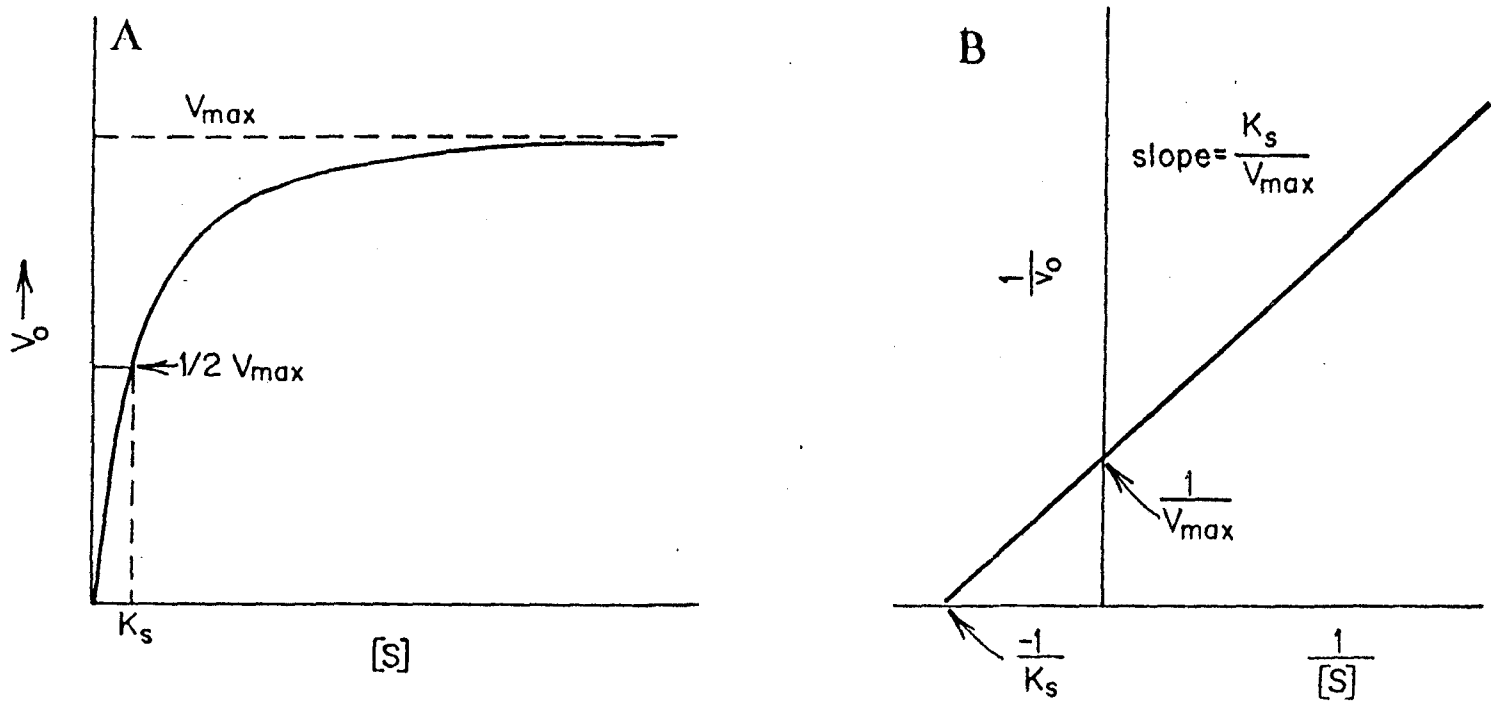

Figure 2. Theoretical relationship between initial reaction velocity and initial substrate concentration (A) and their reciprocals (B; Lineweaver-Burke) for an enzyme-substrate reaction obeying a simple Michaelis-Menten equation. Symbols are defined in text.

since they must also apply to empirical models of nutrient transport and microplankton growth when Michaelis-Menten hyperbolic type kinetics are assumed to hold. These assumptions with reference to the following enzyme (E), substrate (S) and product (P) reaction include:

$$E+S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E+P \qquad (2)$$

1. steady-state conditions, ie $d[ES]/dt=0$;
2. reversible reactions, but k_4 assumed negligible;
3. initial rates (v_o) are used, where $v_o=k_3[ES]$;
4. $[S]>[E]$ so that $[ES]>[S]$, and;
5. K_s is equivalent to k_2+k_3/k_1.

The equation analogous to equation 1 which most models incorporate for descriptions of nutrient transport by microplankton is:

$$V = \frac{V_{MAX}\ S}{K_v + S} \qquad (3)$$

(Caperon, 1967; Dugdale, 1967, 1977; Droop, 1973, 1974), where V is the instantaneous nutrient transport rate, V_{MAX} is the maximum nutrient transport rate, S is the ambient growth-rate-limiting nutrient concentration and K_v is the half-saturation constant numerically equal to substrate concentration at one half V_{MAX}. This expression has been modified by Caperon and Meyer (1972) to include a threshold

value for S (S_t) which is the S below which transport ceases:

$$V = \frac{V_{MAX}\ (S-S_t)}{K_v + (S-S_t)} \qquad (4)$$

NUTRIENT-LIMITED GROWTH KINETICS

Many expressions advanced describing microplankton growth and nutrient transport have been formulated from steady state experiments. Continuous cultures which allow cells to be grown and maintained at steady state for long periods of time have been valuable research tools for studying nutrient-limited microbial growth. A typical continuous culture apparatus (chemostat) is diagramed in Figure 3 with a listing of pertinent parameters presented in Table 1. In chemostat culture, fresh medium containing a limiting concentration of an essential nutrient (S_o) is pumped at a constant flow rate (F) into a culture vessel of volume (Vol.), causing an equal displacement of the culture. The dilution rate (D) is then equal to F/Vol., with the residence time (τ) being the reciprocal of D. Assuming thorough mixing, the microbial population reaches a biomass (X) causing a corresponding reduction of S_o to S, the limiting nutrient concentration in the culture vessel. Each

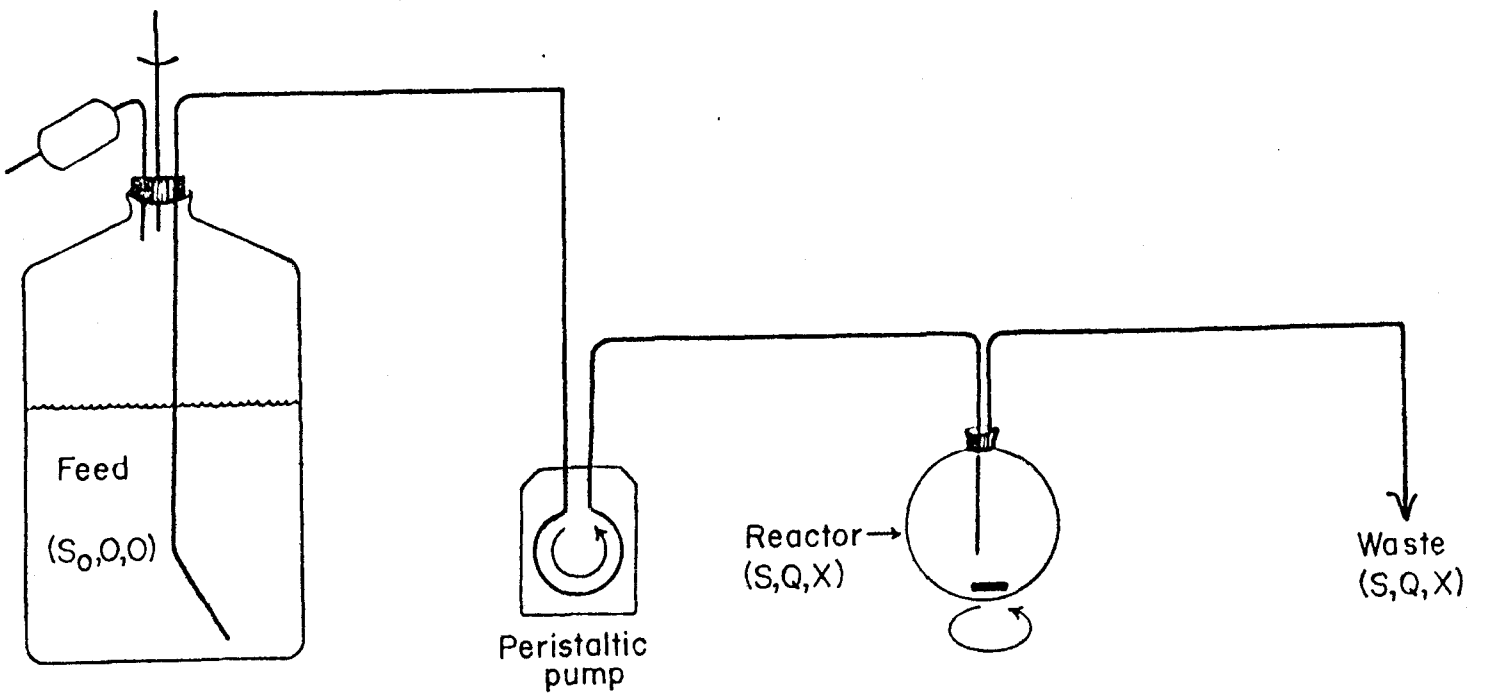

Figure 3. Schematic representation of a typical continuous culture apparatus (chemostat). S_o, influent limiting nutrient concentration; S, ambient limiting nutrient concentration; Q, cell quota; X, cell biomass.

Table 1. Continuous culture parameters.

Symbol	Parameter	Units
F	Flow rate	$l(day)^{-1}$
Vol.	Reactor volume	l
D	Dilution rate (F/Vol.)	day^{-1}
τ	Residence time	day
μ	Growth rate (D)	day^{-1}
S_o	Influent limiting nutrient concentration	nMP
S	Ambient limiting nutrient concentration	nMP
X	Cell biomass	$mg \cdot l^{-1}$
Q	Cell quota	$nMP(mg\ cell)^{-1}$
Y	Cell yield	$\mu g\ cell(nMP)^{-1}$
V	Instantaneous transport rate	$nMP(mg\ cell\text{-}day)^{-1}$
V_n	Net transport rate	$nMP(mg\ cell\text{-}day)^{-1}$
V_t	Gross transport rate	$nMP(mg\ cell\text{-}day)^{-1}$

unit mass of cells, on a population average, contains a quantity of the limiting nutrient, Q, defined as the cell quota.

Mass balance equations can be written for the three variables X, Q and S (Table 2). Under non steady-state (transient) conditions each of these equations are time dependent and thus the relationships on the left side of Table 2 hold. However, at steady-state dX/dt, dS/dt and dQ/dt all equal 0, and thus the relationships on the right side of Table 2 hold. These equations assume no excretion or loss of S by the microbial population. From the conservation equations in Table 2, empirical and descriptive models have been derived which form the framework of established models describing microplankton growth in natural aquatic systems.

EMPIRICAL GROWTH MODELS

Monod (1942) working with bacteria in chemostat culture was the first to show a hyperbolic relationship between specific microbial growth rate and the concentration of an essential growth-rate-limiting nutrient and described it with an expression analogous to the Michaelis-Menten enzyme

Table 2. Mass balance equations for the state variables X, Q and S under transient and steady-state conditions.

transient	steady-state
$dX/dt = \mu X - DX$	$\mu = D$
$dS/dt = D(S_o - S) - VX$	$V = \mu Q$
$dQ/dt = V - \mu Q$	$Q = (S_o - S)/X$

kinetic equation (1):

$$\mu = \frac{\mu_{MAX}\, S}{K_\mu + S} \qquad (5)$$

where μ is the specific growth rate, μ_{MAX} is the maximum value of μ at saturation levels of the essential nutrient, S is the ambient concentration of the growth-rate-limiting nutrient and K_μ is the Monod half saturation constant numerically equal to the substrate concentration at one half μ_{MAX}. In general $K_\mu \neq K_v$. In addition, Monod showed a simple relationship between growth and substrate utilization as shown in the expression:

$$\frac{dX}{dt} = -Y \frac{dS}{dt} \qquad (6)$$

where X is the concentration of organisms and Y is the yield coefficient. Yield is thus the unit mass of cell material produced per unit substrate consumed.

