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BIOENGINEERING ASPECTS OF INORGANIC CARBON SUPPLY TO MASS ALGAL CULTURES

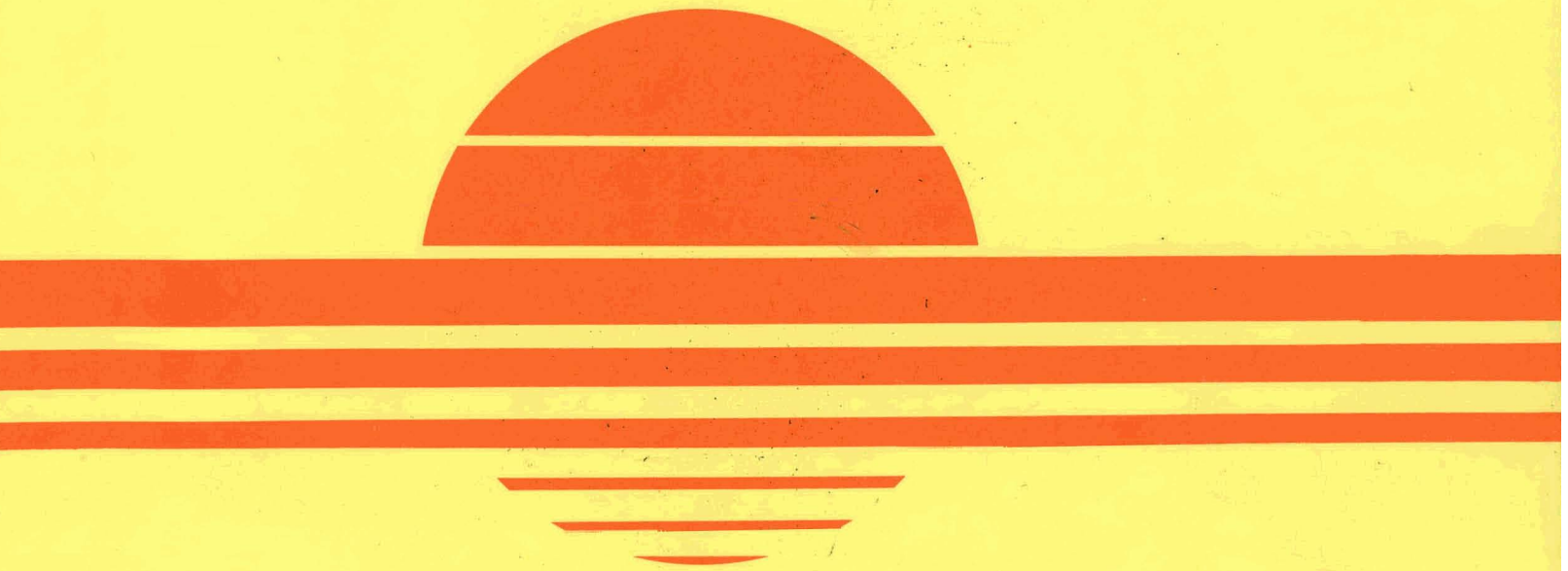
Final Report

By
Joel C. Goldman

June 1980

Work Performed Under Contract No. AS02-78ET20604

Woods Hole Oceanographic Institution
Woods Hole, Massachusetts



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PROJECT SUMMARY

The work included in this report is part of an ongoing study (currently funded by the Solar Energy Research Institute - Subcontract No. XR-9-8144-1) on the inorganic carbon requirements of microalgae under mass culture conditions and covers the period June 1, 1978 through May 31, 1979. It is divided into two parts appended herein. The first part is a literature review on the inorganic carbon chemical system in relation to algal growth requirements, and the second part deals with the kinetics of inorganic carbon-limited growth of two freshwater chlorophytes including the effect of carbon limitation on cellular chemical composition. Additional experiment research covered under this contract was reported in the Proceedings of the 3rd Annual Biomass Energy Systems Conferences, pp. 25-32, "Bioengineering aspects of inorganic carbon supply to mass algal cultures". Report No. SERI/TP 33 285.

APPENDIX A

Literature Review

BIOENGINEERING ASPECTS OF INORGANIC CARBON SUPPLY TO MASS ALGAL CULTURES

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A. Chemistry of Inorganic Carbon and Algal Growth

1. Introduction: Among the many alternative energy sources being considered to meet future demands, the photosynthetic conversion of radiant to useful chemical energy (bioconversion) is receiving widespread attention (Hall, 1976). Clearly, all energy on earth has evolved, in one way or another, from the ultimate source, solar radiation, primarily through the storage over geologic time of fossil fuels. Now the rate of energy consumption far exceeds the rate of supply; the prime determinant, then in deciding the attractiveness of a new alternative source is the rate at which it can be produced. This implies that energy balance for a particular process is favorable, that is, the amount of useful energy produced exceeds the energy expended during production.

The photosynthetic production of energy has particular appeal because it is the most basic of energy-storing and life-supporting processes. One of the major problems, however, is that solar energy, although virtually infinite in total capacity, strikes the earth at a very low flux, about 1.6 or less gr cal/m^2/min., hence requiring very large collection systems for capturing the required energy. The other major problem is that photosynthetic conversion efficiences are very low, and under the most ideal conditions the most efficient plants can convert at best about 10-12% of visible solar radiation to stored chemical energy as organic matter (Radmen and Kok, 1977). In reality, photosynthetic conversion efficiencies of thermal terrestial and aquatic systems are considerably lower, and seldom exceed 1-2%, pri-

marily because other factors such as light availability, nutrients, water, etc. are limiting. In fact, it is estimated that only about 0.1% of the solar energy striking the earth is converted to organically bound energy in the form of plant material (Hall, 1976).

Therefore, it is the prime goal of any bioconversion scheme to maximize possible photosynthetic efficiencies and resulting yields by forcing the only uncontrollable growth factor, light, to be limiting. This means that all the required nutrients for growth must be supplied in excess.

Aquatic plants, primarily micro- and macroalgae, are among the most efficient converters of radient energy, and conversion efficiencies under laboratory conditions with low incident radiation have been reported to be *ca.* 20% (Shelef *et al.*, 1968). In addition, algal cultures can, in theory, be maintained indefinitely and thus are not dependent on seasonal growth. For these reasons mass algal cultures are being considered as candidate bioconversion systems in the ERDA Fuels From Biomass Program (Ward, 1977).

The potential applications for algal cultures are widespread as seen in Fig. 1. Early interest in algal mass culturing centered around the possibility of converting single-celled algae to human, and/or animal, protein supplements (Burlew, 1953) until W. J. Oswald and coworkers at the University of California Berkley expanded on this theme by demonstrating that algal systems could be used for treating wastewater (Oswald *et al.*, 1957) and producing methane via solar energy conversion and anaerobic digestion, as seen in Fig. 1 (Oswald and Golueke, 1960).

The latter concept has been suggested as a dual-functioning process to

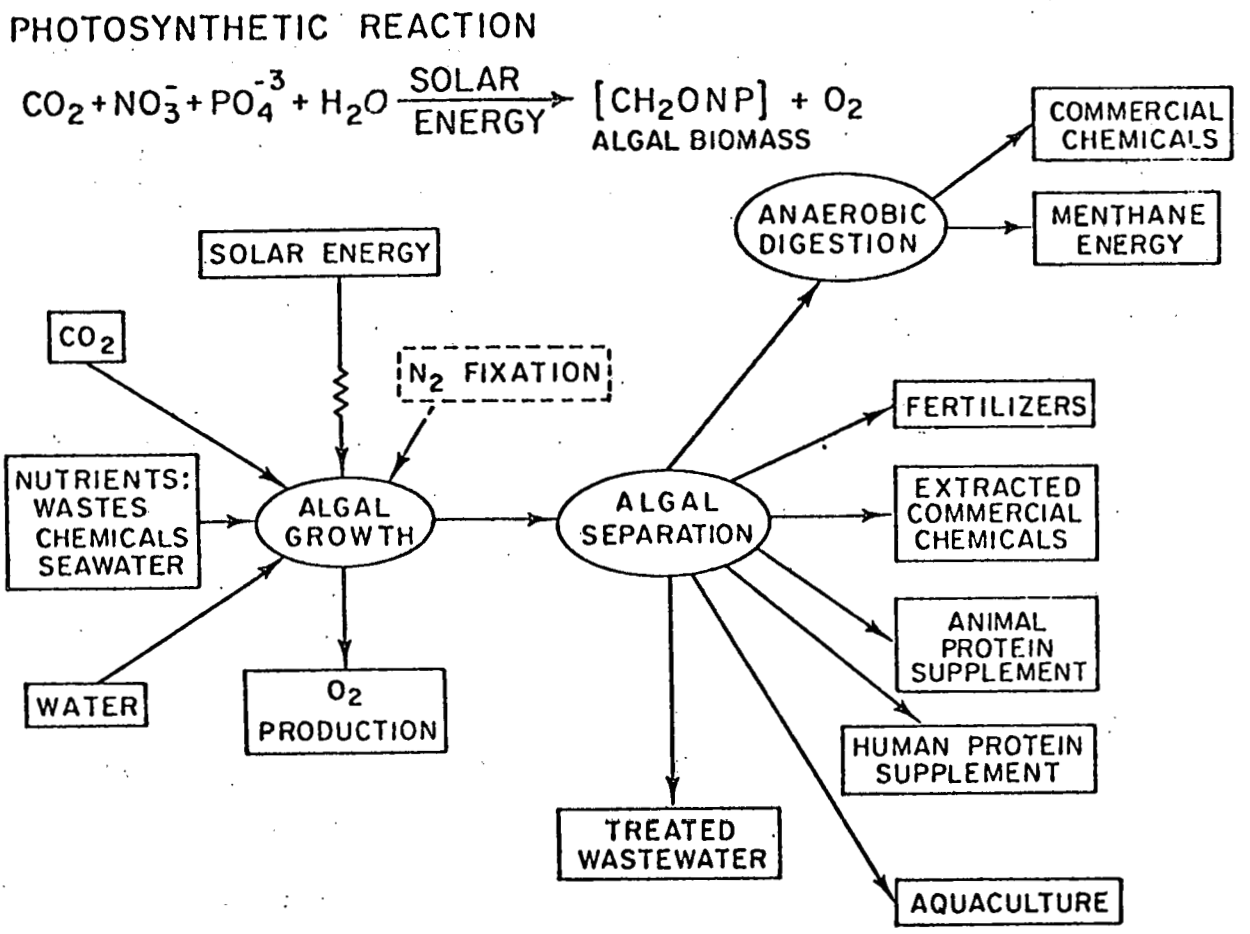

FIGURE 1. PROCESS FLOW DIAGRAM OF ALGAL MASS CULTURES INDICATING MATERIALS REQUIRED AND POTENTIAL APPLICATIONS.

simultaneously treat wastes and produce energy (Benemann *et al.*, 1977). However, as shown by Goldman and Ryther (1977), on a national level the nutrients available from total U.S. domestic waste water discharges, even when completely converted to algal biomass, would represent just a small fraction of the total nutrients required for bioconversion. For example, to produce 1% of the U.S. energy demand projected in 1980 would take a population equivalent of 1 billion to meet the nitrogen requirements in the bioconversion scheme depicted in Fig. 1 (Goldman and Ryther, 1977).

As suggested by Goldman and Ryther (1977) and shown in Fig. 2, any large-scale algal bioconversion process will have to include some form of nutrient recycle to be energy and economically efficient. The open type bioconversion scheme in which nutrients and water are continuously supplied from an external source and eventually wasted is uneconomical and may not be possible due to a lack of available nutrients. Thus, either the semi-closed scheme in which nutrients, and possibly water, are recycled after the methane conversion step, or the completely closed scheme in which both nutrients and water are recycled, will have to be employed if bioconversion is to make a viable contribution to U.S. energy supplies.