Several authors have used the Monod model to successfully describe the nutrient limited growth of organisms in steady state cultures (Paasche, 1973a, 1973b; Goldman et al, 1974; Swift and Taylor, 1974; Brown and Button, 1979). However, a major drawback with the Monod model is that residual nutrient concentrations must be measured. Analytical techniques for many nutrients are inadequate to accurately assess dilute residual nutrient concentrations. Thus the apparent inability of the Monod relationship to ac-

curately describe the nutrient-limited growth of some species may be due to analytical problems and not the basic relationship itself.

A hyperbolic relationship between specific growth rate and internal cellular nutrient concentration (cell quota; Q) has been described by Droop (1968) and Caperon (1968). The general form of this expression is;

$$\mu = \frac{\mu'_{MAX}\ (Q-K_Q)}{Q} \qquad (7)$$

where μ is the specific growth rate, μ'_{MAX} is an extrapolated μ_{MAX}, Q is the cell quota and K_Q the subsistence cell quota. In general, $\mu_{MAX} \doteq \mu'_{MAX}$ with μ_{MAX} being smaller than μ'_{MAX} by the factor $(1-Q_o/Q_{MAX})$. This expression has been modified by Caperon and Meyer (1972) to include a threshold value for Q (Q_o; minimum cell quota):

$$\mu = \frac{\mu_{MAX}\ (Q-Q_o)}{K_Q + (Q-Q_o)} \qquad (8)$$

Equations 6 and 7 are equilvalent only if $K_Q=Q_o$. These hyperbolic relationships have been used to describe phosphate (Fuhs, 1969; Fuhs et al., 1972; Rhee, 1973; Goldman, 1977; Brown and Button, 1979; Robertson and Button, 1979), nitrate (Rhee, 1974, 1978) and ammonia (Thomas and Dodson, 1972) limited chemostat growth of several microorganisms. A major advantage of this relationship is that residual nutrient concentrations do not have to be assessed.

These empirical models which give $\mu=\mu(S)$ and $\mu=\mu(Q)$ describe rectangular hyperbolas. Linearizations, such as the Lineweaver-Burke plot of the Michaelis-Menten hyperbola, can also be applied. Burmaster (1979) has shown the mathematical equivalence of the two hyperbolic growth rate relationships and the nutrient transport relationship (equation 3) under steady-state conditions, and thus suggests each relationship holds if any single relationship holds.

AFFINITY FOR A NUTRIENT

Empirical models based on the previously described relationships have been used to explain observed microplankton biomass, composition and succession in natural aquatic systems (Dugdale, 1967; Caperon, 1967; Eppley and Thomas, 1969; Hecky and Kilham, 1974; Taylor and Williams, 1975; Titman, 1976; Tilman, 1977; Sharp et al., 1979). Constants, such as the half saturation constants K_v and K_μ, derived from empirical models, have been employed to describe a species' competitive ability for a limiting nutrient. Species with large K_v or K_μ values are assumed less efficient at competing for the growth-rate-limiting nutrient than are species with lower K_v or K_μ values. However, kinetic constants such as K_v and K_μ are only useful for comparisons when the assumptions inherent in the derivation of

the hyperbolic relationship hold (see page12) and the hyperbolic maximums, V_{MAX} and μ_{MAX} are identical for all species (Titman, 1976; Tilman, 1977; Healy, 1980).

Another estimate of an organisms ability to compete for a growth-rate-limiting limiting nutrient is a different kinetic constant called affinity (Button, 1978; Brown and Button, 1979). Affinity for a growth-rate-limiting nutrient in dilute solution is the maximum slope of a plot of transport rate versus the external limiting nutrient concentration. It occurs in the vicinity of the origin for a nutrient starved population (Molot and Brown, 1980). Mathematically it is represnted by the expression:

$$a = \lim_{S \to S_t} \left(\frac{\partial V}{\partial S}\right)_{Q \to Q_o} \qquad (9)$$

where a is the affinity, S is the ambient nutrient concentration , S_t is the threshold value for S below which transport rate=0, V is the transport rate, Q is the cell quota and Q_o is the minimum cell quota. Affinity then represents an organisms ability to sequester a nutrient in dilute solution. Since a family of curves is generated when plotting V versus S for various values of Q (Chen, 1974; Brown and Harris, 1978; Rhee, 1978), the maximum affinity (a_{MAX}) is the maximum slope for the famity of curves. A

good approximation of a is the steady-state affinity (a_s):

$$a_s = \frac{\Delta(\mu Q)}{\Delta(S-S_t)} \qquad (10)$$

(Button, 1978). a_s has been successfully used to predict the outcome of competition in dual-species, constant-light, nutrient-limited chemostats (Brown and Button, submitted).

COMPETITION BETWEEN SPECIES FOR ESSENTIAL NUTRIENTS

Although a_s may accurately represent an organism's ability to sequester an essential growth-rate-limiting nutrient in dilute solution and predict the outcome of steady-state nutrient-limited competition experiments, it will not independently describe competition when transient conditions occur. Under transient conditions, nutrient transport and thus growth strategies may vary among organisms (see page 7). Brown et al. (1978) have shown that transient nutrient transport rates (observed when providing a P-starved population of cells with excess P) can exceed the maximum observed steady-state transport rates (μQ) and thus $a_{MAX} > a_s$. Also, as discussed earlier (see page 7), some species are able to temporarily maintain higher Q values under steady-state conditions ($Q_{MAXt} > Q_{MAXs}$; Fuhs, 1972; Rhee, 1974; Brown and Button, 1979). Because of this, under transient conditions, nutrient storage capacity (represented

by R) may be an important kinetic factor. Thus while a_s when coupled with S_t and μ may best describe the competitive abilities of species under steady-state growth conditions, transient growth may best be described by other growth kinetic parameters.

An underlying assumption inherent to empirical models describing microplankton growth is that growth kinetic descriptions obtained from the study of laboratory cultured species are representative of the growth kinetic descriptions of ecologically important species. The assumption is that kinetic characterizations derived from the study of a single species can be extrapolated to taxonomically closely related species. Species, however, are often not chosen for study because of their importance in natural systems, but rather they are chosen based on their amenability to laboratory culture and experimentation. This is particularly true when continuous culturing techniques are used because if organisms stick to growth vessels or have morphologically distinct life phases, complete growth kinetic descriptions can not be made. In addition, taxonomic relationships among plankton are largely based on morphological similarities and thus are not useful for interpreting any physiological similarities that may exist between species.

Fitting data to empirical steady-state models has en-

hanced our understanding of the relationship between organisms and steady-state levels of essential growth-rate-limiting nutrients. However, they have not yet been successfully applied to descriptions of microplankton growth, biomass and composition in natural aquatic systems. Future experimental work, must to a greater extent, consider the physiological responses of organisms to both steady-state and transient environmental conditions.

MATERIALS AND METHODS

MATERIALS

Microorganisms

The microorganisms used in this study were the blue-green algae Synechococcus Nägeli strains A and B; the green algae Selenastrum capricornutum, Scenedesmus quadricauda and Scenedesmus obliquus; a diatom Navicula pelliculosa; and an aquatic heterotrophic yeast Rhodotorula rubra. Table 3 gives the sources of these organisms. Species were chosen to be representive of the taxonomic range of planktonic species typically found in natural aquatic systems. All species were maintained bacteria free at 4°C in PAAP liquid medium (Bartsch, 1971).

Growth Media

The constituents of the basal medium (Table 4) used for these experiments was that described by Bartsch (1971). This medium was modified for the diatom by the addition of 20 $mg \cdot l^{-1}$ sodium metasilicate and for the yeast by the addition of 100 $mg \cdot l^{-1}$ glucose and 100 $mg \cdot l^{-1}$ $(NH_4)_2SO_4$.

Table 3. Microplankton species examined in this study.

Species	Source
Blue-green algae	
Synechococcus Nägeli (strain A)[1] (formerly *Synechococcus cedrorum*)	UTEX[2] 6908
Synechococcus Nägeli (strain B)[1] (formerly *Anacystis nidulans*)	ATCC[3] 27344
Green algae	
Selenastrum capricornutum	Brown and Button, 1979
Scenedesmus quadricauda (Turp.) Breb.	UTEX 76
Scenedesmus obliquus (Turp.)	UTEX 1450
Diatom	
Navicula pelliculosa (breb) Hilse	UTEX 668
Aquatic yeast	
Rhodotorula rubra	Robertson and Button, 1979

[1] see Rippka et al., 1979

[2] University of Texas Culture Collection; Austin, Texas

[3] American Type Culture Collection; Rockville, Maryland

Table 4. Constituents of PAAP medium.

Nutrient	Reagent Source	Nutrient Concentration
		mg/l
N (nitrogen)	$NaNO_3$	14.00
P	K_2HPO_4	0.62
K	K_2HPO_4	1.56
Mg	$MgCl_2$ $MgSO_4 \cdot 7H_2O$	9.68
S	$MgSO_4 \cdot 7H_2O$	6.37
Ca	$CaCl_3 \cdot 2H_2O$	4.01
C	$NaHCO_3$	100.00
Fe	$FeCl_3$	0.11
B	H_3BO_3	0.11
Mn	$MnCl_2$	0.38
Zn	$ZnCl_2$	0.05
Co	$CoCl_2$	0.0012
Cu	$CuCl_2$	0.00001
Mo	$Na_2MoO_4 \cdot 2H_2O$	0.0096
-	$Na_2EDTA \cdot 2H_2O$	-
N (ammonia)[1]	$(NH_4)_2SO_4$	100.00
-	glucose[1]	100.00
Si[2]	$Na_2SiO_3 \cdot 9H_2O$	20.00

[1] added only for experiments with R. rubra

[2] added only for experiments with N. pelliculosa

For batch culture growth experiments full strength PAAP medium was used. After autoclaving , sterile K_2HPO_4 was added to make the medium either phosphate (1 nMP) or nitrogen (20 nMP) limited. Sterile carrier-free orthophosphate (^{32}Pi) was also added to the medium after autoclaving.

For the dilute biomass (0.5-15 mg dry wgt$\cdot l^{-1}$) growth experiments utilizing continuous culturing techniques, a modified PAAP medium was used. The medium was prepared by adding 20% strength phosphate-free PAAP medium to a 20 l pre-conditioned (Brown et al, 1978) glass carboy containing 0.6 g$\cdot l^{-1}$ tris (hydroxymethyl) aminomethane buffer dissolved in 6.5 ml concentrated hydrochloric acid. Prior to autoclaving, K_2HPO_4 was added to bring the Pi concentration to 964 nMP. After autoclaving, the medium was allowed to cool and a sterile solution of $NaHCO_3$ was added to yield a final concentration of 0.1 g$\cdot l^{-1}$. Sterile deionized water was then used to replace any water evaporated during autoclaving. Finally, 1-5 microcuries of sterile carrier free ^{32}Pi was added to the medium reservoir.

Radiophosphorus

Radioactive H_3PO_4 was obtained in 2-5 millicurie quantities from the Amersham Corporation, Des Plains, Illinois

(catalogue #PBS.11A) as carrier free radiolabelled orthophosphate (^{32}Pi). The radiochemical purity is reported to be greater than 98% as ^{32}Pi and less than 1% as pyrophosphate or polyphosphate. The ^{32}Pi was stored frozen until use.

METHODS

Culture Methods

For the batch culture experiments, logarithmically growing cells were inoculated into pre-conditioned 500 ml Erlenmeyer flasks containing either phosphorus or nitrogen limited media. The cells were grown at 25°C under constant illumination of approximately 1.0×10^{16} quanta$\cdot s^{-1} \cdot cm^{-2}$ provided by cool white fluorescent lights.

The continuous culture apparatus used for the dilute biomass experiments consisted of a one-phase system previously described (Brown and Button,1979). All continuous culture experiments were conducted at 25°C under constant illumination of 2.4×10^{16} quanta $s^{-1} \cdot cm^{-2}$ (as measured inside the culture vessel containing a typical dilute biomass concentration) provided by cool white fluorescent lights. The culture vessel was a 500 ml preconditioned, round-bottomed, rubbered-stoppered glass

boiling flask. 5-10 ml of batch culture log-phase cells (2 X 10^6 cells/ml) were inoculated into 100 ml of fresh medium. A peristaltic pump was then started and the vessel filled to begin continuous flow cultivation.

All experiments were performed under aseptic conditions using axenic cultures. Cultures were periodically checked for bacterial contamination by phase and epifluorescent microscopic examination and by plating onto nutrient agar.