The three primary nutrients required for algal growth are the inorganic forms of carbon, nitrogen, and phosphorus. The requirements for these nutrients by algae are reasonably known and the stoichiometric relationship between them is often reported as $C_{106}N_{15}P_1$, after the work of Redfield (1958), in which it was shown that the chemical composition of marine phytoplankton was typically in these proportions. However, it is

ALGAL BIOMASS-ENERGY SYSTEMS

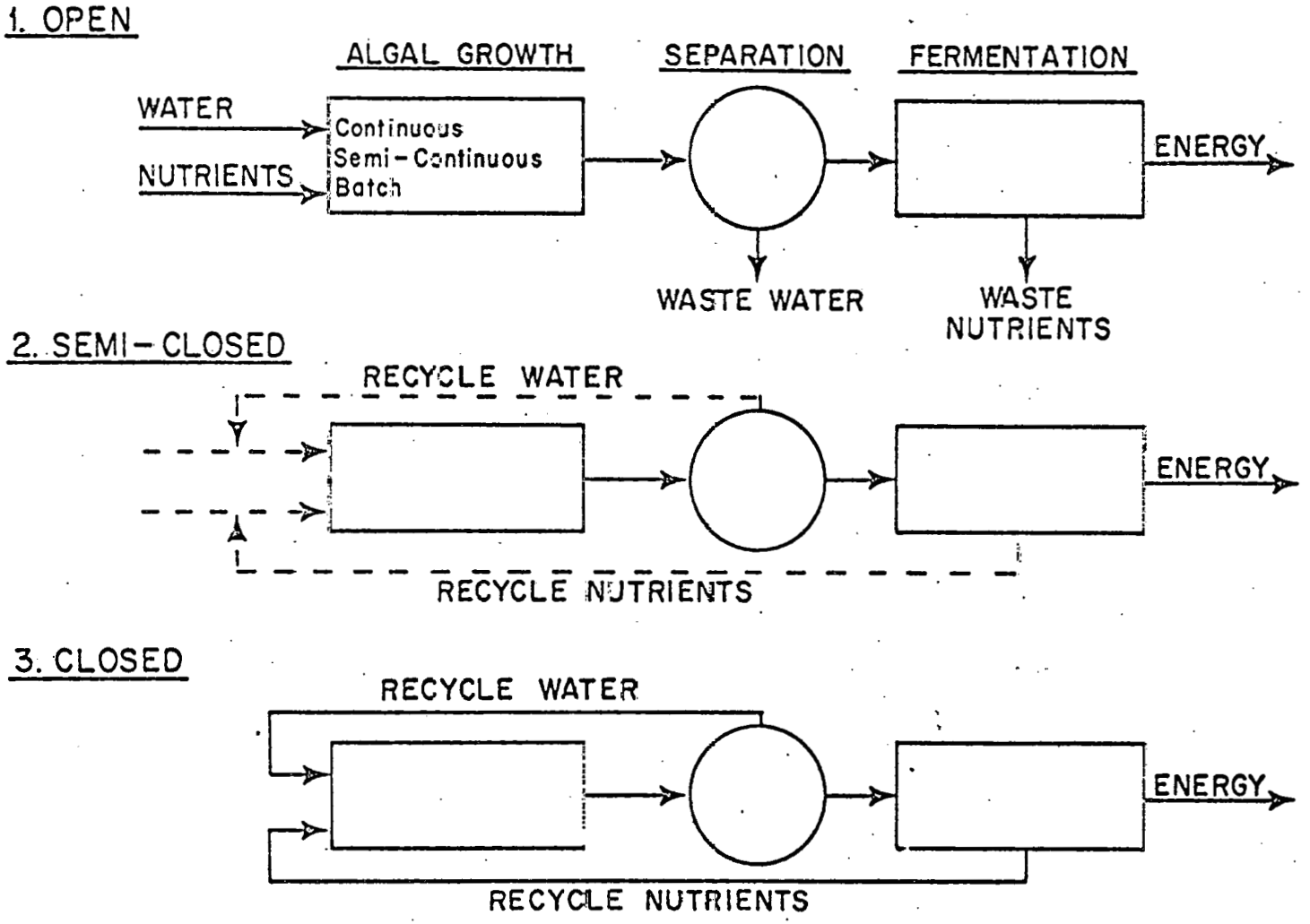

FIGURE 2. ALGAL BIOMASS-ENERGY SYSTEMS - ALTERNATIVE FLOW SCHEMES.

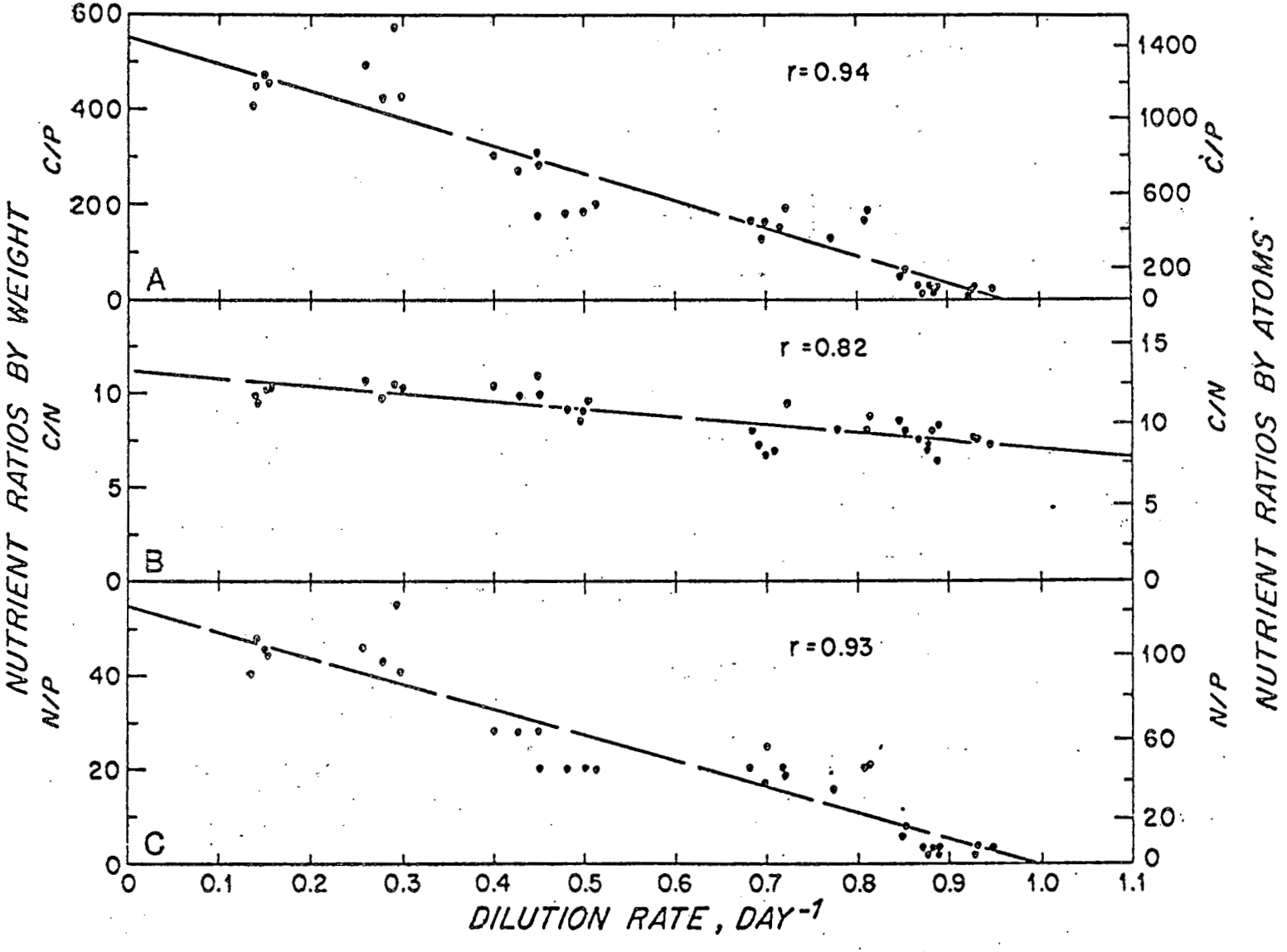

FIGURE 3. EFFECT OF DILUTION RATE (GROWTH RATE) ON NUTRIENT RATIOS FOR *MONOCHRYSIS LUTHERI* GROWN IN PHOSPHORUS-LIMITED CONTINUOUS CULTURES.

now well established that this chemical ratio is achieved only under certain environmental conditions and that the proportions of phosphorus and nitrogen in an algal cell can deviate widely from the above stoichiometry when limitation by one or the other nutrients exists (Perry, 1976). In recent work in the author's laboratory it was demonstrated that under phosphorus-limited growth of the marine chrysophyte *Monochrysis lutheri* in continuous culture the C/P and N/P ratios (by atoms) varied from over 1000 and 100:1 at low growth rates (the region of severe phosphorus limitation) to *ca.* 100 and 10:1 at 95% of the maximum growth rate (the region of non-nutrient limitation) (Fig. 3). Hence, it is clear that under non-nutrient limitation the Redfield equation of $C_{106}N_{15}P_1$ is a good approximation of the chemical composition of algae since the chemical ratios of different algae, both fresh water and marine, appear to vary in the same proportions.

Fortuitously, these are precisely the conditions that are achieved in algal mass cultures when light is made the limiting growth factor. The requirements for carbon, nitrogen and phosphorus in algal mass cultures can then be estimated fairly simply. Assuming that carbon represents about 50% of the organic matter in algae (with a heat of combustion of ∿5.5 cal/gr of ash-free dry weight) (Goldman *et al.*, 1972), the nitrogen and phosphorus requirements in one gram of ash-free algae would be 0.5 x (15/106) = 0.07 gr for nitrogen and 0.5 (1/106) = 0.005 gr for phosphorus. The total requirements for these two nutrients could then be calculated by multiplying the above unit values by the total yield of algae

anticipated, providing a 5-10% excess to ensure that neither nutrient ever becomes limiting. Nitrogen would be supplied as NH_4^+ or NO_3^- and phosphorus as PO_4^{-3}. If these nutrients were to be recycled from the anaerobic digestion portion of a bioconversion process as depicted in Fig. 2, then it would be necessary to ensure that substantial oxidation of the digested residue containing the recycled nitrogen and phosphorus occurred before the nutrients were added back into the algal growth system.

The requirement for inorganic carbon is a far more complex problem, however. Even though the actual quantities of organic carbon produced via photosynthesis can be calculated in the same manner as above, the total amount of inorganic carbon required is much more difficult to calculate. This is because inorganic carbon is distributed among the chemical species CO_2 (aqueous), H_2CO_3 HCO_3^- and CO_3^{-2} in an exceedingly complex chemical equilibrium system which is controlled by two parameters, alkalinity and pH. In natural fresh and marine waters this chemical system constitutes the main buffering system; losses of inorganic carbon through photosynthesis result in the destruction of buffering capacity, leading to a rise in pH, which can adversely affect algal growth in a number of ways (Goldman 1973).

Normally, the transport of CO_2 from the atmosphere cannot keep pace with algal assimilation of CO_2 during intense algal growth and a rise in pH to over 10 is not uncommon in eutrophic natural waters and mass culture systems (Goldman *et al.*, 1972). Thus, in algal mass cultures, to avoid the combined problems of inorganic carbon limitation and pH rise, inorganic carbon as gaseous carbon dioxide is usually supplied via some aeration scheme or by creating sufficient turbulence so that sufficient CO_2 can be

transferred from the atmosphere. Mixing can, to some degree, enhance CO_2 transport from the atmosphere, but, because of the very low concentration of CO_2 in the atmosphere (0.03%), the transport gradient is always small and CO_2 mass transfer is ineffective unless very turbulent mixing is employed. However, mixing is required in algal mass cultures for several other reasons: to prevent settling and subsequent decay of organic matter to prevent thermal stratification, to break down diffusion gradients of essential nutrients which could develop at the cell surface in very intense mass cultures (this is particularly of seaweeds which are large cells and have long diffusion paths), to prevent epiphyte buildup on the surface of seaweeds, and most important, to provide uniform cell exposure to light since self-shading of cells exists in thick cultures.

The technology and resulting economics of providing adequate CO_2 and mixing in algal mass cultures is amazingly undeveloped considering the substantial research effort now underway to mass culture various freshwater and marine algae for bioconversion applications (Ward, 1977). The problem of delineating the requirements for carbon dioxide is exceedingly difficult and the major questions still to be addressed are the relative importance of mixing (for solving non-carbon related problems), pH control, and quantity and source of inorganic carbon necessary for maximizing algal yields. Therefore, the major objective of this proposal is to address the question of inorganic carbon supply to algal mass cultures primarily from a scientific basis. With a firm understanding of the chemical-biological interactions involved in carbon availability, rational decisions can be made regarding

the engineering design of carbon dioxide supply systems for large-scale outdoor cultivation systems.

2. Inorganic Carbon Transformations in the Aquatic Environment: Inorganic carbon utilization by photosynthetic organisms significantly influences the cycling of carbon in the aquatic environment by altering the composition of the chemical species in the CO_2-H_2CO_3-HCO_3^--$CO_3^=$ system, a major component of the buffering system of most natural fresh waters (Weber and Stumm, 1963a, 1963b). Although equilibrium constraints control the total inorganic carbon available for algal growth, kinetic restraints play a dominant role in regulating the instantaneous supply of inorganic carbon from the CO_2 - H_2CO_3 - HCO_3^- - $CO_3^=$ system, from the atmosphere, and from bacterial and other heterotrophic activity.

CO_2 - H_2CO_3 - HCO_3^- - $CO_3^=$ EQUILIBRIUM SYSTEM

Basic Relationships

The CO_2 - H_2CO_3 - HCO_3^- - $CO_3^=$ system is governed by the content of atmospheric CO_2 and the total alkalinity of a water, and can be described by the following equilibrium expressions (all constants used to describe the CO_2 - H_2CO_3 - HCO_3^- - $CO_3^=$ system are the most widely accepted values, as presented in the comprehensive review by Kern (1960):

(1) Ionization of water*

$$H_2O \rightleftharpoons H^+ + OH^-;\ K_w = (H^+)(OH^-) = 10^{-14} \text{ @ } 25^oC \quad . \quad . \quad . \qquad (1)$$

*Parenthesis indicate moles/liter

(2) Solubility of CO_2 in H_2O

$$CO_2 \text{ (gas)} \rightleftharpoons CO_2 \text{ (aq)}; \quad CO_2 = k_{CO_2} P_{CO_2} \quad \ldots\ldots\ldots\ldots (2)$$

in which

$k_{CO_2} = 10^{-1.5}$ moles/atmosphere @ 25°C

$P_{CO_2} = 10^{-3.5}$ for typical atmospheric air

(3) Hydration of CO_2

$$CO_2(aq) + H_2O \rightleftharpoons H_2CO_3; \quad K_{\text{hyd}} = \frac{(CO_2)}{(H_2CO_3)} \simeq 600 \quad \ldots\ldots\ldots (3)$$

Because at equilibrium (CO_2) is much greater than (H_2CO_3), the reaction $(H_2CO_3)^* = (H_2CO_3) + (CO_2)$ (aq) can be considered valid and $(H_2CO_3)^*$ will hereafter be considered to be equal to the sum of the dehydrated and hydrated forms of CO_2.

(4) First ionization of $(H_2CO_3)^*$

$$H_2CO_3^* \rightleftharpoons H^+ + HCO_3^-; \quad K_1' = \frac{(H^+)(HCO_3^-)}{(H_2CO_3)^*} = 4.45 \times 10^{-7} \text{ @ } 25^oC \quad \ldots (4)$$

in which

K_1' = Apparent dissociation coefficient

(5) Second ionization of $(H_2CO_3)^*$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^=; \quad K_2 = \frac{(H^+)(CO_3^=)}{(HCO_3^=)} = 4.6 \times 10^{-11} \text{ @ } 25^oC \quad \ldots (5)$$

(6) Total alkalinity of most natural waters (in equiv./liter)

$$\text{Tot. Alk. (ALK)} = (HCO_3^-) + 2(CO_3^=) + (OH^-) + (NH_3)$$
$$+ (H_2PO_4^-) + 2(HPO_4^=) + 3(PO_4^{\equiv}) + B(OH)_4^- - (H^+) \quad \ldots\ldots (6)$$

(H^+) can be neglected for $(H^+) < 10^{-4}$ (pH 4)

The molar concentration of all three carbon species making up the equilibrium system can be described in terms of the pH and alkalinity of a given system by the following equations:

$$C_T = (H_2CO_3)^* + (HCO_3^-) + (CO_3^=) \quad \ldots\ldots\ldots\ldots\ldots (7)$$

in which

C_T = Total molar concentration of inorganic carbon

$$(H_2CO_3)^* = \frac{(H^+)}{K_1'} \left[\frac{(ALK) - (OH^-) + (H^+)}{1 + \frac{2K_2}{(H^+)}} \right] \quad \ldots\ldots\ldots (8)$$

$$(HCO_3^-) = \frac{(ALK) - (OH^-) + (H^+)}{1 + \frac{2K_2}{(H^+)}} \quad \ldots\ldots\ldots\ldots\ldots (9)$$

$$(CO_3^=) = \frac{K_2}{(H^+)} \left[\frac{(ALK) - (OH^-) + (H^+)}{1 + \frac{2K_2}{(H^+)}} \right] \quad \ldots\ldots\ldots (10)$$

The concentrations of the three species can also be expressed as a function of the total inorganic carbon concentration (C_T) and the pH (independent of alkalinity concentration) by equations similar to Equations 8-9.

$$(H_2CO_3)^* = C_T \alpha_o = C_T \left[1 + \frac{K_1'}{(H^+)} + \frac{K_1' K_2}{(H^+)^2} \right]^{-1} \quad \ldots\ldots (11)$$

$$(HCO_3^-) = C_T \alpha_1 = C_T \left[1 + \frac{(H^+)}{K_1} + \frac{K_2}{(H^+)} \right]^{-1} \quad \ldots\ldots\ldots \quad (12)$$

$$(CO_3^=) = C_T \alpha_2 = C_T \left[1 + \frac{(H^+)^2}{K_1 K_2} + \frac{(H^+)^2}{K_2} \right]^{-1} \quad \ldots\ldots\ldots \quad (13)$$

in which

$$\alpha_o = (H_2CO_3)^*/C_T$$

$$\alpha_1 = (HCO_3^-)/C_T$$

$$\alpha_2 = (CO_3^=)/C_T$$

Thus,

$$\alpha_o + \alpha_1 + \alpha_2 = 1 \quad \ldots\ldots\ldots \quad (14)$$

For simplicity activity effects are not considered in these equations, but can easily be included and for seawater are substantial and must be considered.

A distribution diagram based upon Equation 7 and Equations 11-13, showing the relative proportions of all three species as a function of pH (independent of alkalinity concentration), is presented in Fig. 4.

Equilibrium with Atmospheric CO_2

When the $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ is in equilibrium with atmospheric CO_2, the concentration of free CO_2 in solution remains constant for all pH values, while the concentration of the bicarbonate and carbonate species changes as a

function of the total carbon alkalinity.

The equilibrium pH of a natural water can be described by combining and rearranging Equations 1 through 6. The following equations, developed by Weber and Stumm (1963a) and by Thomas and Trussell (1970), show this relationship:

$$(H^+) = \frac{K_w + K_1' (H_2CO_3)^* + \sqrt{[K_w + K_1' (H_2CO_3)^*]^2 + 8 (ALK) K_1' K_2 (H_2CO_3)^*}}{2 (ALK)} \quad \ldots\ldots\ldots\ldots (15)$$

or

$$pH = -\log_{10} \left[\frac{K_w + K_1' (k_{CO_2} P_{CO_2}) + \sqrt{[K_w + K_1' k_{CO_2} P_{CO_2}]^2 + 8(ALK) K_1' K_2 k_{CO_2} P_{CO_2}}}{2 (ALK)} \right] \quad \ldots\ldots\ldots\ldots (16)$$

These equations describe situations in which the solution CO_2 concentration is in equilibrium with atmospheric CO_2--situations rarely encountered, as most natural waters in contact with the air are supersaturated with CO_2 (Stumm, 1964). Morton and Lee (1968a) showed that thermal stratification plays an important role in maintaining vertical CO_2 gradients in some natural waters. Higher CO_2 concentrations, and correspondently lower pH values, are found nearer the bottom where bacterial activity predominantes. Hutchinson (1957) extensively described the $H_2CO_3^* - HCO_3^- - CO_3^=$ equilibrium system in lakes and similarly suggested that natural water bodies are often supersaturated with CO_2. Continually occurring complex interactions, such as biological activity and heterogeneous chemical reactions, greatly affect the CO_2 concentration present in a water body (Hutchinson, 1957). That most natural

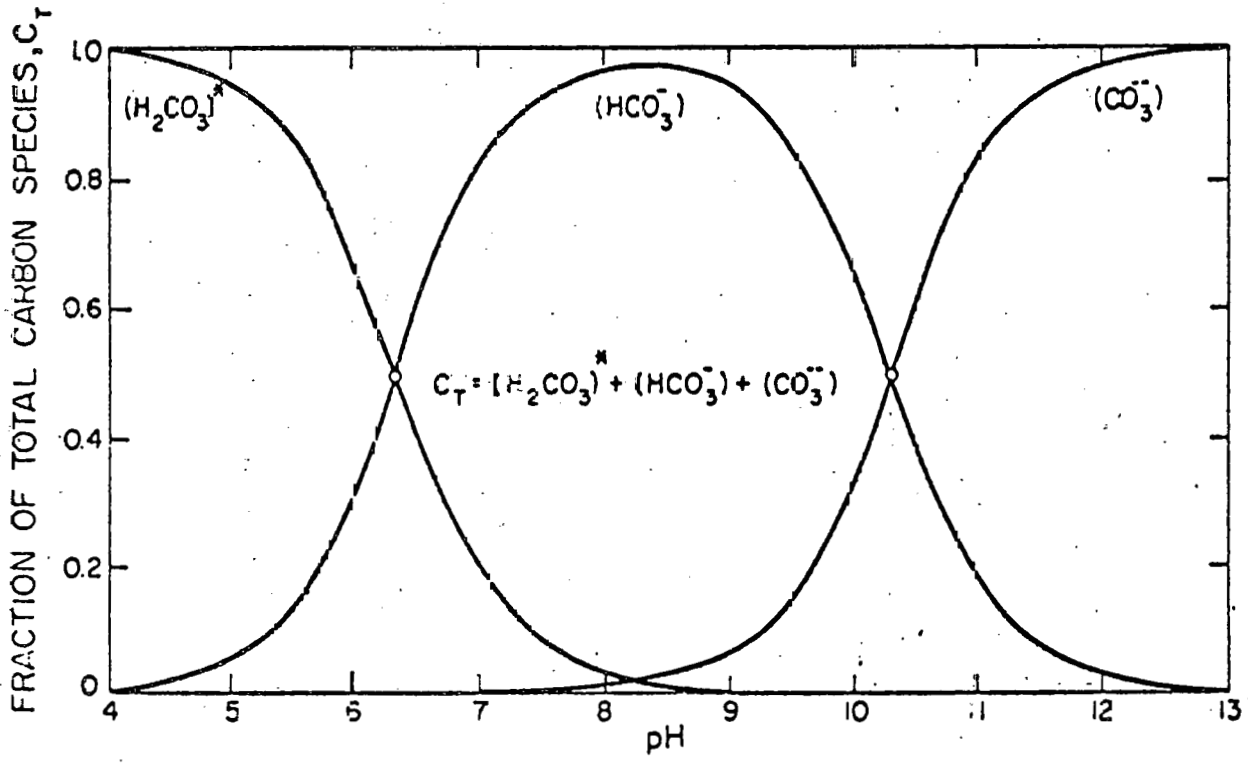

FIGURE 4. EFFECT OF pH ON DISTRIBUTION OF INORGANIC CARBON SPECIES IN $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ SYSTEM

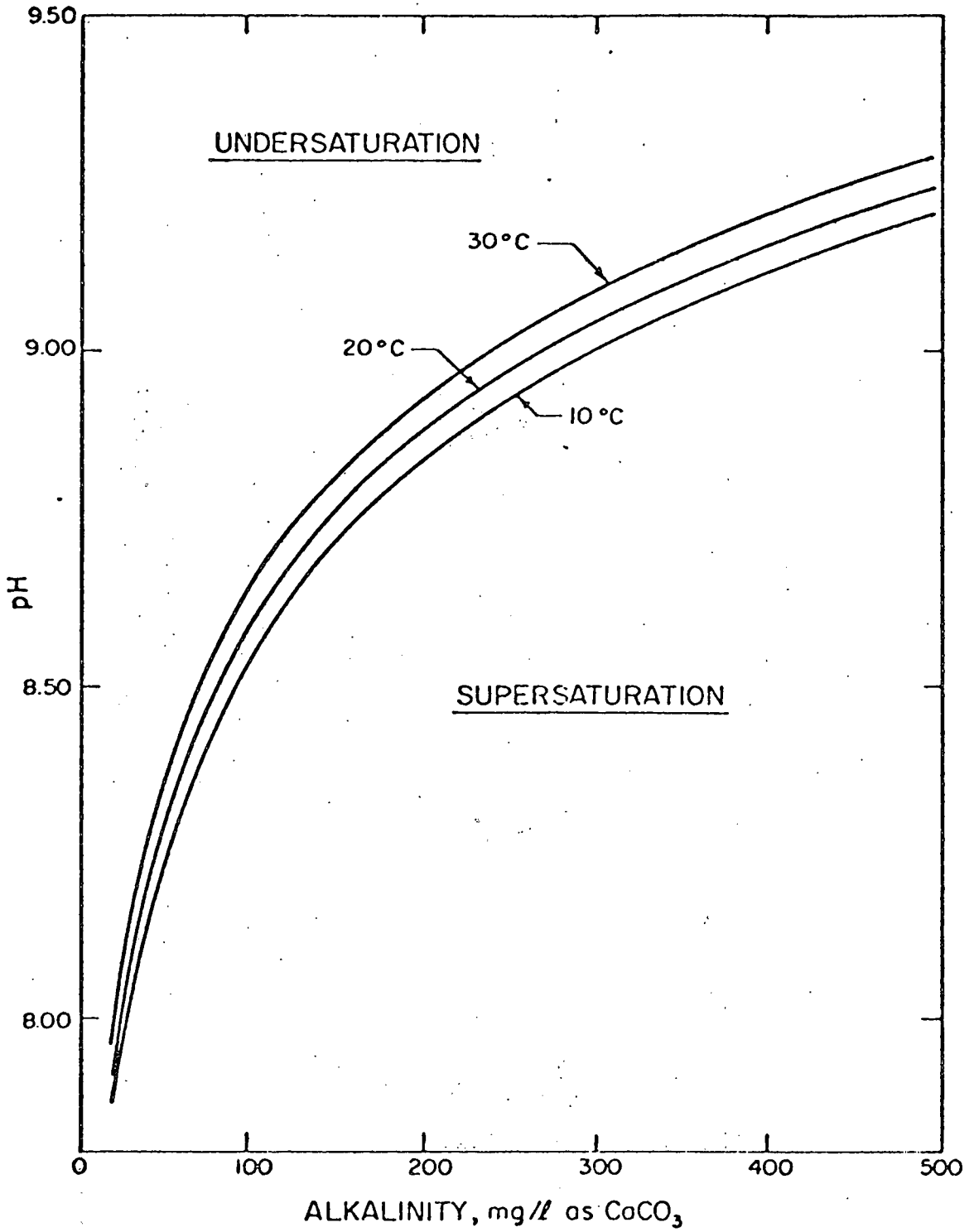

FIGURE 5. EFFECT OF ALKALINITY ON EQUILIBRIUM pH IN AQUATIC SYSTEM EXPOSED TO THE ATMOSPHERE (CO_2 = 0.03%). FROM THOMAS AND TRUSSELL (1970).

waters are supersaturated with CO_2 can readily be seen from a plot of Equation 16 shown in Fig. 5 (Thomas and Trussell, 1970). All combinations of pH and alkalinity falling above the equilibrium curve demonstrate undersaturation, whereas those values below the curve indicate supersaturation. As an example, for a water with a bicarbonate alkalinity of 100 mg/ℓ as $CaCO_3$ equilibrium would be reached at a pH of about 8.6. As will be demonstrated in a later section, very few natural waters with this concentration of alkalinity achieve such a high pH except during intensive algal activity.