Sampling

To sample the batch cultures 5-10 ml were aseptically removed. Immediately after removal, a portion of the sample was filtered through a 0.22 micrometer (μm) membrane filter to effect rapid separation of the cells from the medium. A portion of the filtrate and a non-filtered portion were analyzed immediately for biomass (non-filtered) and phosphorus (both) analyses.

Due to the large size variation among the organisms studied, different sampling techniques had to be employed to ensure rapid separation of the cells from the medium in the continuous culture experiments. For S. Nägeli strains A and B and S. obliquus the sampling procedure was identical to that used by Brown and Button (1979), except that 0.22 μm membrane filters were used to separate cells from the

medium. S. quadricauda was sampled by clamping off the continuous culture effulent line and quickly removing 15-20 ml of culture using a sterile syringe. Immediately after removal, 10 ml of the sample were filtered through a 0.22 µm membrane filter of which 8 ml of the filtrate were frozen for later phosphorus analyses. A portion of the remaining filtrate and a non-filtered portion were immediately analyzed for biomass (non-filtered) and phosphorus (both) concentrations.

Biomass Analysis

Approximately 10 ml of a non-filtered sample were needed for each biomass analysis. Cell number and median cell volume were determined using an electronic particle counter and sizer. Dry weights were estimated for each species from dry weight conversion factors calculated from conventionally determined dry weights (membrane filtered cells were dried 24 hr at 65°C under 15 inches mercury vacuum; the weight of the dried filter was measured before and after filtering), and then applying dry weight conversion factors (presented in Table 5) for each species.

Phosphorus Analyses

Total P (P_t) and solution P (P_{sol}) were determined by

Table 5. Dry weight conversion factors.

Species	Conversion Factor
	$mg(\mu m^3\ cell)^{-9}$
Blue-green algae	
S. Nägeli (strain A)	0.52
S. Nägeli (strain B)	0.55
Green algae	
S. capricornutum	0.33[1]
S. quadricauda	0.33
S. obliquus	0.23
Diatom	
N. pelliculosa	0.80[2]
Aquatic yeast	
R. rubra	0.41

[1] Brown and Button, 1979

[2] Sullivan, 1979

adding 1 ml of non-filtered and filtrate samples respectively to triton-X toluene scintillation cocktails and counting the radiophosphorus label with a liquid scintillation counter. Since the ^{32}Pi added was carrier free, the percent ^{32}P in any fraction reflected the percent ^{32}P in that fraction (Kuenzler and Ketchum,1962). Cell phosphorus (P_{cell}) was determined as the difference of $P_t - P_{sol}$ since $P_t = P_{sol} + P_{cell}$.

For the continuous culture experiments, P_{sol} was further separated into molybdate reactive and molybdate unreactive fractions by treating 2 ml of the 0.22 µm filtrate with 1.8 mg ammonium molybdate in 0.2 ml of 0.2 N H_2SO_4 containing 0.04 mg antimony potassium tartrate (catalyst). The molybdate reactive fraction was partioned into isoamyl alcohol. Radioactivity in untreated filtrate (P_{sol}), in three combined isoamyl alcohol extracts containing the molybdate reactive complex (Pi), and in the aqueous phase containing the unreactive fractions (P_{nex}), were analyzed as above. Since the radiochemical purity was <2% polyphosphate and pyrophosphate combined, when P_{sol} was <2%, P_{nex} could not be measured accurately. This is because any polyphosphate and pyrophosphate contaminants not hyrolyzed by organisms would remain in the unreactive fraction.

Pi concentrations of continuous culture media were also colorimetrically determined using a modified (Eisenreich,

1975) Murphy and Riley (1962) technique at the completion of each experiment to ensure that feed Pi levels remained chemically stable for the entire experiment (up to 6 months).

Kinetic Measurements

Batch culture parameters

The following growth kinetic parameters were determined by growing the organisms in batch culture; apparent maximum growth rate (μ_{MAX}), maximum and minimum cellular phosphorus concentrations (Q_{MAXt} and Q_o respectively) and the coefficient of luxury consumption (R). The apparent μ_{MAX} was determined using regression analysis of exponential (ln) increase of cell dry weight versus time. Cell quota (Q) is a measure of the nutrient (e.g., phosphorus) content per cell dry weight (Droop,1968). Q_{MAX} was determined as the maximum Q measured during early-logarithmic growth of nitrogen-limited cells (Q_{MAXt}). Q_o was determined as the minimum Q measured in stationary phase cells growing in phosphorus-limited batch cultures. R was determined from the ratio of Q_{MAXt} to Q_o (Tilman and Kilham,1976).

Continuous culture parameters

Considering the population of cells within the culture

vessel rather than individual cells, the dilution rate (D) represents the specific growth rate (μ; Novick and Szilard, 1950). At steady state, the net specific Pi transport rate (V_n) is equal to the product of μQ. Q_{MAXs} was determined from the maximum Q expected (by extrapolation) at μ_{MAX} when Pi was the growth-rate-limiting factor. A complete listing and description of pertinant continuous culture parameters is given in Table 1.

RESULTS

BATCH CULTURE

Table 6 shows apparent maximum growth rates (μ_{MAX}), maximum and minimum cellular phosphorus concentrations (Q_{MAXt} and Q_o respectively), coefficients of luxury consumption (R) and median cell volume ranges for all of the microplankton species studied. The apparent μ_{MAX} represents the highest observed growth rate for each species studied. μ_{MAX} was calculated from the slope of the ln increase of dry weight versus time and not the slope of the ln increase of cell numbers versus time. Calculations of cell quota were also based on dry weights rather than cell number (see Droop, 1979). Q_{MAXt} is the maximum value of Q under transient conditions when P is not growth-rate-limiting while Q_o is the minimum value of Q when P is growth-rate-limiting. Figure 4 shows the relationship between Q and time after inoculation for both phosphorus-limited and nitrogen-limited batch cultures of S. Nägeli (strain A). Results from batch cultures of all other organisms studied were similar to Figure 4. Table 6 also gives coefficients of luxury consumption (the ratio of Q_{MAXt} to Q_o) which represent each

Table 6. Batch (transient) culture growth kinetic parameters.

Species	Apparent μ_{MAX}	Q_{MAXt}	Q_o	R	Median Volume Range
	day^{-1}	nMP(mg cell)$^{-1}$	nMP(mg cell)$^{-1}$	-	μm^3
Blue-green algae					
S. Nägeli (strain A)	1.0	987	14	70.5	1-3
S. Nägeli (strain B)	1.4	950	15	63.0	1-3
Green algae					
S. capricornutum	1.2[1]	2260[1]	40[1]	56.5	50-130
S. quadricauda	2.0	960	100	9.6	850-1400
S. obliquus	0.6	2433	173	14.0	50-130
Diatom					
N. pelliculosa	1.6	6873	124	55.0	20-80
Aquatic yeast					
R. rubra	5.8[2]	1397	145	9.6	30-80

[1] Brown and Button, 1979

[2] Robertson and Button, 1979

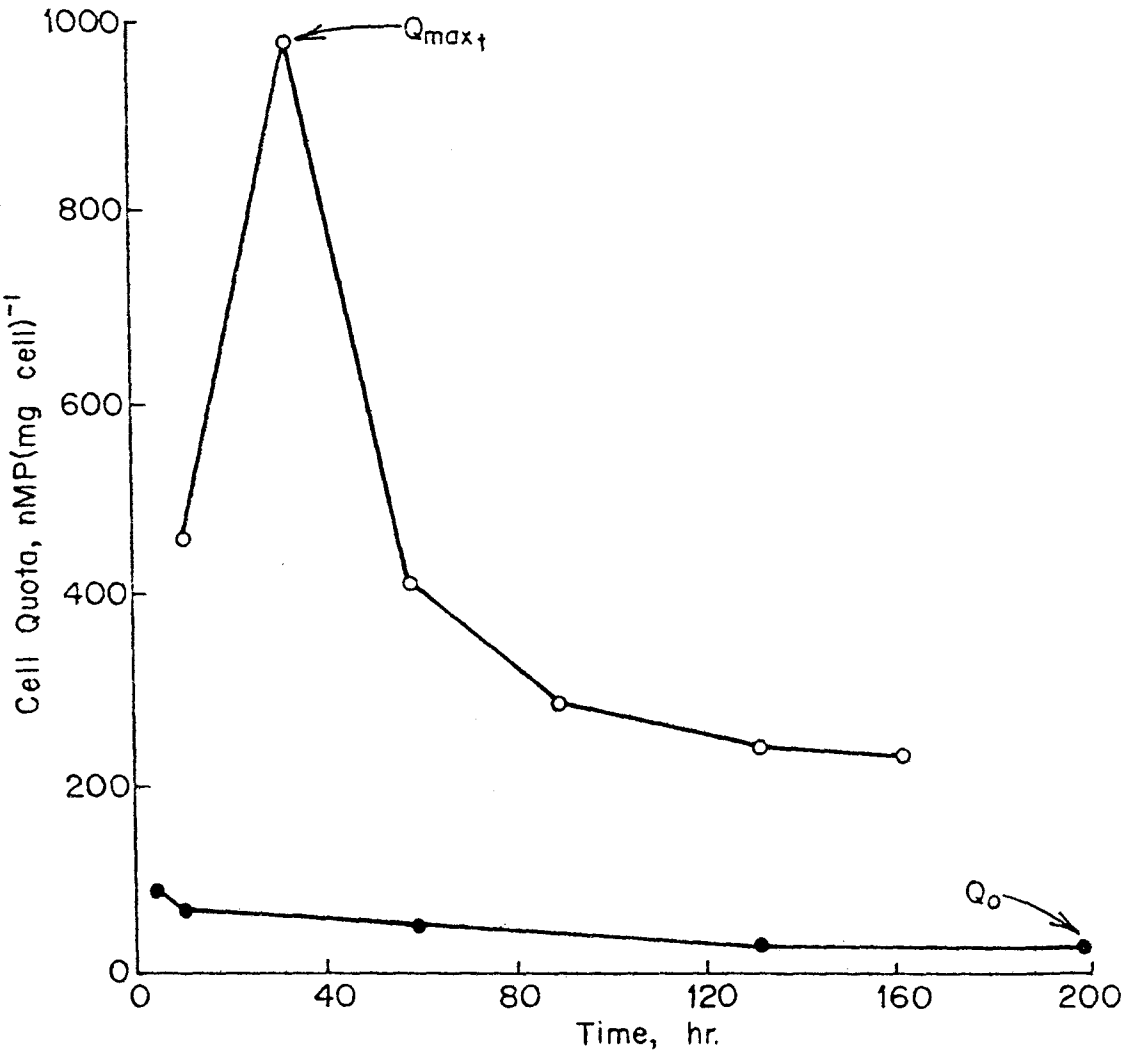

Figure 4. Phosphorus cell quota as a function of time for S. Nägeli (strain A) in nitrogen-limited (o——o) and phosphorus-limited (•——•) batch cultures.

organism's ability to transport and store P above its minimum requirements (Tilman and Kilham, 1976). Cell volume is known to be variable depending on many environmental conditions such as degree of nutrient limitation, growth rate and phase of growth cycle (Robertson and Button, 1979). The observed cell volume ranges for each species are given in Table 6.

CONTINUOUS CULTURE

Kinetic Descriptions of S. Nägeli(strain A) and S. quadricauda

Figures 5 and 6 show the relationships between external phosphate (Pi) concentration and growth rate (D) for S. Nägeli (strain A) and S. quadricauda respectively. External Pi concentrations of 4 nMP for S. Nägeli (strain A) and 138 nMP for S. quadricauda occured at one half the apparent μ_{MAX}.