With respect to CO_2 equilibrium in the oceans, the Pacific Ocean is said to be undersaturated to a large degree, whereas the Indian Ocean and the South Atlantic near the equator are said to be supersaturated (Horne, 1969). Keeling (1968) developed a map of the surface distribution of CO_2 in the oceans, showing supersaturation at the equator and undersaturation toward the poles. Kelley and Hood (1969) reported that CO_2 concentrations in the North Pacific Ocean and the Bering Sea are greatly affected by currents and river discharges. The supersaturation with dissolved CO_2 at the entrance to Puget Sound in Washington was equivalent to an atmospheric CO_2 concentration of 0.09 percent. Park *et al.* (1969) showed that the dissolved CO_2 concentration of the Columbia River was greatly in excess of equilibrium values. Other rivers in the region displayed a similar characteristic (Park *et al.*, 1970).

CO_2 Transport From the Atmosphere

The rate of CO_2 exchange between the atmosphere and a natural water is

governed by the pH and alkalinity (Equation 16 and Fig. 5). The main driving force in CO_2 exchange is the difference between atmospheric and aqueous CO_2 concentrations. Only when the water is undersaturated with respect to CO_2 (as represented by the region above the curve in Fig. 5) can CO_2 enter the water from the atmosphere. Hence, CO_2 transport into a natural water becomes increasingly important as the pH rises for a given alkalinity.

The rate of CO_2 transfer across a gas-liquid interface is described by Fick's law:

$$dC/Adt = K\,\Delta C = (1/R_L)\,\Delta C \quad \ldots\ldots\ldots \quad (17)$$

in which

dC/Adt = CO_2 flux across the gas-liquid interface in mass of inorganic carbon (as CO_2) transported per unit time.

K = Transfer coefficient in distance per unit time.

R_L = Resistance to transfer ($1/K$), specific for a given liquid and given hydrodynamic conditions.

ΔC = Difference in carbon concentration (as CO_2) between the gas and liquid phases in mass per unit volume.

The rate of transfer is dependent not only on the main driving force, ΔC, but also on the value of R_L, whose value increases as liquid turbulence decreases, and approaches a maximum as the water approaches stagnation.

The rate of CO_2 transport is complicated by the reaction of CO_2(aq) with HCO_3^- and $CO_3^=$, because atmospheric CO_2 dissolving in water containing bicarbonate alkalinity equilibrates with the $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system.

CO_2(aq) may disappear on entering the system and the driving force, ΔC, will be larger than if CO_2 was nonreactive. This enhancement only occurs when the CO_2 transport rate is slower than the CO_2 reaction rate between CO_2 and either HCO_3^- of $CO_3^=$. The value of R_L determines the conditions that govern the added contribution of CO_2 reactivity. Bolin (1960), Kanwisher (1963), Hoover and Berkshire (1969), and Quinn and Otto (1971) generally conclude that at the pH of sea water ($\sim$ 8.1), and with the turbulence usually present, CO_2 chemical reactivity play a relatively minor role. However, in natural fresh waters, particularly those lakes protected against wind action, the value for R_L is undoubtedly much higher than in the oceans, and chemical reactivity may play an important role in providing CO_2 from the atmosphere.

In an algal growth situation the uptake of inorganic carbon provides an additional mechanism by which CO_2 transport into the liquid phase can be enhanced. The uptake of CO_2 by algae, like CO_2 reactivity, tends to keep ΔC high, particularly at high pH values at which solution-free CO_2 concentrations are small. Schindler (1971) reported that atmospheric CO_2 provided between 73-200 percent of the inorganic carbon utilized by algae during a bloom period at high pH in an experimental Canadian Shield lake. This finding supported Schindler's hypothesis that carbon is not a limiting nutrient in natural waters, because even though the lake was extremely low in inorganic carbon and was fertilized with additional phosphorus and nitrogen, atmospheric carbon provided enough carbon for a bloom to develop. Thus, it appears that under conditions of high pH, low alkalinity, and intense algal activity, atmospheric CO_2 can supply substantial quantities of CO_2 for algal

growth. This finding is dramatically opposed to the views of Lange (1967, 1970) and Kuentzel (1969), who completely reject atmospheric CO_2 as a major source of carbon for algal growth.

Alkalinity and Buffering Capacity

The operational definition of alkalinity for most natural waters, defined by Equation 9, implies that the $H_2CO_3^* - HCO_3^- - CO_3^=$ system is the major buffering system of fresh waters. In sea water suspended silicates and, to a lesser extent borates, are contributors to the alkalinity and buffering capacity (Sillen, 1961; Garrels, 1965). Fresh waters normally contain quite low concentrations of these constituents, as well as other weak bases such as phosphates and ammonia, which could also contribute to a water's total alkalinity.

Thus, the apparent major buffering system of a fresh water is composed of the $H_2CO_3^* - HCO_3^- - CO_3^=$ system. However, the homogenous $H_2CO_3^* - HCO_3^- - CO_3^=$ buffering system, although an important component of the overall buffering capacity of a natural water, is itself greatly affected by the complicated network of heterogeneous and biological reactions continually occurring in the aquatic environment as the whole system tends toward, but seldom reaches, equilibrium (Weber and Stumm, 1963a). Heterogeneous reactions involving solid phases, such as clays and other minerals, may be the principal buffering agents in fresh waters; however, there is a dearth of information in this area and further research is required (Stumm, 1964, 1967; Bricker and Garrels, 1967; and Bostrom, 1967).

$CaCO_3$ Equilibrium

Although the literature dealing with the purely chemical aspects of the $CaCO_3$ equilibrium in natural waters is extensive, surprisingly little attention has been given to the role of $CaCO_3$ in phytoplankton activity. The chemistry of $CaCO_3$ is extremely complex. $CaCO_3$ exists in a number of polymorphic and hydrated forms, though the two principal forms found in the sediments underlying natural waters are calcite and aragonite (Bricker and Garrels, 1967). Many natural waters are supersaturated both with respect to calcite and aragonite (Bricker and Garrels, 1967), and many areas of the oceans are in equilibrium with calcite (Weyl, 1961; Schmalz and Chave, 1963; Dietrich, 1963; Siever *et al.*). The Pacific Ocean is unsaturated with respect to calcite except at its surface (Peterson, 1966); ion pair formation, particularly between magnesium and carbonate ions, strongly influences $CaCO_3$ equilibrium in this and other ocean waters (Morton and Lee, 1968b).

In natural fresh waters $CaCO_3$ saturation is not as common as in ocean waters. Kramer's (1967) studies of $CaCO_3$ formation in the Great Lakes indicated, that for the observed temperatures, waters in Lake Erie and Lake Ontario are mostly unsaturated. Morton and Lee (1968a) found Lake Mendota in Wisconsin to be unsaturated with respect to $CaCO_3$ in the bottom layers and supersaturated in the surface waters.

Weber and Stumm (1963a, 1963b) and Kleijn (1965) showed the effect of heterogeneous equilibrium between solid $CaCO_3$ and carbonate species on the buffering capacity of a natural water. A water in equilibrium with solid $CaCO_3$ is considerably more buffered than the same water in the absence of

solid $CaCO_3$. It follows that the water in its heterogeneous natural environment is more strongly buffered than the same water studied in the laboratory in the absence of solids. Thus, factors other than the homogeneous $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system help control the buffering capacity of these waters, and further complicate the role of solid $CaCO_3$ as a source of carbon for the growth of algae. For example, the observation that marl forming plants such as *Charophytes* become encrusted with $CaCO_3$ during growth, and yet are dominant in waters saturated with $CaCO_3$, seems to indicate that at least some plants are able to extract sufficient CO_2 from $CaCO_3$ saturated waters (Forsberg, 1965). Thermodynamically it would take considerable energy to cause the CO_2 from solid phase $CaCO_3$ to become available for algal growth without the addition of H^+ according to the following equations:

$$CaCO_3(s) \rightleftharpoons Ca^{++} + CO_3^= \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (18)$$

$$CO_3^= + 2H_2O \rightleftharpoons CO_2 + 2OH^- + H_2O;$$

$$K_{eq} = \frac{(OH)^2\ (H_2CO_3)}{(CO_3^=)} = \frac{K_w^2}{K_1'\ K_2} \simeq 5 \times 10^{-8} \quad \ldots\ldots\ldots\ldots (19)$$

The reaction in Equation 19 is strongly favored to the left. Therefore, the utilization of CO_2 would lead to a rise in pH and actually lead to a decrease in available inorganic carbon for growth; the following reaction would then predominate at a pH greater than 6.5:

$$Ca^{++} + 2HCO_3^- \rightleftharpoons \underset{\text{(algal uptake)}}{CO_2} + \underset{\text{(precipitate)}}{CaCO_3} + H_2O$$

$$K_{eq} = \frac{(CO_3^=)\ (H_2CO_3)}{(HCO_3^-)^2} = \frac{K_2}{K_1'} \simeq 1 \times 10^{-4} \quad \ldots\ldots\ldots\ldots (20)$$

Hutchinson (1957), applying the fact that the carbonate in natural limestone contains no ^{14}C, observed that only a relatively few hard water lakes have a lower ^{14}C content than the atmosphere. Therefore, most of the carbon in lake systems is of modern origin, and thus enters the aquatic system as allochthonous material, or directly from the air, and little CO_2 would be available from $CaCO_3$.

In the time span of a transient algal bloom the solid phase $CaCO_3$ system may not have any effect on the availability of carbon, because the rate at which $CaCO_3$ goes into and out of solution is slow at pH values typical of natural waters. Thus, the maintenance of the heterogeneous equilibrium may not keep pace with the changes in the $H_2CO_3^* - HCO_3^- - CO_3^=$ system caused by photosynthetic activity. Also, other factors present in a natural water may accelerate or hinder $CaCO_3$ precipitation. Chave (1965, 1970) and Chave and Suess (1967, 1970) showed that organic compounds coat $CaCO_3$ precipitates, thus preventing their return to solution under otherwise favorable conditions. Others, such as Oppenheimer (1961) and Greenfeld (1963), indicated acclerated $CaCO_3$ precipitation in the presence of bacteria. Simkiss (1964) showed the inhibitory effect of certain organophosphates on the precipitation of $CaCO_3$ in sea water. Eyster (1958) found a similar effect on $CaCO_3$ precipitation by inorganic phosphates. He suggested that formation of marl deposits is associated with solution phosphate deficiencies. Stumm and Leckie (1970) demonstrated the buildup of hydroxylapatite on solid $CaCO_3$ surfaces, stressing this point as a major factor controlling the resolubilization of phosphates in natural waters. Paasche (1963, 1964) and Steemann Nielsen (1966) reported

in detail on the mechanisms governing $CaCO_3$ precipitation due to the growth of the marine coccolithophorid, *Coccolithus huxleyi*, showing that HCO_3^- was the source of $CaCO_3$ and that CO_2 was the major form of inorganic carbon being assimilated by the algae (see Equation 20).

Because of the complexity and nonequilibrium effects involved in solution and precipitation of $CaCO_3$ solids, their role in the total $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system with respect to the availability of inorganic carbon for algal growth is difficult to assess. One would surmise that $CaCO_3$ solids would have a small effect, but further research is required to define the exact mangitude of its role in providing carbon for algal growth.

Reaction Rates

Because most algae use CO_2 as their major carbon source, it is possible that the chemical transformation rates of the ionic forms of inorganic carbon to free CO_2 could be limiting for algal growth. This concept is a key point in any discussion of carbon limitations in mass cultures; yet, there is almost a complete absence of information on this subject in the literature. In the following sections the literature on rates of conversion of inorganic carbon species, especially those involving $CO_2(aq)$, and the possible role of carbonic anhydrase, is discussed in relation to algal growth rates.

Chemical Reaction Rates. Kern (1960) reviewed the literature on CO_2 hydration and dehydration and pointed out that, whereas the reactions described in Equations 4 and 5 are relatively instantaneous, the rates of hydration and dehydration of CO_2 (Equation 4) are relatively slow.

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-; \text{ Instantaneous.} \quad (4)$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^=; \text{ Instantaneous.} \quad (5)$$

$$CO_2(aq) + H_2O \rightleftharpoons H_2CO_3; \; k_{H_2CO_3} = 26.6 \text{ sec}^{-1} \text{ @ } 25^\circ\text{C.} \quad (3)$$

Hood and Park (1962) claimed that the slowness of CO_2 dehydration favored direct bicarbonate utilization by certain marine phytoplankton, but Watt and Paasche (1963) and Steemann Nielsen (1963) effectively disputed this claim.

Dehydration of carbon dioxide can occur in one of two ways, depending on the pH. For pH values below 8 the reaction described in Equation 3 predominates. At pH values greater than 10, dehydration occurs largely by the following reaction (Kern, 1960):

$$HCO_3^- \rightleftharpoons CO_2 + OH^- \quad (21)$$

Between pH values of 8 and 10 both methods of dehydration are significant. Also in this pH range the normal dehydration of H_2CO_3 to CO_2 and H_2O is catalyzed by OH^- (Kiese and Hastings, 1940).

The rate constants for the dehydration reaction in Equation 6 were studied in detail (Faurholt, 1925; Brinkmann *et al.*, 1933; Roughton, 1941; Scheurer *et al.*, 1958; Sirs, 1958). Roughton (1941), using a thermometric method, determined the rate constant for the dehydration process according to Equation 6 for various temperatures. His rate constant values, plotted in Fig. 6, show a strong temperature dependence, with a value of 26.6 sec^{-1} at 25°C. Rabinowitch (1951) reported the rate constant at 25°C for the dehydration reaction in Equation 24 to be 0.47×10^{-4} sec^{-1}, considerably slower

than in Equation 3, whereas Kern (1960) reviewed the literature and found it to be 2×10^{-4} sec^{-1}.

On the basis of these rate constant values it is possible to examine an algal growth situation in which the rate of dehydration of H_2CO_3 could possibly become a rate-limiting step in algal growth. Rabinowitch (1951) developed a hypothetical situation in which the following conditions prevalied: (1) A pH of over 10; (2) a HCO_3^- concentration of 0.02 M; and (3) only the reaction in Equation 21 took place. He calculated that a maximum of 9×10^{-7} mole CO_2/1 - sec would be available for algal growth. He then considered that a 0.1 percent (by volume) concentration of algae would be under non-limiting light intensity capable of photosynthesizing at a maximum rate of 3.3×10^{-7} mole CO_2/1-sec. Thus, almost three times more CO_2 would be available than would be required by this concentration of algae. The numbers used by Rabinowitch for these calculations may be completely unrealistic when applied to a typical bloom condition in an eutrophic natural water. To show that Rabinowitch's calculations are conservative, similar calculations will be made, based on the following assumptions:

(1) Total bicarbonate alkalinity is equal to 50 mg/ℓ as $CaCO_3$;

(2) The bloom has progressed to the point at which the pH has been raised to 10, so that CO_2 is derived directly from HCO_3^- (Equation 21);

(3) The algal concentration, X, at this point in the bloom is 5 mg/ℓ;

(4) The specific growth rate of the algae, μ, is 0.3 day^{-1};

(5) The carbon content of the algal cells is 50 percent of the total algal dry weight.

Thus, the change in algal concentration with time, dX/dt, can be described as:

$$\frac{dX}{dt} = \mu X = 1.5 \text{ mg/}\ell - \text{day} = 1.74 \times 10^{-5} \text{ mg/}\ell\text{-sec.} \quad \ldots \quad (22)$$

and the change in carbon, C, per unit time transformed into algal biomass is:

$$\frac{dC}{dt} = 0.5\ (\mu X) = 8.7 \times 10^{-6} \text{ mg/}\ell\text{-sec.} \quad \ldots \quad (23)$$

Converting the carbon content to an equivalent molar concentration of bicarbonate (HCO_3^-), we have:

$$\frac{d(HCO_3^-)}{dt} = 7.24 \times 10^{-10} \text{ moles/}\ell\text{-sec.} \quad \ldots \quad (24)$$

Fifty mg/l of $CaCO_3$ alkalinity equals 61 mg/ℓ of HCO_3^- alkalinity, or 1×10^{-3} M of HCO_3^-. Using Rabinowitch's value of 0.47×10^{-4} sec^{-1} for the dehydration rate constant, the ratio of the rate of carbon dioxide available to the rate of carbon dioxide utilized at 25°C is approximately 65:1. With Kern's dehydration rate constant of 2×10^{-4} sec^{-1}, the ratio is approximately 275:1. Under the conditions described the dehydration step is definitely not rate limiting.

To further illustrate this point, a family of curves was constructed comparing the ratio of the rate at which CO_2(aq) was made available by dehydration to the rate at which carbon was utilized by algae with the specific growth rate at varying algal concentrations. These plots, shown in Fig. 7, represent a wide range of eutrophic conditions and approach those that might be present in mass cultures, and were based on assumptions similar to those used in the

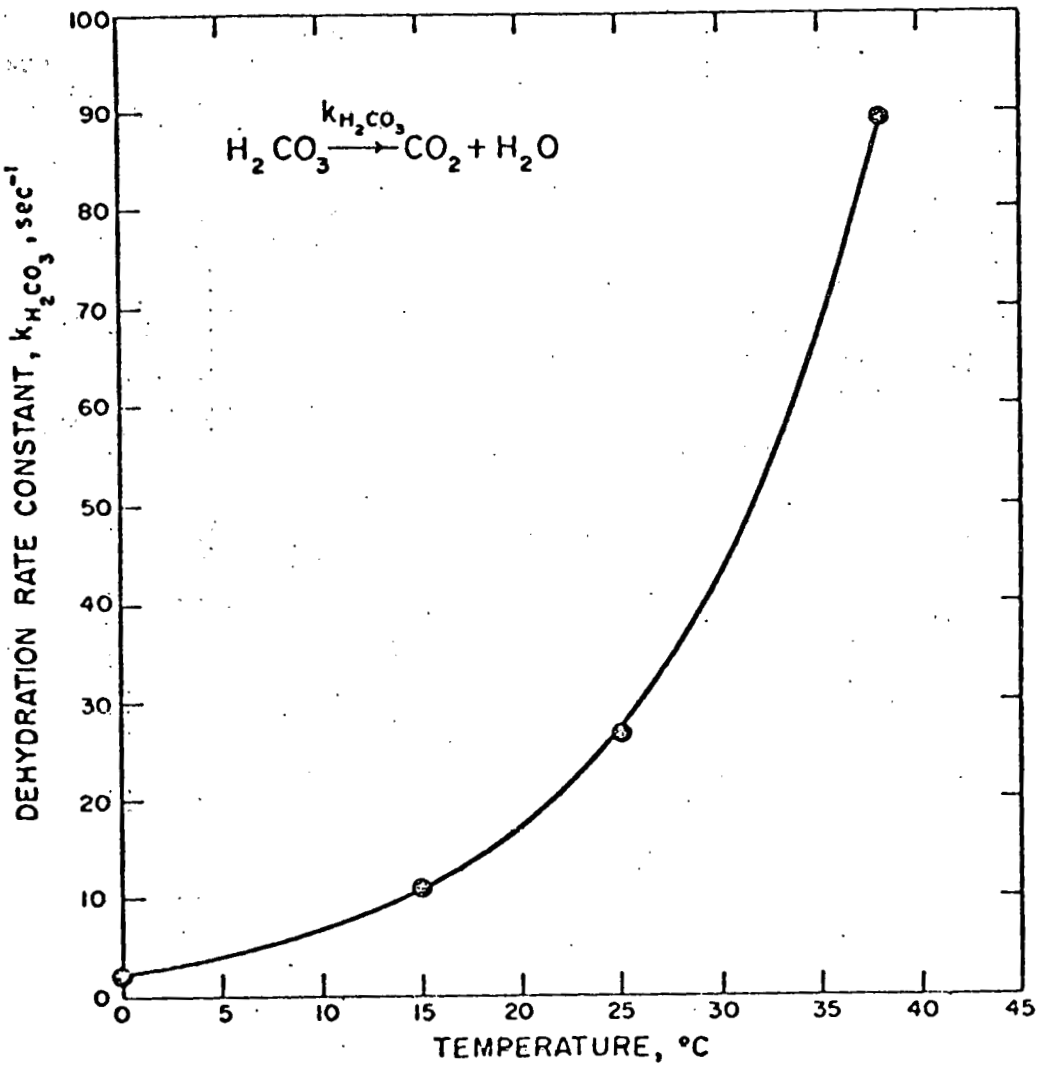

FIGURE 6. EFFECT OF TEMPERATURE ON DEHYDRATION RATE CONSTANT FOR CARBONIC ACID. FROM DATA OF ROUGHTON (1941).

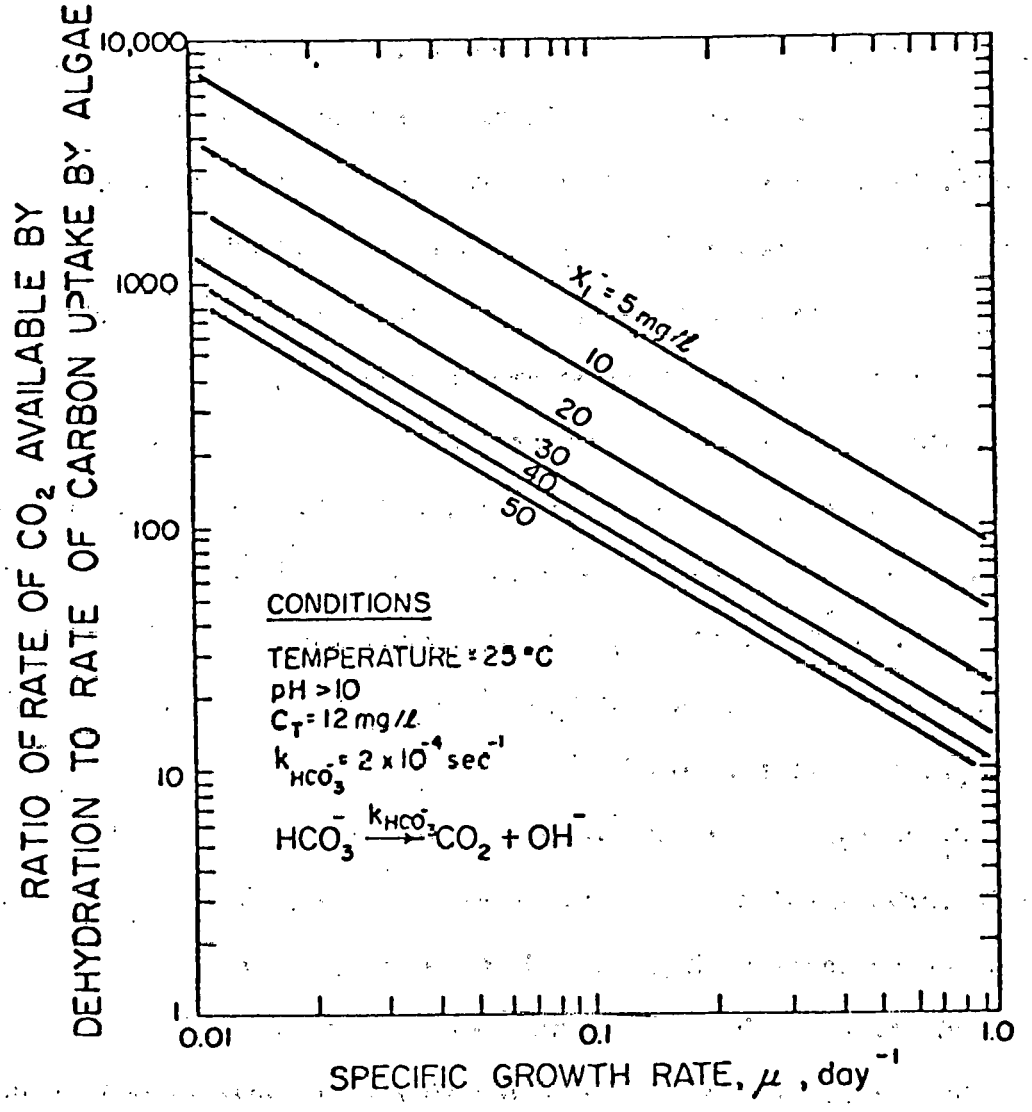

FIGURE 7. COMPARISON OF RATE OF CO_2 AVAILABLE THROUGH DEHYDRATION REACTION AT HIGH pH TO CARBON UPTAKE RATE BY ALGAE FOR VARYING SPECIFIC GROWTH RATES (μ) AND ALGAL CONCENTRATIONS (X_1).

previous example. Even under the most severe bloom conditions described in these curves--50 mg/ℓ of algae and a specific growth rate of 1.0 day^{-1}--the rate at which $CO_2(aq)$ is made available by the dehydration reaction is over 8 times as great as the rate at which it is used by the algae. Even if the available inorganic carbon level were reduced to 3 mg/ℓ then there would still be twice as much $CO_2(aq)$ available than as required. However, for mass algal cultures both algal standing stocks and growth rates are high in order to maximize yields, and without supplementary CO_2 the dehydration reaction could conceivably become rate-limiting.

Even though the crudeness of these calculations is recognized (no accounting was made for temperature and activity affects, nor was CO_2 transport from the atmosphere considered) the results are believed to be conservative because algal blooms usually start at pH values considerably lower than 10 where the faster dehydration reaction, $H_2CO_3 \rightleftharpoons CO_2 + H_2O$, predominates, making HCO_3^- more readily convertable to CO_2.

If CO_2 dehydration is not rate limiting then algal growth rates in an inorganic carbon-limited situation should be a function of the total inorganic carbon concentration (C_T), and not of any one of its components (e.g. $CO_2(aq)$, HCO_3^-), even though only one form of carbon may be assimilated. Shown in Fig. 8 are three ways in which inorganic carbon may be made available for algal growth. In the first system the only source of inorganic carbon is gaseous CO_2. Bicarbonate alkalinity is absent. This system is usually found in laboratory cultures in which excess CO_2 is sparged into the medium.

The second system contains bicarbonate alkalinity, and is equilibrated

with a gas phase of CO_2. In this system which is typical of mass cultures the equilibrium pH is a function of bicarbonate alkalinity, the partial pressure of the sparged CO_2, and the carbon assimilation rate by the algae. Because algal assimilation of inorganic carbon can possibly take place in any of the carbon forms, the adjustment of the $H_2CO_3^* - HCO_3^- - CO_3^=$ system is dependent solely on the pH and alkalinity. The pH, in turn, is controlled by the rate of carbon assimilation by the algae and the sparging rate. Regardless of whether $CO_2(aq)$ or HCO_3^- is the form of carbon being taken up by the algae, the effect on pH will be the same. This concept is often disregarded by algal researchers.

The third system, containing only bicarbonate alkalinity as the inorganic carbon source, represents most natural waters. CO_2 from the atmosphere, although being the ultimate regulator of pH in many natural waters, plays a small role in providing inorganic carbon for algal growth at the neutral pH values at which most blooms originate, because it diffuses slowly, and because the concentration gradient between the gas and liquid phases is small. This is evidenced by the commonly observed rise in pH in natural waters during a bloom, as HCO_3^- and $CO_3^=$ provide the bulk of the available inorganic carbon.

The Role of Carbonic Anhydrase

The presence of carbonic anhydrase, an enzyme that catalyzes the dehydration of CO_2, is another factor strengthening the argument against $CO_2(aq)$ production by the reactions of Equations 3 and 21 as being a rate-limiting step for carbon utilization by algae.