Growth rate has also been related to Q and cell yield (Y; Droop, 1968; Button et al., 1973; Rhee, 1973; Chen, 1974). The relationships between Q and dilution rate and Y and dilution rate for S. Nägeli (strain A) and S. quadricauda are given in Figures 7 and 8 and Figures 9 and 10 respectively. Cell yield is defined as the unit mass

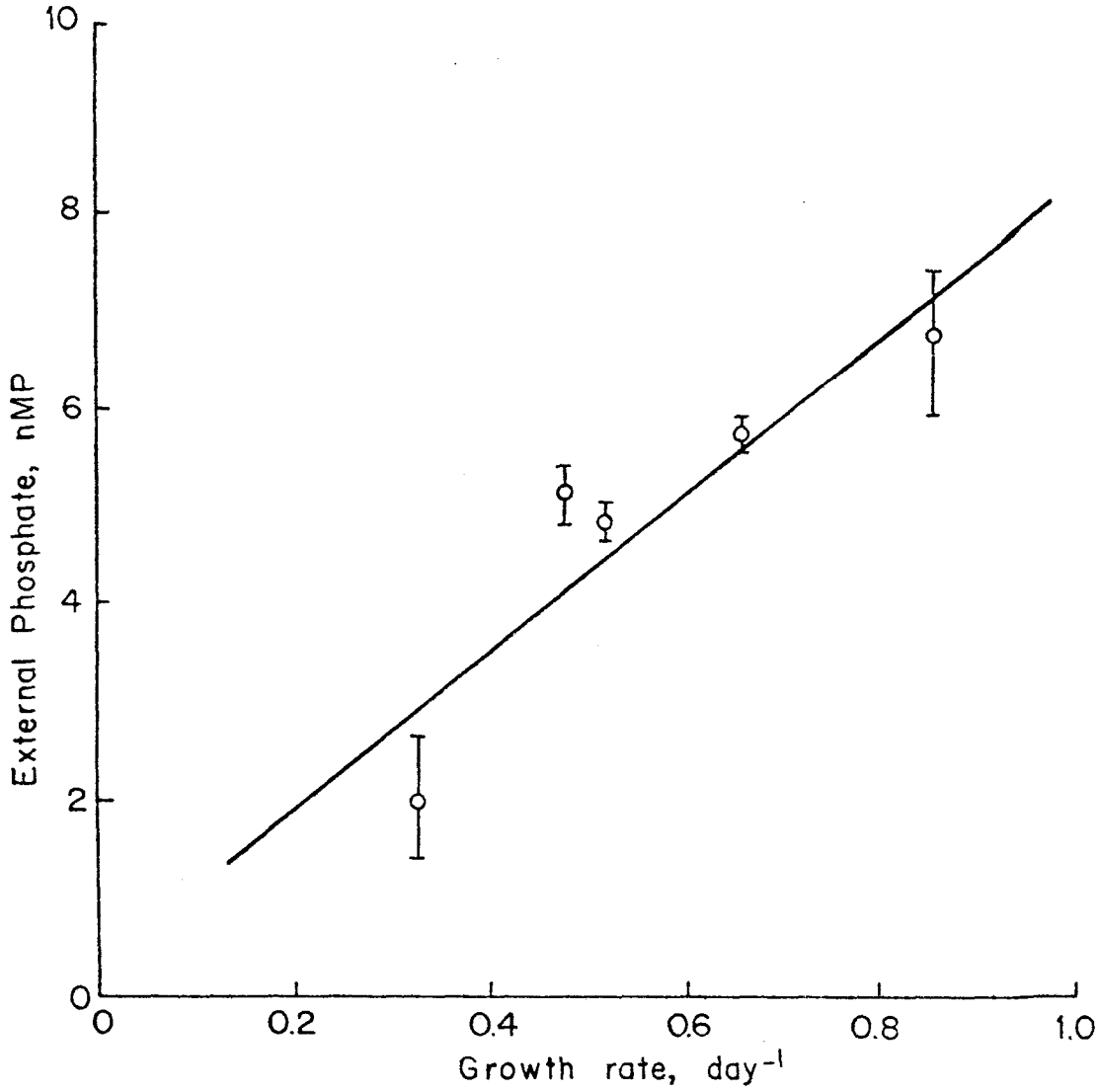

Figure 5. Steady-state external phosphate concentration as a function of growth rate (μ) for S. Nägeli (strain A).

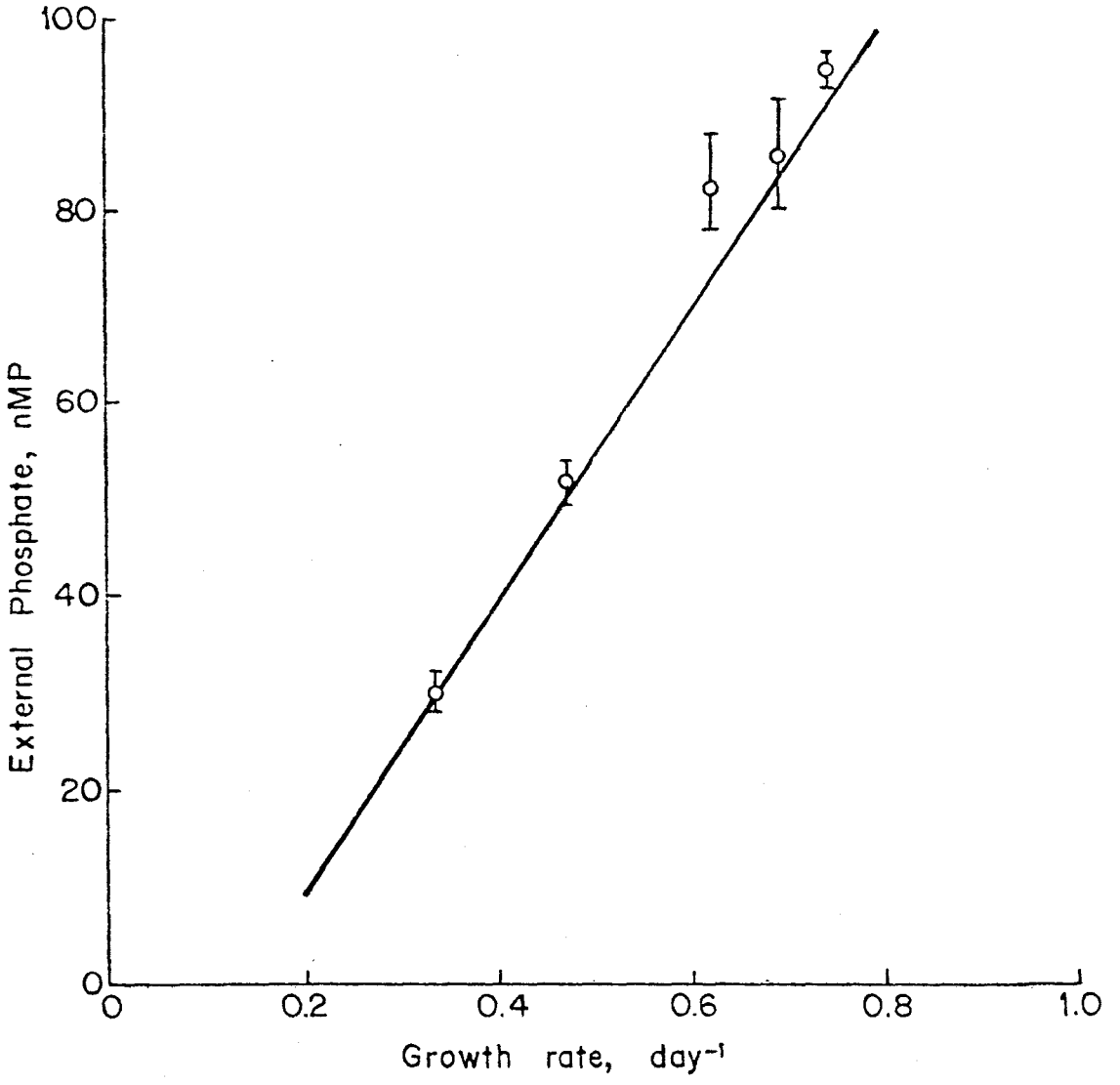

Figure 6. Steady-state external phosphate concentration as a function of growth rate (μ) for S. quadricauda.

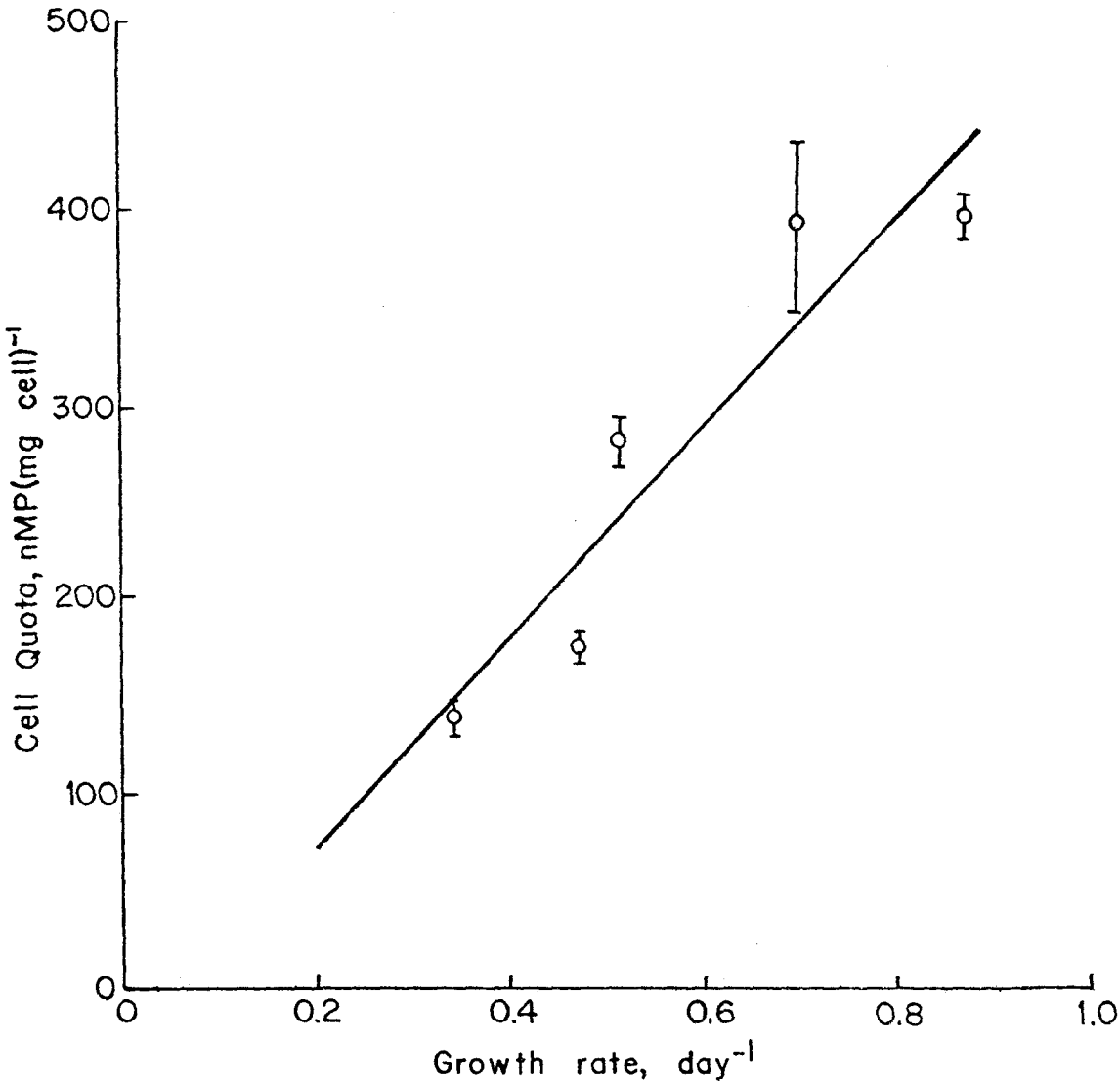

Figure 7. Steady-state cell quota as a function of growth rate (μ) for S. Nägeli (strain A).