Carbonic anhydrase, which catalyzes H_2CO_3 dehydration during human respiration (Meldrum and Roughton, 1933), has a very high activity, being able to double this dehydration when present in minute concentrations of 1 μg/ℓ or less (Edsall and Wyman, 1958). Although its role in human respiration is quite well defined, little information is available on how it affects H_2CO_3 dehydration in algal systems. Both Steemann Nielsen and Kristiansen (1949) and Osterlind (1950b) tried to explain direct HCO_3^- utilization in certain aquatic plants and algae by demonstrating a lack of this enzyme. However, the enzyme was found in all species they examined, whether or not they used HCO_3^-, and they could not draw positive conclusions regarding its role. Litchfield and Hood (1964) demonstrated that the enzyme was present in the cellular protoplasm of 11 fresh water and marine algae. Nelsen *et al.* (1969) found that the carbonic anhydrase concentration in *Chlamydomonas* was 20 times greater when the cells were grown on 0.03 percent CO_2 than on 1 percent CO_2, suggesting that its activity becomes more important at pH values higher than those predicted by equilibrium with atmospheric CO_2. It is also possible that the enzyme mediates the direct dehydration of HCO_3^- (Equation 21). Graham and Reed (1971) proposed that the enzyme may play a role in regulating the intracellular proton gradient produced during photophosphorylation. Thus, once CO_2 entered the cell the enzyme would catalyze the hydration reaction of Equation 21 and produce H^+.

Although there is no direct evidence that carbonic anhydrase is extracellular, Berger and Libby (1969) suggested that its presence in the oceans

could be a significant factor in equilibrating atmospheric CO_2 with the CO_2 content of sea water. Krishnamurty (1969) reported the possibility of metal carbonate complexes aiding in this enzymatic process.

Longmuir *et al.* (1966), Enns (1967), Ward and Robb (1967) and Broun *et al.* (1970) demonstrated enhanced CO_2 transport as bicarbonate ions across artificial membranes containing a solution of bicarbonate and carbonic anhydrase. The development and successful demonstration of such transport systems in artificial membranes is suggestive of the role of carbonic anhydrase in algal photosynthesis. If the enzyme is indeed embedded in the outer cell membrane, then it could play a major role in carrying CO_2 and/or HCO_3^- across the membrane, so that CO_2 could be directly available for incorporation into the Calvin cycle and HCO_3^- could be dehydrated to provide additional CO_2. Because the Calvin cycle operates in chloroplasts, transport of CO_2 to/and across the chloroplast membrane would also be required. Perhaps carbonic anhydrase is also present in this membrane.

The specific role of carbonic anhydrase in inorganic carbon uptake by algae in natural waters is still unanswered. The very fact that its presence has been demonstrated in many algal systems suggests that it may be intimately involved in providing carbon for algal growth when the free CO_2 content of a water is low. It would appear that to effectively influence inorganic carbon uptake carbonic anhydrase would have to be extracellular or a surface-bound enzyme.

Effect of Ionic Strength

Ionic strength greatly influences the composition of the $H_2CO_3^*$ - HCO_3^- -

$CO_3^=$ system. Morton and Lee (1968a) calculated an activity coefficient of 0.74 for $CO_3^=$ in Lake Mendota in Wisconsin (ionic strength = 0.0045). Similarly, Park *et al.* (1970) found the ionic strength of the Columbia River to be 0.0018, giving an activity coefficient for $CO_3^=$ of 0.83. Because of these results, activity rather than concentration must be considered in any generalized formulation of the $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system, particularly for sea water (Berner, 1965), as well as for fresh waters.

The increasing effect of ionic strength on activity with increasing valency can be seen in Fig. 9, in which the strong dependence of $CO_3^=$ activity relative to the monovalent ions is demonstrated (Klotz, 1964).

Effect of Temperature

Langelier (1946) and Dye (1952) discussed the importance of temperature on the equilibrium constants of the H_2CO_3 - HCO_3^- - $CO_3^=$ system. As shown in Figure 10, the acid-base equilibrium constants increase, while the solubility coefficient of CO_2 decreases with increasing temperature (Harned and Scholes, 1941; Harned and Davis, 1943; Harned and Bonner, 1945; Harned and Owen, 1958).

ALGAL GROWTH AND pH CHANGES

The extraction of CO_2 through algal assimilation at a rate faster than it can be replaced through transport from the atmosphere, from respiration, from fermentation processes, and from dissolution of carbonate containing solids leads to an increase in pH. This rise in pH can affect algal growth in the following ways:

(1) A change in the carbon species: Aqueous CO_2 concentration is reduced,

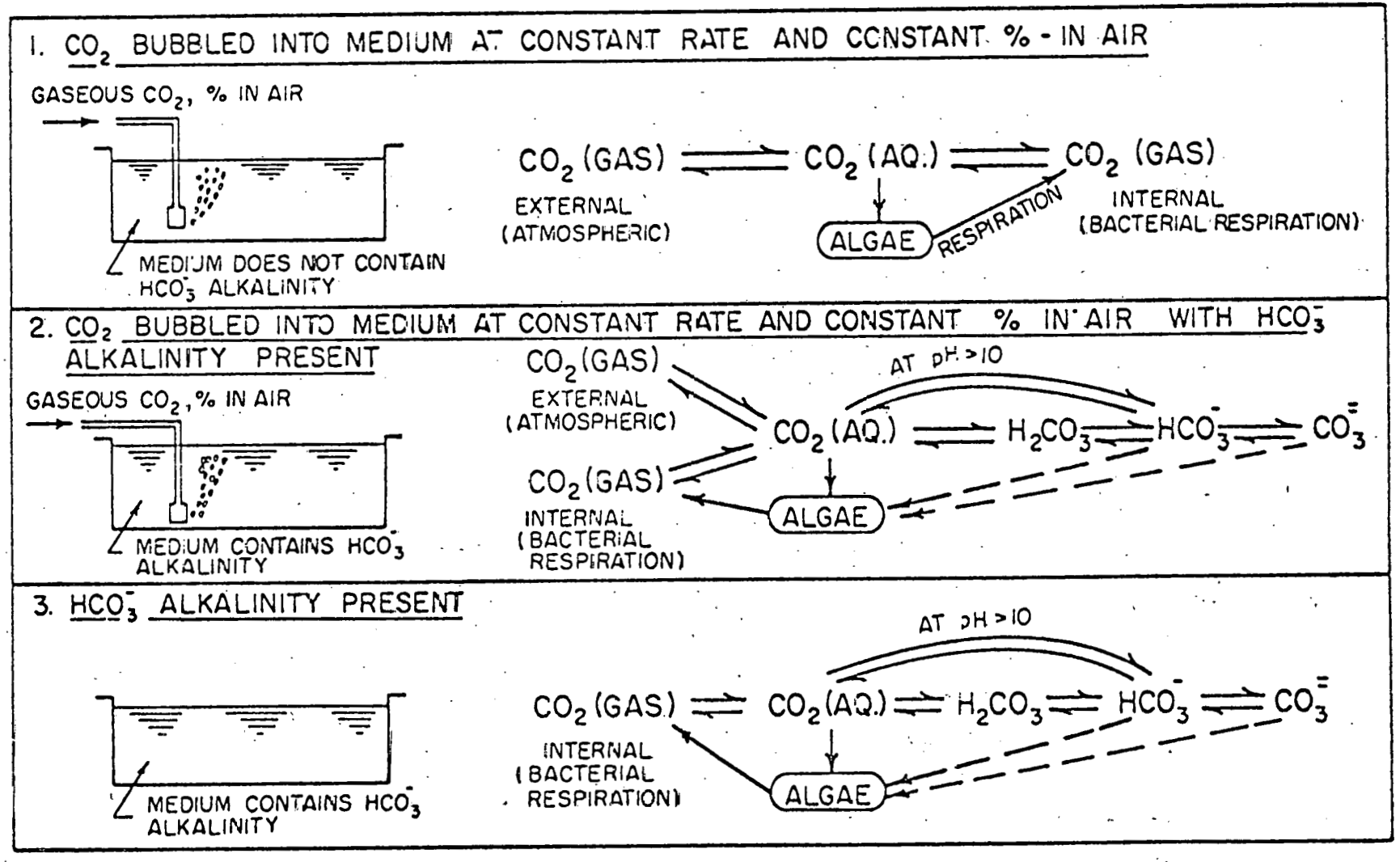

FIGURE 8. THREE ALGAL GROWTH SYSTEMS: INTERACTION OF ALGAL GROWTH WITH $H_2CO_3^* - HCO_3^- - CO_3^=$ EQUILIBRIUM SYSTEM IN PRESENCE OF GASEOUS CO_2 AND/OR BICARBONATE ALKALINITY.

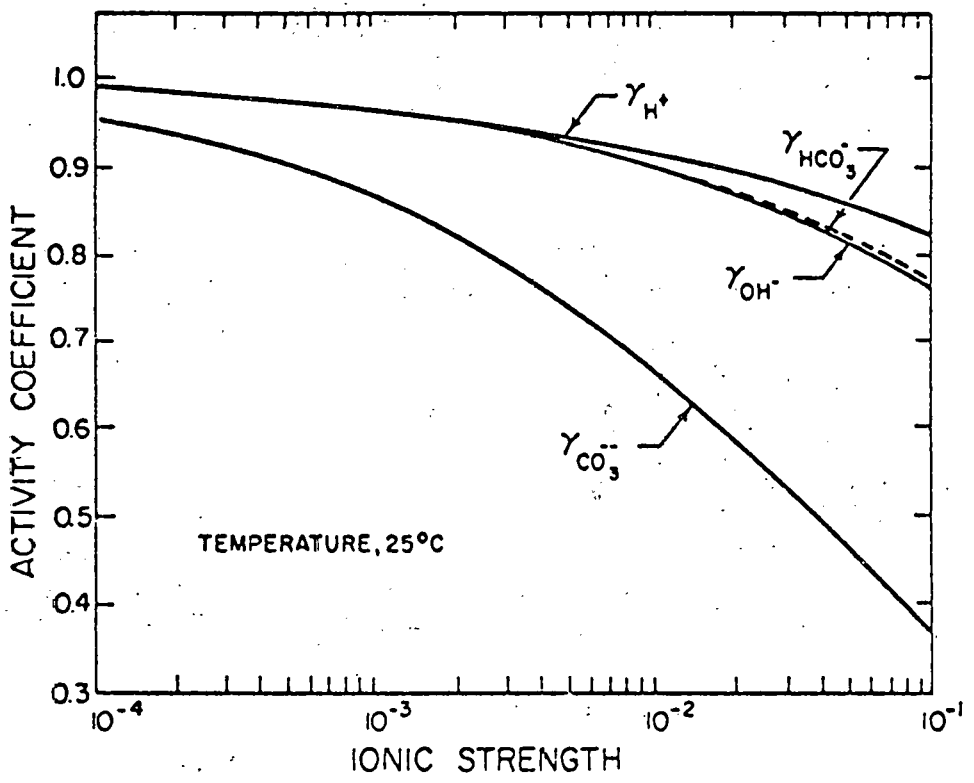

FIGURE 9. EFFECT OF IONIC STRENGTH ON ACTIVITY COEFFICIENTS FOR COMPONENTS OF $H_2CO_3^* - HCO_3^- - CO_3^=$ SYSTEM. FROM DATA OF KLOTZ (1964).

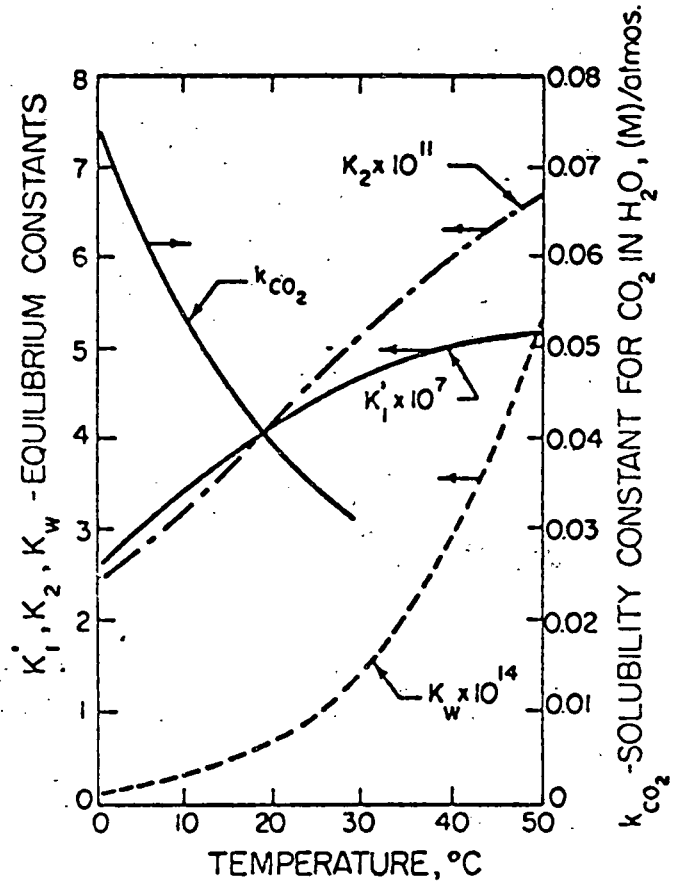

FIGURE 10. EFFECT OF TEMPERATURE ON EQUILIBRIUM CONSTANTS AND SOLUBILITY COEFFICIENT IN $H_2CO_3^* - HCO_3^- - CO_3^=$ SYSTEM. FROM DATA OF HARNED AND SCHOLES (1941), HARNED & DAVIS (1943), HARNED & BONNER (1945), & HARNED & OWENS (1958).

and HCO_3^- and $CO_3^=$ become predominant. Research to date indicates that certain algae growing autotrophically can use only aqueous CO_2 as their carbon source; others, notably blue-green algae dominate in higher pH environments and may be able to take up HCO_3^- and even $CO_3^=$ directly (Jackson, 1964), and then convert it to aqueous CO_2 for assimilation via the Calvin cycle, as shown by Cooper *et al.* (1969). A controversy exists today over whether or not these ions are taken up directly by green algae (Raven, 1970), and by blue-greens (Holm-Hansen, 1967).