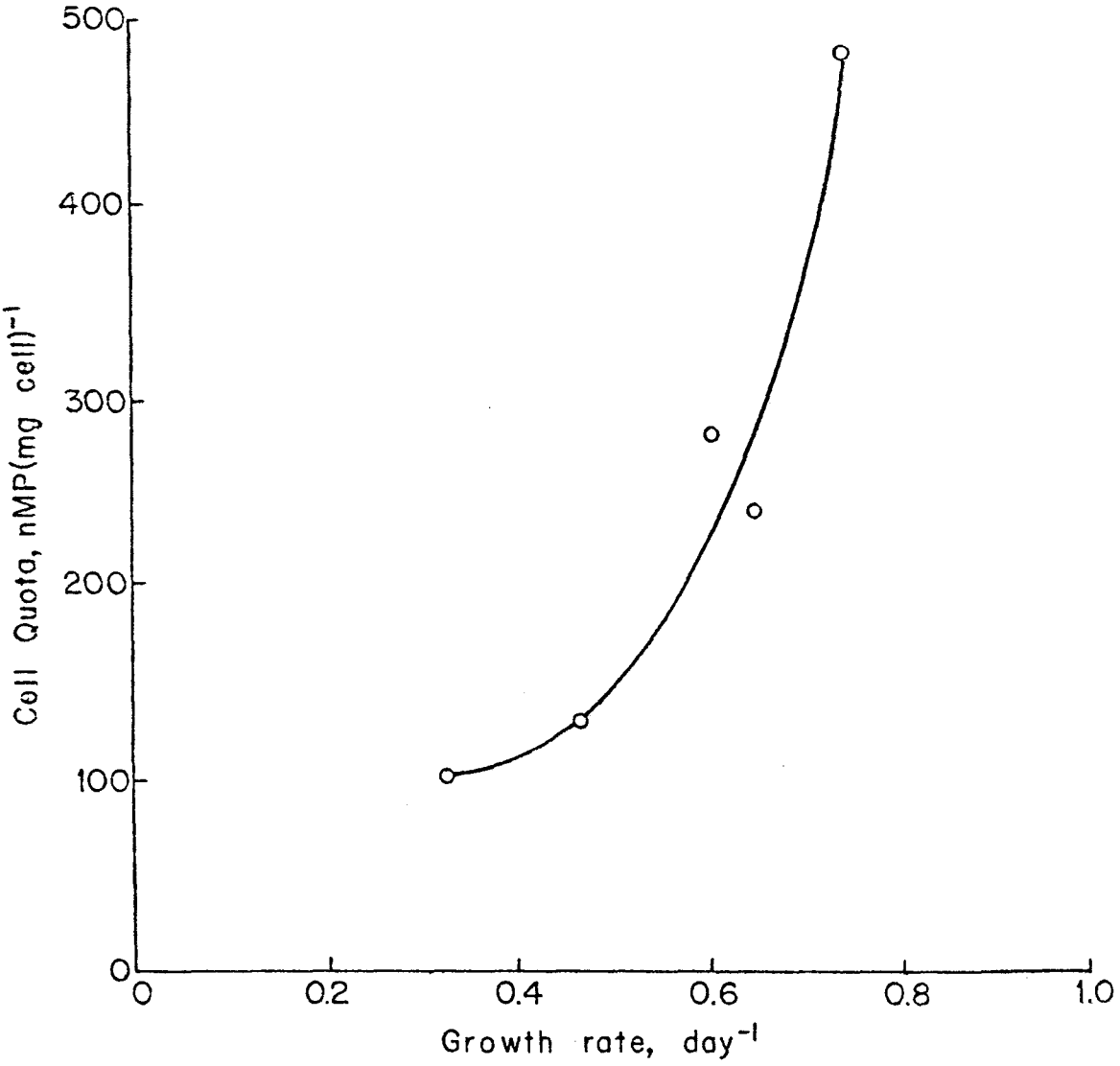

Figure 8. Steady-state cell quota as a function of growth rate (μ) for S. quadricauda. Error bars fall within diameter of points.

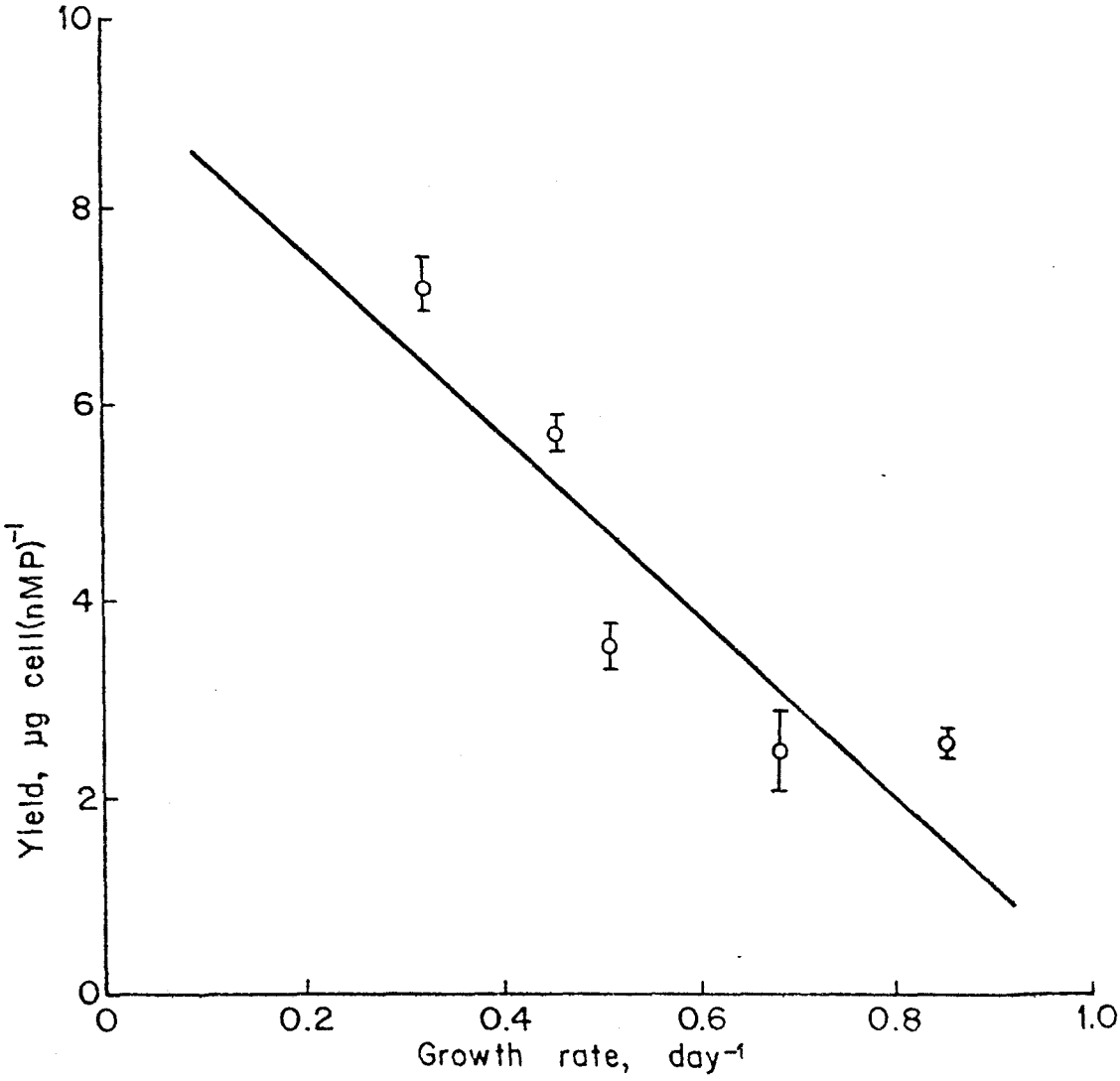

Figure 9. Steady-state cell yield as a function of growth rate (μ) for S. Nägeli (strain A).

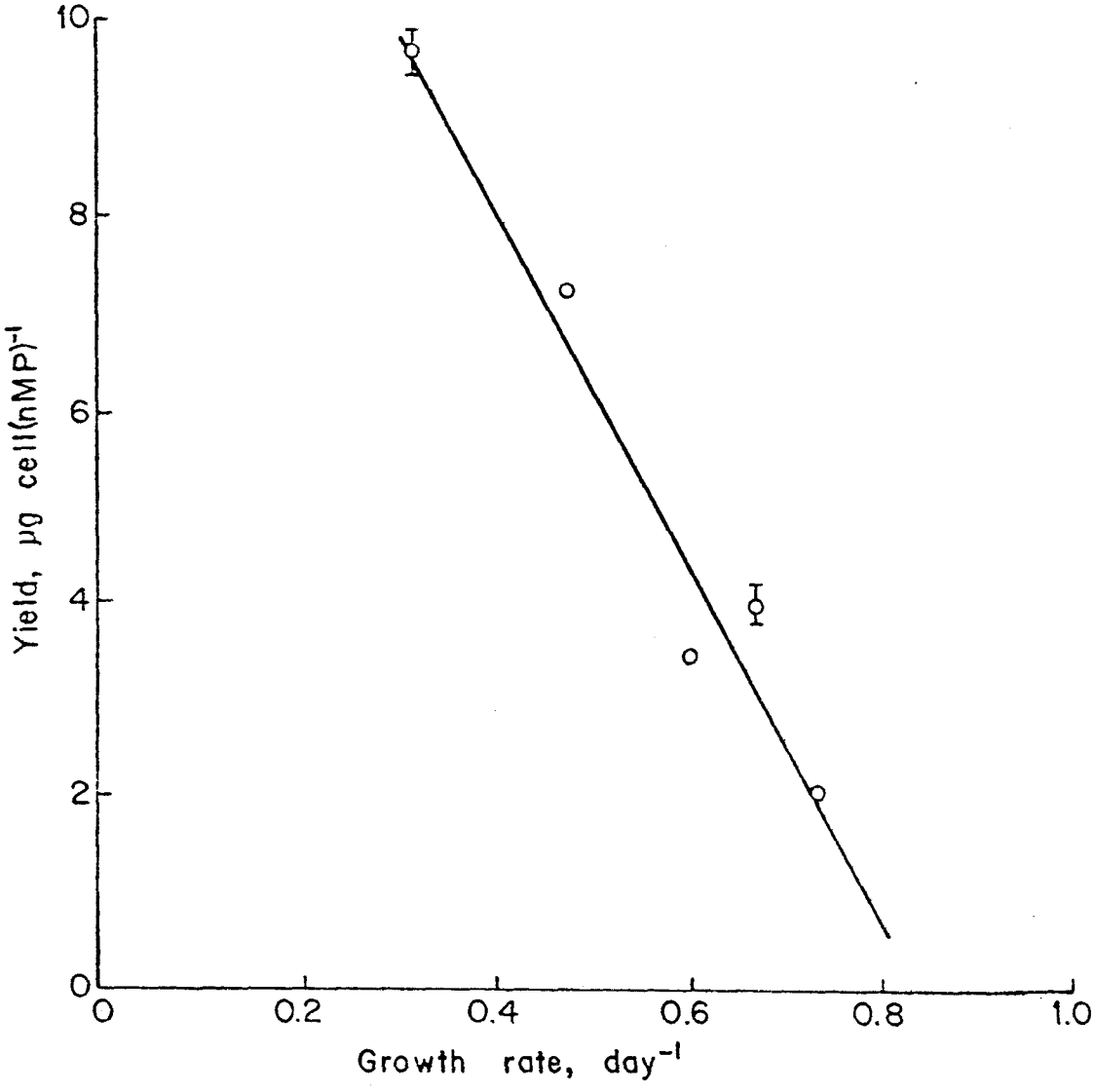

Figure 10. Steady-state cell yield as a function of growth rate (μ) for S. quadricauda. Error bars fall within diameter of points unless otherwise noted.

of cell matter produced per P consumed and is mathematically defined as (van Uden, 1969):

$$Y = \frac{X}{(P_t - Pi)} \tag{11}$$

where X is the unit mass of cell material (dry weight). Since the reciprocal of equation (9) gives:

$$\frac{1}{Y} = \frac{P_t}{X} - \frac{Pi}{X} \tag{12}$$

and since:

$$Q = \frac{P_{cell}}{X} = \frac{P_t}{X} - \frac{Pi}{X} - \frac{P_{nex}}{X} \tag{13}$$

then:

$$\frac{1}{Y} = Q + \frac{P_{nex}}{X} \tag{14}$$

Excreted P products on a dry weight basis never exceeded 4% of Q for both organisms and thus within experimental error (which, because of possible ^{32}P contamination, could have ranged from 15-100% of solution P depending on D), cell quota is equal to reciprocal cell yield.

The relationship between net steady-state transport rate (V_n) and Q is shown in Figures 11 and 12 for both species. Steady-state affinities for P can be appoximated from the slopes of the plots in Figures 11 and 12. These steady-state affinities are 58 l(mg cell-day)$^{-1}$ for S. Nägeli (strain A) and 4 l(mg cell-day)$^{-1}$ for

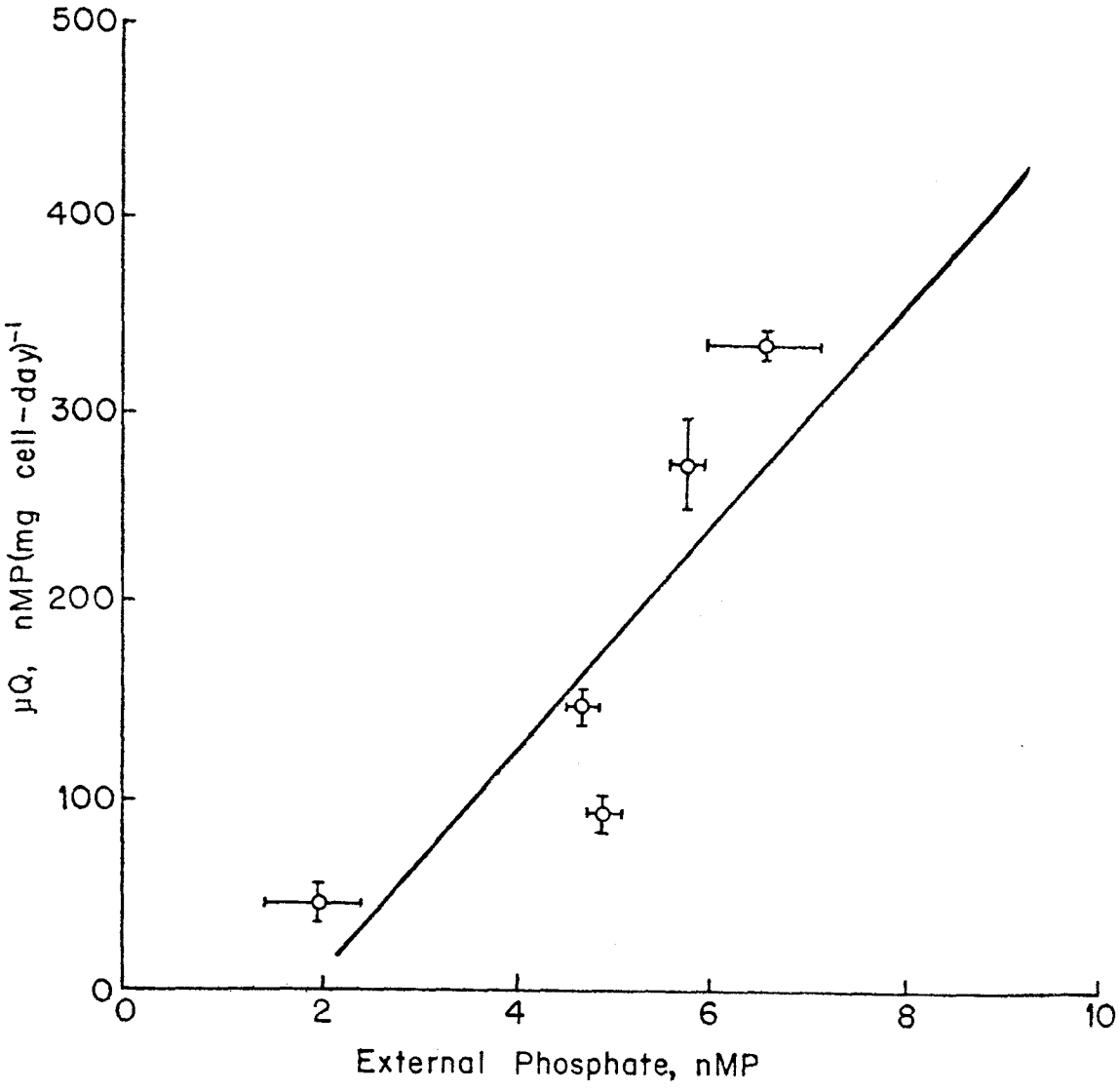

Figure 11. Net steady-state transport rate (μQ) as a function of external phosphate concentration for S. Nägeli (strain A).

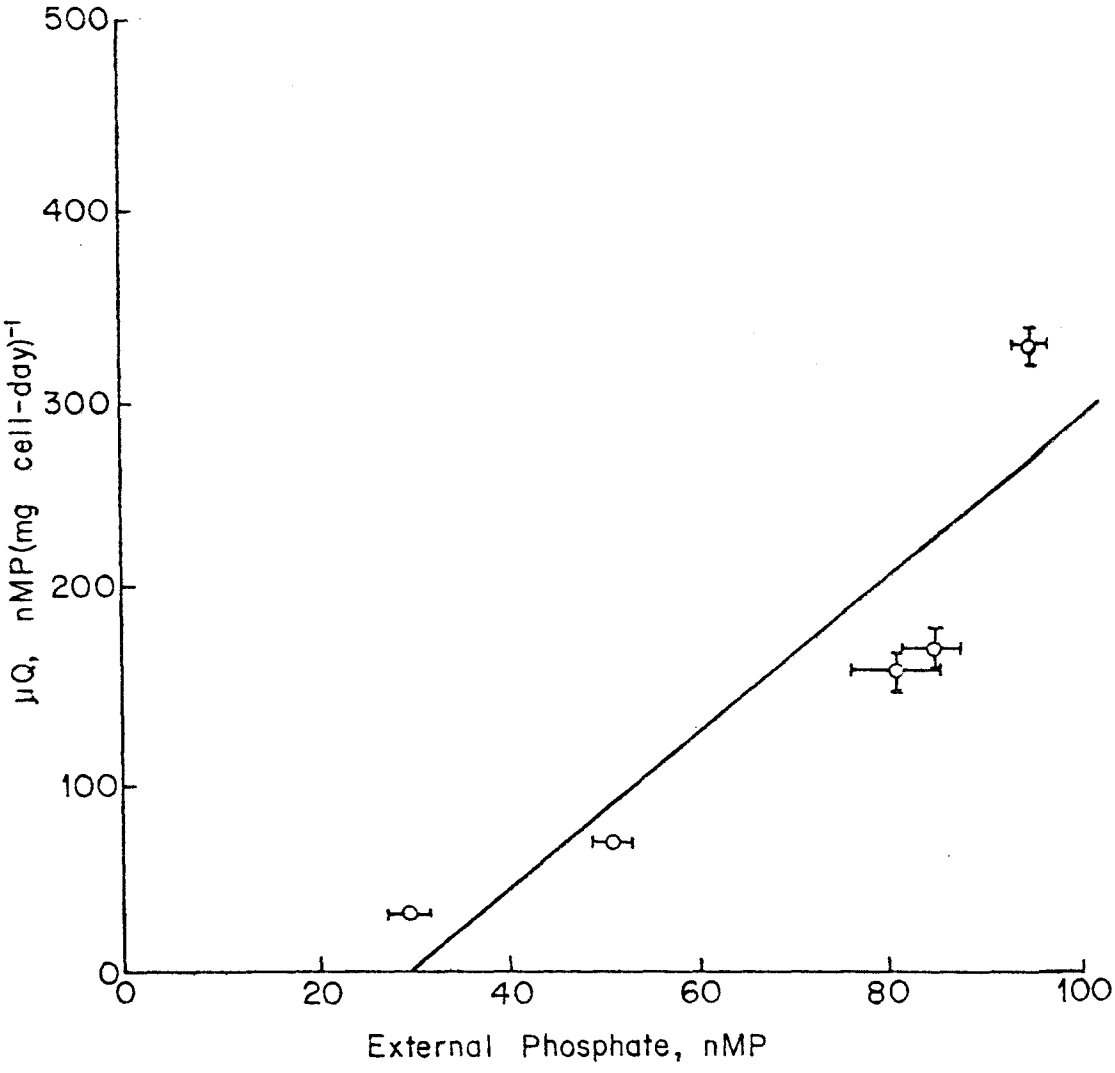

Figure 12. Net steady-state transport rate (μQ) as a function of external phosphate concentration for S. quadricauda.

S. quadricauda. Extrapolations of the lines presented in Figures 11 and 12 yield concentration intercepts. These intercepts give Pi thesholds (Pi concentrations below which $V_n=0$). These threshold values are 30.0 nMP and 1.8 nMP for S. quadricauda and S. Nägeli (strain A) respectively.

The gross transport rate (V_t) at steady-state is:

$$V_t = \mu\left(\frac{P_t-Pi}{X}\right) \tag{15}$$

As previously shown, the net transport rate (V_n) is equal to μQ at steady-state so that:

$$V_n = \mu Q = \mu\left(\frac{P_{cell}}{X}\right) \tag{16}$$

and since:

$$\frac{P_{cell}}{X} = \frac{P_t-Pi-P_{nex}}{X} \tag{17}$$

then:

$$V_n = \mu\left(\frac{P_t}{X} - \frac{Pi}{X} - \frac{P_{nex}}{X}\right) \tag{18}$$

As discussed earlier, P_{nex}/X is neglible for both species, therefor, within experimental error, V_t is approximately equal to V_n at steady state. This agrees with the data for the green alga Selenastrum capricornutum (Brown and Button, 1979). However, V_t may not be equivalent to V_n for all organisms or for all nutrients when excreted products are significant (Robertson and Button, 1979).

The relationships between μQ and Q are presented in Figures 13 and 14 for both species. Such relationships have been used to describe nutrient-limited growth kinetics when the external concentration of the growth-rate-limiting nutrient cannot be measured (see Droop, 1968). The X intercept is a kinetic parameter called Q_o (Droop, 1968). The X intercepts are 98 nMP(mg cell)$^{-1}$ and 80 nMP(mg cell)$^{-1}$ for S. Nägeli (strain A) and S. quadricauda respectively.

Kinetic Data for S. Nägeli (strain B), S. obliquus and S. carpricornutum.

Table 7 shows kinetic parameters obtained from growing S. Nägeli (strain B) and S. obliquus at several growth rates. Neither species could be completely described in this study due to their specific morphological characteristics. S. Nägeli (strain B) adheres to reactor vessels and S. obliquus exhibits a sexual life cycle. These traits complicate accurate assessments of biomass and maintenance of steady states. If P_{nex}/X is negligble for both S. Nägeli (strain B) and S. obliquus, V_t is approximately equal to V_n for both species. The steady-state affinity for S. Nägeli (strain B), based on μQ versus Pi data for three growth rates is 38 l(mg cell-day)$^{-1}$ with a correlation coefficient of 0.97. Since kinetic data for only a single growth rate