(2) Solubility: The solubility of other essential nutrients, particularly phosphorus, iron, and trace elements is decreased (most commonly by the formation of solids). Algal growth could then become limited by one or more precipitated, and hence unavailable, elements.

(3) Metabolic effects: In as much as all enzymes have pH optima, extreme pH values affect the metabolic mechanisms of all living organisms, and algal growth could be altered at the higher pH values found in some active algal systems.

Biological Effects on pH

Biological activity can alter the pH of a natural water in many ways. In Table I, taken from Weber and Stumm (1963a), is depicted the various biological reactions that may alter pH. For example, Berner *et al.* (1970), demonstrated that sulfate reduction and oxidation of organics in marine sediments increased alkalinity, and hence buffering capacity, through the production of HCO_3^-. Because many of these reactions are localized in the

TABLE I

BIOLOGICALLY MEDIATED REACTIONS AFFECTING pH IN NATURAL WATER SYSTEMS*

Process	Reaction	Effect on pH
Photosynthesis	$6(CO_2) + 6(H_2O) \rightarrow (C_6H_{12}O_6 + 6O_2)$	Increase
Respiration	$(C_6H_{12}O_6 + 6O_2) \rightarrow 6(CO_2) + 6(H_2O)$	Decrease
Methane Fermentation	$(C_6H_{12}O_6 + 3(CO_2) \rightarrow 3(CH_4) + 6(CO_2)$	Decrease
Nitrification	$(NH_4^+) + 2(O_2) \rightarrow (NO_3) + H_2O + 2(H^+)$	Decrease
Denitrification	$5(C_6H_{12}O_6) + 24(NO_3) + 24(H^+) \rightarrow 30(CO_2) + 12(N_2) + 42(H_2O)$	Increase
Sulfide Oxidation	$(HS^-) + 2(O_2) \rightarrow (SO_4^=) + (H^+)$	Decrease
Sulfate Reduction	$(C_6H_{12}O_6) + 3(SO_4^=) + 3(H^+) \rightarrow 6(CO_2) + (HS^-) + 6(H_2O)$	Increase

*From Weber and Stumm (1963a)

sense that they occur only in certain portions of an aquqtic ecosystem (i.e. photosynthesis in the photic zone, reduction processes in axonic portions of the bottom sediments), gradients of decreasing pH with increasing depth are very often found in natural waters. These gradients can be maintained by thermal stratification. With the onset of spring and fall overturn, relatively equal pH levels are produced for short times throughout the volume of the water body. But, the formation of new thermal gradients, together with the continuous biological activity, again causes pH gradients to form (Lee and Hoadley, 1967).

Effects of Nitrogen Transformations. The effect of actively growing algae on the pH of surface waters can be better understood by an analysis of the complete photosynthetic reaction. In any such analysis one should consider the assimilation of all nutrients and the formation of complete products. The photosynthetic equation (the first reaction of Table I) should thus be expanded from its simplified form.

As an example, the assimilation of nitrogen may increase or decrease the pH of a water, depending on the form of nitrogen being assimilated. If ammonium (NH_4^+) is used the pH will tend to decrease, whereas the utilization of nitrate (NO_3^-) will tend to cause a rise in pH. This phenomenon has been observed by a number of researchers (Trelease and Trelease, 1935; Cramer and Myers, 1948; Davis *et al.*, 1953). Cramer and Myers (1948), working with *Chlorella*, proposed the following stoichiometric equations to describe this situation:

Nitrate Assimilation

$$1.0\ (NO_3^-) + 5.7\ (CO_2) + 5.4\ (H_2O) \xrightarrow{LIGHT} (C_{5.7}H_{9.8}O_{2.3}N_{1.0}) + 8.25\ (O_2) + 1.0\ (OH^-) \quad \ldots\ldots\ldots\ldots\ldots\ldots (25)$$

Ammonium Assimilation

$$1.0\ (NH_4^+) + 7.6\ (O_2) + 17.7\ (H_2O) \xrightarrow{LIGHT} (C_{7.6}H_{8.1}O_{2.5}N_{1.0}) \quad \ldots\ldots (26)$$

Every mole of NO_3^- assimilated results in the formation of one mole of OH^-, thus raising the pH. NH_4^+ assimilation, on the other hand, leads to the production of one mole of H^+ for every mole of NH_4^+ assimilated, with a resulting decrease in pH. These effects were clearly shown by Brewer and Goldman (1976).

Effects of Carbon Transformations. Algae may utilize CO_2 generated from one or more of four sources in a natural water: (1) CO_2 diffused from the atmosphere; (2) the respiration of heterotrophic forms; (3) anaerobic fermentation; and (4) bicarbonate alkalinity. The first three sources provide a direct supply of CO_2. The alkalinity, on the other hand, provides CO_2 by a continual readjustment of the concentrations of the various carbon sources making up the $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system as shown by the following equations:

$$2(HCO_3^-) \rightleftharpoons (CO_3^=) + (H_2O) + (CO_2) \quad \ldots\ldots\ldots\ldots (27)$$

$$(HCO_3^-) + (H_2O) \rightleftharpoons (H_2O) + (CO_2) + (OH^-) \quad \ldots\ldots\ldots (28)$$

$$(CO_3^=) + (H_2O) \rightleftharpoons (CO_2) + 2(OH^-) \quad \ldots\ldots\ldots\ldots (29)$$

Typically, the pH of natural waters at or near equilibrium with air is about 8.3, and HCO_3^- is the major ion (see Fig. 6). As CO_2 is extracted from

solution by growing algae at a pH of approximately 8.3, additional carbon dioxide is provided through these reactions. The principal reactions are described by Equations 27 and 28, with the reaction in Equation 27 predominating. As the pH increases, $CO_3^=$ becomes the major carbon species and it, too, can be converted directly to CO_2 by a hydration process as shown in Equation 29. This reaction similarly results in a pH rise. It is not uncommon to have pH values as high as 10 to 11 in active algal systems such as waste stabilization ponds (Caldwell, 1946; Allen, 1955; Golueke *et al.*, 1962; Pipes, 1962; Beck *et al.*, 1969). This rise in pH demonstrates that the CO_2 supplied from the first three sources mentioned is either unavailable (i.e., diffusion gradients) or insufficient to meet the demands of the growing algae, and that a further demand is placed on the bicarbonate alkalinity through a readjustment of the $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system. Therefore, when the free CO_2 content of a water is insufficient to meet the demand of the algae, the HCO_3^- and $CO_3^=$ forms can continually supply free CO_2 for algal utilization. Deuser (1970) showed that the carbon utilized by the diatom *Chaetoceros curvisetum* during intense algal activity in the Black Sea was derived from inorganic carbon species other than free CO_2 after the initial free CO_2 was depleted.

Only a portion of the total inorganic carbon can be extracted during intense algal activity in a natural water principally buffered by the $H_2CO_3^*$ - HCO_3^- - $CO_3^=$ system. If all the HCO_3^- and $CO_3^=$ were converted to CO_2 and OH^- as described by Equations 30-32, the pH would approach a very high value dependent on alkalinity. Metabolic inhibition of algal growth usually occurs at a pH between 10 and 11, and thus places an upper limit on the amount of CO_2 available from HCO_3^- and $CO_3^=$.

No changes in the total alkalinity of the system occur when free CO_2 is utilized by algae, as there is no change from electrical neutrality (Sawyer and McCarty, 1967). This would also hold true for direct HCO_3^- utilization if OH^- replaced HCO_3^- to maintain a charge balance, as depicted by Equation 28.

However, changes in the total alkalinity occur due to nitrogen assimilation (Equations 25 and 26). Nitrate uptake causes an increase in total alkalinity through OH^- production and leads to a decrease in alkalinity. Similarly, as the pH rises due to algal growth, salts of $CO_3^=$ and eventually OH^- will begin to precipitate out of solution with a corresponding reduction in alkalinity. Also, the concentration of other minor alkalinity components such as phosphates could be affected by uptake during algal growth, but such effects on alkalinity will be relatively minor in the normal case.

Effect of pH on Solubility of Essential Algal Nutrients.

The solubility of a number of essential algal nutrients is greatly affected by pH changes. The solubility of key nutrients such as iron, phosphorus, and trace elements (i.e. manganese, molybdenum, zinc, etc.) are controlled by pH and the concentrations of other ions such as hydroxide, calcium, and magnesium. Stumm and Morgan (1970) present a detailed discussion of the chemical equilibria that determines the availability of these nutrients. For most natural waters the concentrations of the multivalent cations are controlled by the solubility of their hydroxides and carbonates. As can be seen in Fig. 11, the solubility of a number of trace elements, of iron (both the ferric and ferrous forms), of magnesium, and of calcium is controlled by pH. For cations

that form sparingly soluble carbonates the pH determines whether the hydroxide or the carbonate compounds controls metal ion solubility. As an example, the solubility of ferrous iron in a water with a carbonate concentration of 2×10^{-4} M is controlled by carbonate for a pH value less than 9.5, and by hydroxide at a pH above this value.

Although complex formation is not considered in Fig. 11, in a complete analysis of solubility one should consider the formation of all soluble and insoluble compounds. As an example of the important role that complex formation plays in determining the solubility of a nutrient, complexes of ferric iron in pure water have a minimum solubility at a pH of approximately 8.5. This solubility increases with increasing or decreasing pH because ferric iron can form a number of soluble hydroxide complexes such as $FeOH^{+2}$, $Fe(OH)_4^-$, and $Fe(OH)_2^+$ (Fig. 12). However, over the entire pH range in which algal growth occurs (6-11) ferric iron is highly insoluble, the maximum soluble concentrations of iron complexes being, at the most, 1-2 µg/ℓ.

Surprisingly little attention has been given to the role of iron as a limiting nutrient in intense cultivation systems. Perhaps the difficulty in making accurate determinations of iron concentration in the µg/ℓ range, in differentiating between soluble and colloidal iron, in understanding the interrelationships between iron and organic matter, and in understanding the difficult chemistry of iron has discouraged researchers from studying this important nutrient.

The solubility of phosphorus in natural waters and mass cultures is largely controlled by pH and calcium concentration. The equilibrium solid calcium phos-

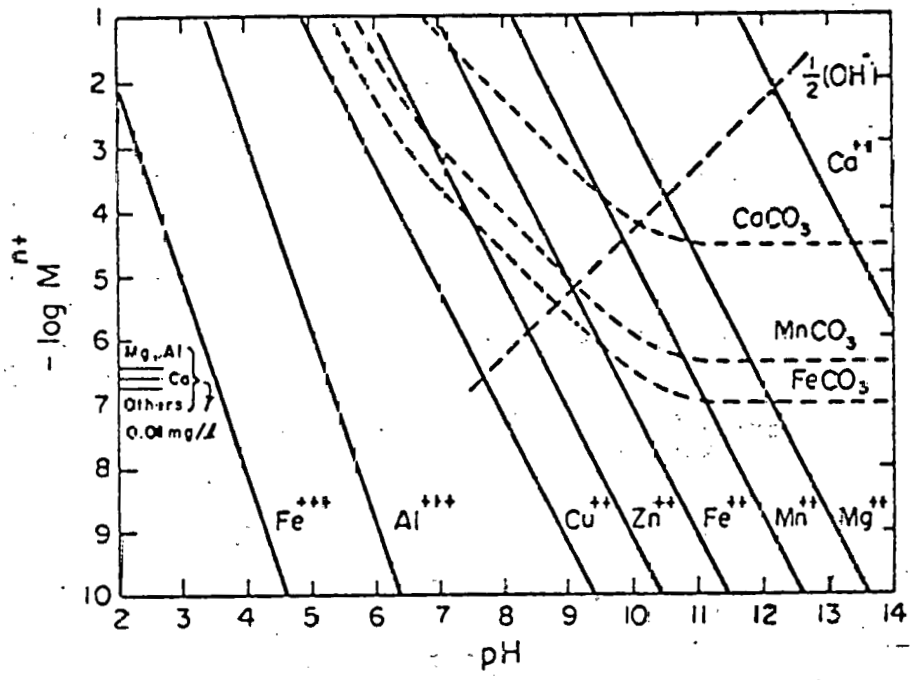

FIGURE 11. LOGARITHMIC CONCENTRATION DIAGRAMS FOR SOLUBILITIES OF HYDROXIDES AND CARBONATES. FROM STUMM AND MORGAN (1970)

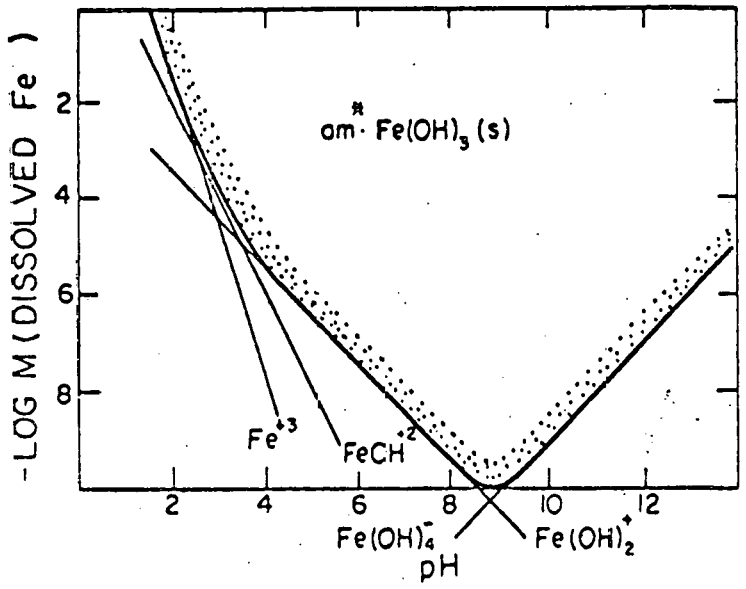

FIGURE 12. LOGARITHMIC CONCENTRATION DIAGRAM FOR SOLUBILITY OF AMORPHOUS $Fe(OH)_3$. FROM STUMM AND MORGAN (1970).