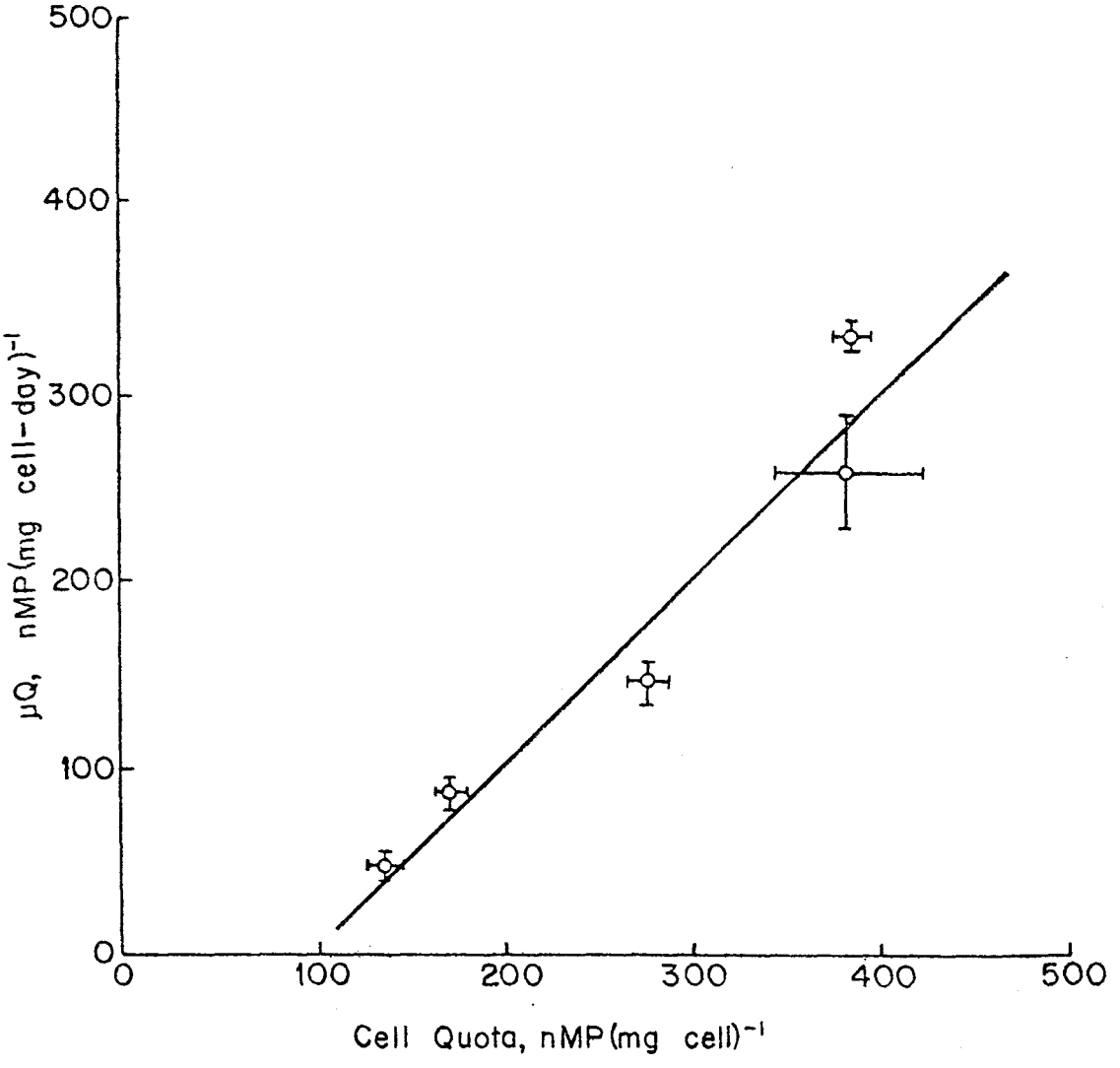

Figure 13. Net steady-state transport rate (μQ) as a function of cell quota (Q) for S. Nägeli (strain A).

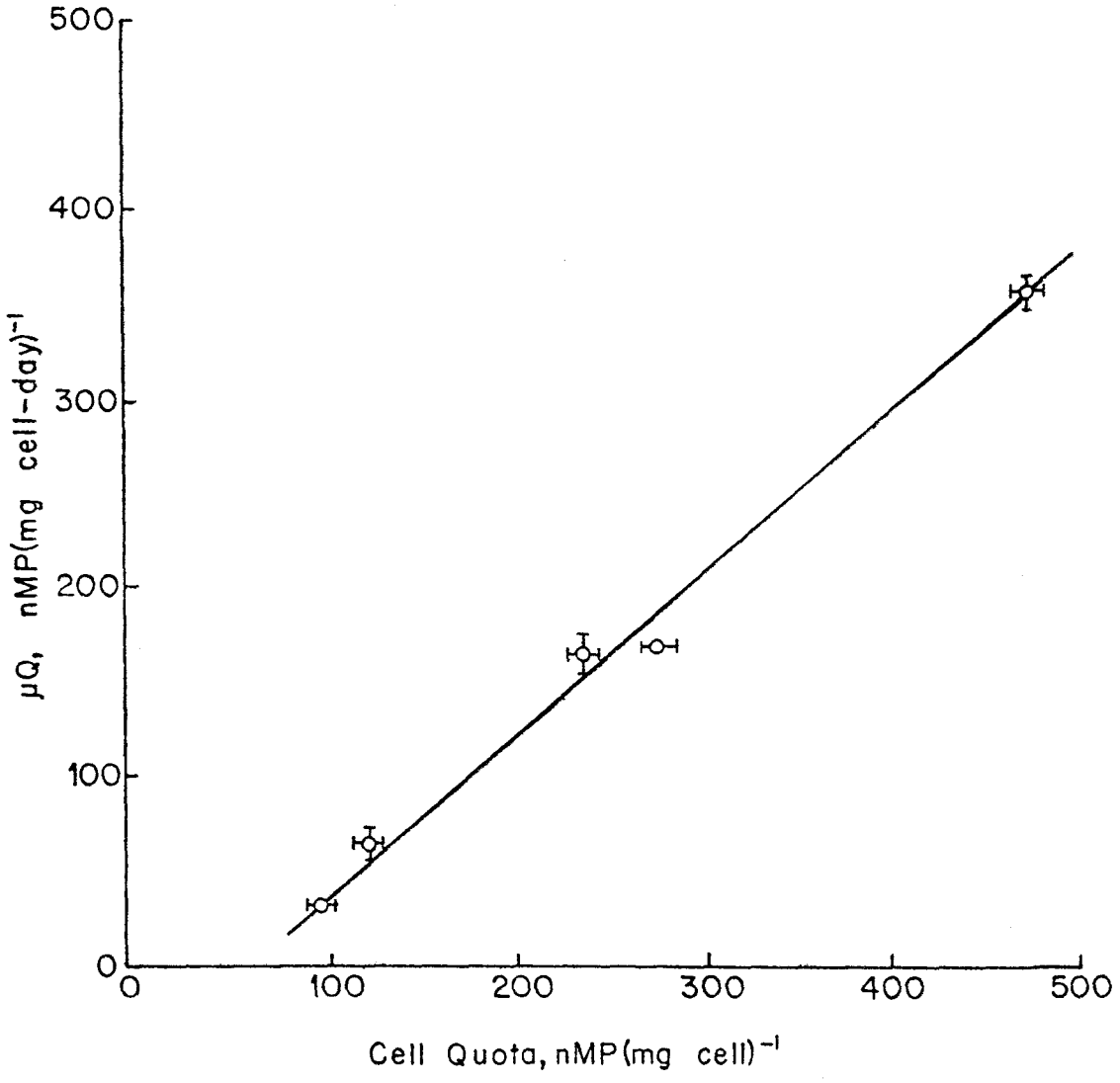

Figure 14. Net steady-state transport rate (μQ) as a function of cell quota (Q) for S. quadricauda.

Table 7. Steady-state growth kinetic parameters for S. Nägeli (strain B) and S. obliquus.

Parameter	Units	S. Nägeli (strain B)			S. obliquus
μ	day^{-1}	0.336	0.392	0.432	0.420
P_t	nMP	500.00	500.00	1000.00	964.00
P_s	nMP	9.95	5.35	14.63	5.10
Pi	nMP	2.39	2.41	4.32	2.75
P_{nex}	nMP	7.56	2.94	10.32	2.35
P_{cell}	nMP	490.05	494.65	985.40	958.90
X	$mg \cdot l^{-1}$	3.00	3.10	3.40	6.76
Q	nMP(mg cell)$^{-1}$	116.50	160.00	289.80	142.00
Y	μg cell(nMP)$^{-1}$	0.0060	0.0063	0.0034	0.0070
V_n (μQ)	nMP(mg cell-day)$^{-1}$	55.90	62.30	125.20	59.64
V_t	nMP(mg cell-day)$^{-1}$	70.20	63.10	126.50	59.72

were obtained for S. obliquus, a steady-state affinity could not be computed. However, a minimum measure of this organism's affinity was estimated from $\mu Q/S$. For S. obliquus $\mu Q/S$ is 22.7 l(mq cell-day)$^{-1}$ at μ=0.42 day^{-1}.

Steady-state growth kinetic data were also obtained at one growth rate for both carbon (bicarbonate; μ=0.46 day^{-1}) and nitrogen (nitrate; μ=0.69 day^{-1}) limited S. capricornutum continuous cultures. ^{32}P labelled Pi was used in these experiments, thus phosphorus cell quota (Q_p) could be monitered. The values for Q_p under steady-state carbon and nitrogen limited growth are 208 and 452 nMP(mg-cell)$^{-1}$ respectively.

Competition in Continuous Culture

Figure 15 shows the results of a dual species Pi-limited continuous culture of S. Nägeli (strain A) and S. quadricauda. The experiment was begun by injecting S. quadricauda into a continuous culture of S. Nägeli (strain A) at a growth rate of 0.336 day^{-1}. The S. Nägeli (strain A) was not maintaining a stable population and was declining in biomass. The first response observed was a biomass increase in S. quadricauda indicating no inhibition from the low undectable biomass of S. Nägeli (strain A). However, as predicted by the steady-state growth descrip-

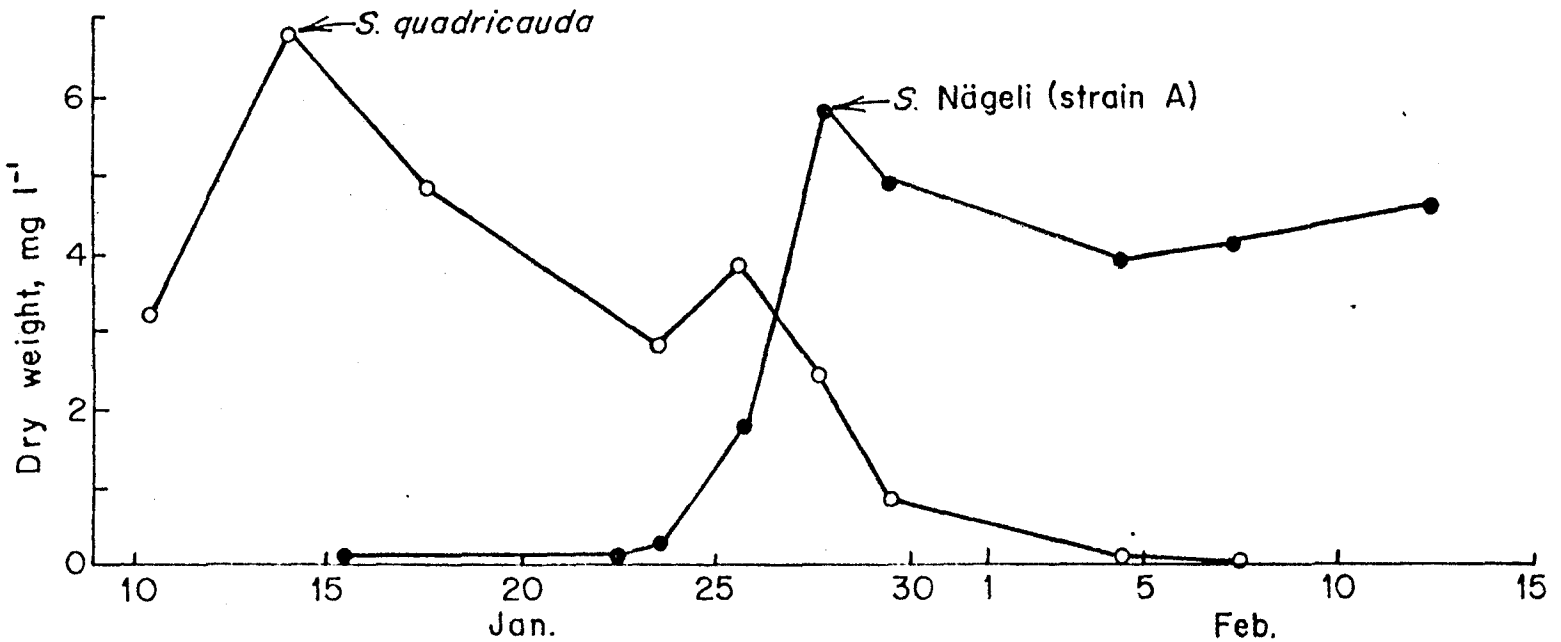

Figure 15. Cell dry weights as a function of time for a Pi-limited, dual-species continuous culture of S. Nageli (strain A) (•—•) and S. quadricauda (o—o).

tions of both organisms, S. Nägeli (strain A) eventually displaced S. quadricauda. The original declining biomass of S. Nägeli (strain A) may have been caused by either an inhibiting factor in the vessel which was removed by S. quadricauda or an essential micronutrient provided by S. quadricauda. Neither possibility was tested.

DISCUSSION

CONTINUOUS CULTURE

The continuous culture results give kinetic descriptions of the steady-state Pi-limited growth of S. Nägeli (strain A) and S. quadricauda and some kinetic information about S. Nägeli (strain B) and S. obliquus. Since empirical kinetic models are frequently used to describe the type of data reported in this thesis, the Monod (1942) model and the modified cell quota model of Droop (Caperon and Meyer, 1972) were both used to describe the steady-state Pi-limited growth of S. Nägeli (strain A) and S. quadricauda. Table 8 shows the calculated growth kinetic parameters computed by data fit to both the Monod and modified Droop (Caperon and Meyer, 1972) models. Both models provided a fair description of the steady-state growth of these two species. It was not possible to use these models to describe the limited amount of data from S. Nägeli (strain B) and S. obliquus continuous cultures.

Affinity (a; not half saturation constant) is an index of the substrate collection ability of microorganisms. The steady-state affinity (a_s) is a good approximation of

Table 8. Computed growth kinetic parameters for S. Nägeli (strain A) and S. quadricauda.

Species	Monod Model		Modified Droop Model*	
	K_μ	μ_{MAX}	k_Q	μ'_{MAX}
	nMP	day^{-1}	nMP	day^{-1}
S. Nägeli (strain A)	8.1	1.54	74.0	0.90
S. quadricauda	126.80	1.70	27.0	0.75

* based on Q_o values calculated via extrapolation from Figures 13 and 14

affinity for a growth-rate-limiting nutrient (Law and Button, 1978). The steady-state Pi affinities for S. Nägeli (strain A) and S. quadricauda are 58 l(mg cell-day)$^{-1}$ and 4 l(mg cell-day)$^{-1}$ respectively. An estimate of a_s for Pi for S. Nägeli (strain B) based on three steady-state growth rates is 38 l(mg cell-day)$^{-1}$ (r=0.97). Since kinetic data for only a single growth rate was obtained for S. obliquus, a value for a_s for Pi could not be computed. However, an approximation was calculated from $\mu Q/S$ for the measured growth rate (μ=0.42 day^{-1}). This value is 22.7 l(mg cell-day)$^{-1}$. The a_s values for Pi found for these organisms compare with reported a values for Pi of 2.8 l(mg cell-day)$^{-1}$ and 30.7 l(mg cell-day)$^{-1}$ for the green alga S. capricornutum (Brown and Button, 1979) and the aquatic heterotrophic yeast R. rubra (Robertson and Button, 1979) respectively. When growing in mixed populations the microplankton species with the highest a_s for the growth-rate-limiting nutrient should exclude its competitors if steady-state conditions exist. Dual species continuous culture experiments have confirmed that this does occur (E. J. Brown, unpublished data).

Phosphorus cell quota was measured in two continuous culture experiments with S. capricornutum when P was not the growth-rate-limiting factor. When carbon (bicarbonate) and nitrogen (nitrate) limited growth, Q for P (Q_p) approximated

the Q_{MAXs} for P found by Brown and Button (1979). Under these conditions, Q_p did not approach Q_{MAXt} for P. This suggests that neither carbon nor nitrogen limitation has a significant effect on the steady-state transport and accumulation of P. Rhee (1974) similarily found that the maximum Q_p under nitrogen limitation approximated Q_{MAXs} for P under steady-state Pi-limited conditions. Thus, it appears that although cells growing under steady-state conditions are capable of transporting and accumulating non-limiting nutrients above basal requirements, this storage does not approach the maximal transient storage capacity (as measured with R).

BATCH CULURE

When transient (non-steady state) conditions exist, competition dependent factors can not be directly related to steady-state kinetics. Brown et al. (1978) have shown that transient transport rates observed when providing a P-starved population of cells with excess phosphorus may exceed the maximum observed steady-state transport rates, thus suggesting that a_s may underestimate the maximum affinity observed under transient conditions. Fuhs et al. (1972), Rhee (1974) and my data show that cell phosphorus pool concentrations under transient conditions reach a maximum

(Q_{MAXt}) at a higher concentration than observed at μ_{MAX} under steady-state conditions (Q_{MAXs}). Q_{MAXt} for S. Nägeli (strain A) and S. quadricauda are 987 and 960 nMP(mg cell)$^{-1}$ respectively. Q_{MAXs} for S. Nägeli (strain A) is 506 nMP(mg cell)$^{-1}$. A value for Q_{MAXs} for S. quadricauda could not be calculated due to a curvilinear μ versus Q plot (Fig. 3).

Such results suggest that different metabolic mechanisms may control cell-nutrient levels, transport rates and growth rates when transient rather than steady-state conditions exist. While transient transport rates for low concentrations of Pi are diffucult to measure experimentally, they have been shown to be regulated by cellular P levels (Rhee, 1973, 1974; Chen, 1974; Argast and Boos, 1980) which in turn are ultimately controlled by the cellular storage capacity. Thus, the coefficient of luxury consumption (R) is a useful index of transport capacity and ability to compete under transient conditions. R was first defined by Droop (1974, 1975) and later by Tilman and Kilham (1976) as a measure of nutrient storage capacity. It is the maximum observed ratio of Q_{MAX} to Q_o, and is thus dimensionless. Notice that the maximum value for R occurs at Q_{MAXt}/Q_o and not Q_{MAXs}/Q_o. The coefficients of luxury consumption for P for all organisms studied are presented in Table 6. These compare to reported values of 82 and 6.6 for

Asterionella formosa and Cyclotella meneghiniana respectively (Tilman and Kilham, 1976). R values are calculated from Q values standardized to unit cell mass rather than cell numbers. Dry weight per cell is a multiple function of volume, density, growth rate and degree of nutrient limitation. In order for comparisons of nutrient storage capacity among organisms to be valid, all of these factors must be taken into consideration.

COMPETITION IN CONTINUOUS CULTURE

Many complex models, usually based on Monod or Droop relationships, have been advanced to predict the outcome of competition among microplankton for limiting nutrients (Dugdale, 1967; Caperon, 1968; Eppley and Thomas, 1969; Hecky and Kilham, 1974; Taylor and Williams, 1975; Lehman et al., 1975; Titman, 1976; Tilman, 1977). In these models, half saturation constants are used to assess relative competitive abilities and thus to predict species dominance when various nutrients limit growth. However, half saturation constants are not always good indicators of competitive abilities for limiting nutrients (Healy, 1980). In addition, most of the models are based on steady-state enzyme type kinetics and thus are bound to the same conditions assumed to be valid for a single enzyme-substrate reaction (see page 12). Since

most natural systems are rarely at steady-state for long periods of time and since nutrient transport and nutrient limited growth are not single enzyme-substrate reactions, steady-state based models fail to accurately describe microplankton growth. Any attempt to predict microplankton biomass, composition and succession in natural aquatic systems as related to a growth limiting nutrient, must consider both steady-state and transient levels of that nutrient. Relative competitive abilities for microplankton species when steady-state growth conditions exist are best described by the steady-state affinity (a_s) coupled to the threshold concentration for the growth-limiting nutrient (S_t) and the apparent maximal growth rate (μ_{MAX}). Transient growth is best described by transient affinities (if they can accurately be measured) coupled to luxury storage capacity (as measured through R) , subsistence cell quota (Q_o) and apparent μ_{MAX}.

The P-dependent growth kinetic data for S. Nageli (strain A) and S. quadricauda indicate that S. Nägeli (strain A) should outcompete S. quadricauda for P when both steady-state and transient conditions exist, if no other factors (such as allelopathy) are involved. S. Nägeli (strain A) was shown to outcompete S. quadricauda in Pi-limited dual species continuous culture (Figure 15). Since elimination does not occur under batch culture condi-

tions, the outcome of competition under transient conditions is diffucult to gauge. However, in dual species batch cultures, S. Nägeli (strain A) does predominate.

PHYSIOLOGICAL PROFILES

From the continuous and batch culture results, it is possible to construct partial physiological profiles for S. Nägeli (strains A and B) and S. quadricauda. These profiles are useful for determining the ecological niche that each species occupies in an environment. Kuenen and Harder (1980) suggest that an ideal oligotroph would exhibit a physiological profile with such characteristics as a high surface to volume ratio, a high affinity for nutrient transport and nutrient transport coupled to stored polymer utilization. On this basis, S. Nägeli (strains A and B) exhibit physiological characteristics that more closely resemble those of an oligotroph than does S. quadricauda. Therefor, the blue-green alga should be most competitive in nutrient poor environments (if the environment is P-limited, as many aquatic systems are). A large proportion of the biomass in an oligotrophic marine environment has been recently reported to consist of small unicellular blue-green algae (Johnson and Sieburth, 1979). S. quadricauda on the other hand should predominate in eutrophic and/or tran-

siently eutrophic environments. This green alga has been found to dominate the phytoplankton community of a hypereutrophic pond (Vincent, 1980).

TAXONOMIC/PHYSIOLOGICAL SIMILARITIES

An inherent assumption of models predicting microplankton biomass, competition and succession in natural aquatic systems is that growth kinetic descriptions obtained from the study of laboratory cultured species will represent growth of taxonomically closely related ecologically significant species. However, taxonomic relationships are based largely on morphological similarities and may not be useful for predicting physiological similarities that may exist among species.

Overall my results show, that as a group, the microplankton species studied exhibit relative uniformity in their phosphorus dependent growth kinetic characteristics. In comparison to other major groups of microorganisms, such as Escherichia coli and other gut microbes, the microplankton species I studied have much higher affinities and yields and have lower Q_o values for P. Within the group of microplankton species studied, however, variability did exist, and this variability did not always occur along taxonomic lines. For example, while the two blue-green species

studied exhibited close physiological similarities in their P-dependent growth kinetic characteristics (both transient and steady-state), the two Scenedesmus species studied differed significantly in both their transient and steady-state P-dependent growth kinetic characteristics. In addition, S. quadricauda differed significantly from the Scenedesmus sp. Rhee (1973, 1974) studied. Therefor, it is not accurate to extend growth kinetic descriptions of easily cultured organisms to predictions of the biomass and composition of taxonomically related species in natural aquatic systems.

IMPLICATIONS

A major focus of limnological and oceanographic research has been to gain an understanding of the fundamental processes responsible for controlling microplankton biomass, composition and succession in natural aquatic systems. Microplankton production has been traditionally related to the concentration of an essential factor (most often an essential nutrient) to steady-state models. Fitting data to these steady-state models has indeed enhanced our understanding of the relationship between microplankton production and limiting nutrient concentrations. Several attempts have also been made to apply these

empirical models to natural systems using laboratory microcosms (Jassby et al., 1977), large scale outdoor batch (DeNoyelles and O'Brien, 1978) and continuously fertilized (Malone et al., 1978) culture studies. The approach used in these xenic outdoor culture studies is to observe, over time, changes in various parameters as caused by nutrient enrichment and/or nutrient ratio modification to growing microplankton species. However, these attempts have had limited success in accurately predicting observed events. Until more is known about microplankton eco-physiology, it is unreasonable to assume that steady-state empirical models are a good approximation of microplankton growing in natural aquatic systems which are often subject to periodic nutrient perturbations. Further experimental work must, to a greater extent, consider the physiological responses of microplankton to both steady-state and transient environmental conditions (see Burmaster, 1978).

Knowledge of the processes regulating the microplankton community in natural aquatic systems has many applications. It will be useful be predicting microplankton biomass, composition and succession in natural aquatic systems, thereby providing a means in controlling nuisance species and their blooms. It will also be utilized in devising management schemes for maximal production of desirable microplankton species in artifical upwelling areas and fish production

ponds. Further, the ability to model and understand a natural system would allow accurate assessments of perturbation effects to that system. This would be of value for assessments of the potential effects of substances such as pesticides, petroleum, mine slag, cannery wastes, etc... added to natural aquatic systems.

SUMMARY

In this thesis I have described the P-limited growth of several aquatic microplankton species, living in P concentrations that are typical of many natural aquatic systems. Because natural systems are subject to periodic fluctuations in P-levels, both steady-state (via continuous culture) and transient (via batch culture) growth were described. From the detailed growth kinetic analyses of S. Nägeli (strain A) and S. quadricauda, I was able to predict the competitive abilities of these organisms when P-limited. In addition to describing competitive abilities, I used growth kinetic descriptions and morphological characteristics to construct partial physiological profiles for several of these species. However, I found that these physiological profiles could not be extrapolated to other taxonomically related species. Discovering the factors that regulate the microplankton community in natural systems provides further insight into eutrophication processes, fishery management and aquatic system modelling.

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