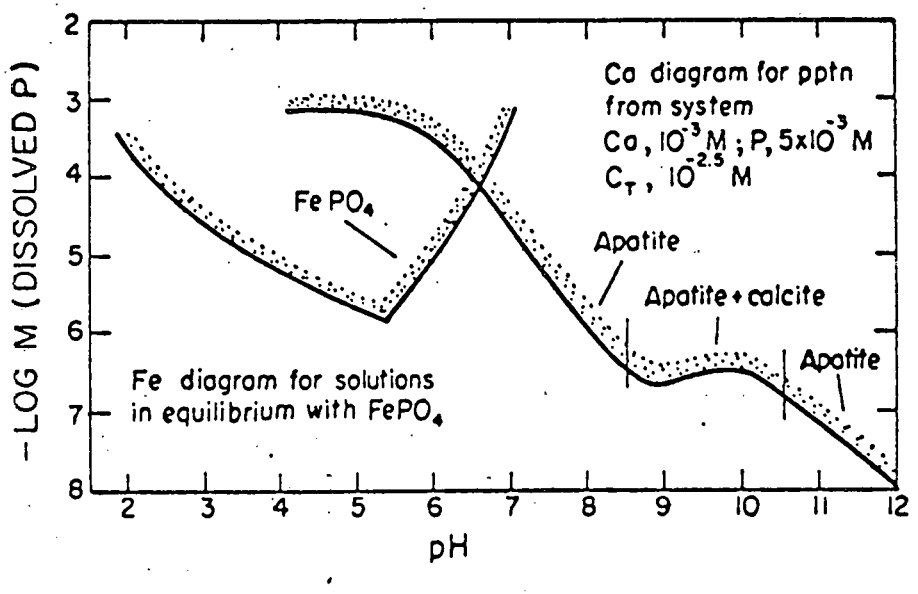

FIGURE 13. LOGARITHMIC CONCENTRATION DIAGRAM FOR SOLUBILITY OF Fe AND Ca PHOSPHATES. FROM JENKINS ET AL. (1971).

phate phase in typical natural fresh waters is hydroxylapatite ($Ca_5OH(PO_4)_3$). This solid forms slowly, and generally its equilibria does not control the phosphate concentrations of natural fresh waters. The formation of such compounds as metastable precursors to hydroxylapatite, hydrolysis products on apatite surfaces, carbonate apatites, and metastable phases such as beta-tricalcium phosphate, make possible the existence of steady-state phosphate concentrations far in excess of the 6 µg/ℓ one would predict from the equilibrium of pure hydroxylapatite (pK = 57) with pure water.

An example of the influence of pH on phosphorus availability can be seen in Fig. 13, in which equilibrium solubility diagrams for iron and calcium are presented, showing the effect of calcite on hydroxylapatite solubility over a wide pH range. Although the solubility curves shown in this figure describe a simplified aqueous system not truly representative of natural waters, they do serve to illustrate the complexity of phosphorus chemistry.

From the foregoing examples it is apparent that the $H_2CO_3^* - HCO_3^- - CO_3^=$ system, through its influence on pH, plays a major role in controlling the solubility, and hence the availability, of a number of essential algal nutrients.

pH Effects on Algal Growth

Hydrogen ion concentration exerts a profound effect on algal growth and metabolism, largely through its effect in the protonation and deprotonation of key enzyme systems. Algae of various species show different pH optima for growth, and it is plausable that these are due to the effect of H^+ on various enzymes in these organisms. Evidence exists that microbial cells

have negatively charged outer membrane surfaces, resulting in the attraction of H^+ from the bulk fluid. The cell surface pH is thus lower than in the bulk fluid, causing the observed pH optima for surface enzyme reactions to be shifted to the alkaline region, as compared to the lower observed pH optima for the same enzyme reaction in solution (McLaren and Packer, 1970; Katchalski *et al.*, 1971). The lowered pH at the algal cell surface undoubtedly results in an increase in the CO_2(aq) concentration in this region over that observed in the bulk fluid. Because cell surface pH is a function of the cell surface isoelectric point - distinct for each algal species - it is possible that different algal species, though seeming to have different pH optima for growth, may in fact, at these observed pH optima values, have closer surface pH values and resulting inorganic carbon species distribution. No studies have been attempted to examine the effect of cell surface pH on algal growth, although Kolin (1955) and Ives (1959) studied the electrical properties of several algae while investigating methods for their removal from the liquid phase.

On the other hand, King (1970) suggested that pH, through its control of the free CO_2 concentration, played a major role in regulating the distribution of algal species in natural waters. Although this hypothesis has never been thoroughly examined, pH may play a very important role in the selection of certain algal species in natural waters for other reasons, presently unknown. McLachlan and Gorham (1962), Jackson (1964), and Holm-Hensen (1967) pointed out the blue-green algae appear to be favored by a more alkaline environment in natural waters. Allen (1953) demonstrated species change in

oxidation ponds with increases in pH from the neutral to the alkaline regions.

Paasche (1964) and Swift and Taylor (1966) claimed to isolate the effects of pH on the growth of marine coccolithophorids. Paasche (1964) showed that carbon uptake in the coccolithophorid *Coccolithus huxleyi* was highest at a pH of 7.5. Swift and Taylor (1966) showed an optimum pH of 7.8 for cell division of the coccolithophorid *Cricosphaera elongata.*

Zabat (1970) grew *Chlorella pyrenoidosa* (high temperature strain) in a phosphorus-limited continuous culture, and showed a decrease in cell yield with increasing pH values between 7.0 and 8.25. Emerson and Green (1938), on the other hand, could show no change in *Chlorella* photosynthetic rates over a pH range from 4.6 to 8.9.

Soltero and Lee (1967), in demonstrating an automatic pH control device for algal cultures, gave evidence that optimum growth of *Scenedesmus* occurred at a pH of 7. This was in contrast to the work of Witt and Borchardt (1960) and Gates and Borchardt (1964) who could show little change in the growth of *Scenedesmus* over a wide pH range, although best growth was observed at a pH of 8.3. Brown (1969), when growing *Scenedesmus* on agricultural tile drainage (alkalinity = 350 mg/ℓ as $CaCO_3$) in which pH was controlled with "Good" organic acid buffers (Good *et al.*, 1966), achieved best growth at a pH of 8.4 (as compared to other tested pH values of 6.15, 7.5, and 10.5), in agreement with the results of Witt, Gates and Borchardt.

Gerloff *et al.* (1952) showed that the maximum yield of the blue-green alga, *Microcystis aeruginosa,* cultured in an unbuffered medium, occurred at a pH of 10. Rand and Nemerow (1965) presented similar results for the same

species, while McLachlan and Gorham (1961) and McLachlan (1962) observed little change in growth in a pure culture over a pH range of 6.5 to 10 in a well-buffered medium. When they tried to grow this alga together with *Scenedesmus* at a pH of 7.4 they obtained less than one-third of the growth reached by the blue-green alga in pure culture. There appeared to be a definite competitive effect at the lower pH, indicating that only at the higher pH values would the blue-green alga predominate. Eberly (1967) reported that in batch cultures of *Oscillatoria agardhii,* another blue-green algae, those cultures with the highest initial pH values (up to 10) reached the exponential phase earliest, but that all cultures eventually reached the same level of maximum biomass.

The optimum pH for several enzymes in the Calvin cycle was studied by Preiss *et al.* (1967) and Bassham *et al.* (1968). Preiss *et al.* (1967) found that by increasing the magnesium concentration the pH optimum for fructose diphosphatase activity, an enzyme of the Calvin cycle, was decreased. Bassham *et al.* (1968) found similar effects of magnesium on the pH optimum of ribulose diphosphate carboxylase.

Ouellet and Bensen (1952) showed that the initial incorporation of CO_2 in *Scenedesmus* switched from three-carbon compounds to four-carbon compounds when the pH was raised rrom the acid to the alkaline regions. pH control of enzyme activity was felt to be the main factor controlling the shift in carbon compound synthesis.

More recently, Goldman *et al.* (1974) demonstrated that under inorganic carbon limitation growth rates of two freshwater green algae were controlled

by the total inorganic carbon concentration and that even within a small range of pH between 7.1-7.6 half-saturation coefficients for growth increased with increasing pH. Gavis and Ferguson (1975) expanded on this concept by developing a model to account for any mass transport (or diffusion) limitations that might exist at high pH when CO_2 concentrations are very low and CO_2 is the only form of inorganic carbon available. In the study of Goldman *et al.* (1974) no attempt was made to determine which form of inorganic carbon was utilized since the imposed growth conditions were such that the rate of CO_2 provided from HCO_3^- via Equations 3 and 21 were always greater the rate at which inorganic carbon was assimilated by the test algae. Hence, the effect of total inorganic carbon on growth rates was indistinguishable from any of the inorganic carbon species. This situation more than likely would not occur in mass cultures if HCO_3^- was the only source of inorganic carbon. In addition, unless good mixing is established the mass transfer of CO_2 to an algal cell could become limiting, as suggested by Gavis and Ferguson (1975).

B. Previous Mass Culture Studies on CO_2 Effects:

It has long been recognized that gaseous CO_2 must be supplied to algal mass cultures to optimize yields. Cook (1951) in one of the earliest mass culture experiments with *Chlorella* suggested that a 5% CO_2 mixture with air bubbled into the culture optimized inorganic carbon requirements. The early Japanese workers, developed both the "outdoor bubbling technique" (Morimura *et al.*, 1955) and the "open circulation method" (Kanazawa *et al.*, 1958) for optimizing the supply of CO_2 to algal cultures, but found that, because the

culture surface was exposed to the atmosphere, "an enormously large quantity of CO_2-enriched air is required for aeration, and a considerable part of CO_2 is wasted without being utilized by algal cells." Similarly, Oswald and co-workers at Berkeley, in their early experiments with wastewater-grown freshwater algae, found substantial inorganic carbon limitations existing in their cultures that could be overcome by enrichment with CO_2-air mixtures bubbled into the cultures (Ludwig *et al.*, 1951, Oswald *et al.*, 1953). In the German mass culture experiments at Dortmund, Germany (Söeder, 1976) only about one-half the CO_2 supplied through aeration was actually assimilated by the algae. In none of these studies was any attempt made to optimize the efficiency of CO_2 use by consideration of the interrelationships between the CO_2 - HCO_3^- - CO_3^{-2} chemical equilibrium system controlled by the alkalinity present and the added gaseous CO_2 as they were affected by inorganic carbon assimilation during photosynthesis.

Pipes (1962), in a laboratory study showed for inorganic-carbon limited growth of algae in continuous culture, that there was a linear relationship between steady state algal concentration and cell residence period for a fixed rate of CO_2-air addition. This was the first attempt to optimize the addition of CO_2 to a mass algal culture. Unfortunately however, no consideration was given to the role of alkalinity in regulating the availability of the CO_2.

Some attempts to add very high CO_2 in air mixtures have resulted in apparent toxicity effects (Steeman-Neilson, 1955, Sorokin, 1962, Brown, 1971 Shelef, 1976). Others (Tew *et al.*, 1962, Fowler, *et al.*, 1972) have demon-

strated no adverse affects on *Chlorella* using virtually 100% CO_2 additions. An important factor not considered in any of these studies is the rate of addition and the efficiency of diffusion.

Work at the Trebon mass culture laboratories in Czechoslovokia was addressed towards answering some of the fundamental questions of CO_2 mass transfer and diffusion in liquid cultures of growing algae (Necas and Chotsky, 1969; 1970), and although significant technological advances were made in these studies, once again no accounting was made for the effects of alkalility and pH on the efficiency of CO_2 transfer and use.

With marine systems the requirements for gaseous CO_2 are not as severe as with freshwater cultures because of the substantial alkalinity (two milliequivalents/liter) and resulting total inorganic carbon concentration (25 mg/ℓ) present in seawater. Yet, in even these systems inorganic carbon limitation can exist and high culture pH levels can occur if supplementary CO_2 is not provided. Goldman and Ryther (1975) showed that very strong mixing to enhance CO_2 transport from the atmosphere was required in mass cultures of marine diatoms grown on wastewater-seawater mixtures to meet the full inorganic carbon requirements of the algae and prevent the pH from rising to growth-inhibiting levels; even still, mid-day pH values rose to over 10 as CO_2 derived from HCO_3^- present was utilized along with the CO_2 added during mixing. No attempt was made to add CO_2-enriched air and control the pH, however.

The growth or seaweeds in mass culture presents serious possibilities of inorganic carbon limitation (Jackson, 1977). Little is known about the

inorganic carbon requirements of macroalgae although Jolliffe and Tregunna (1970) showed that photosynthetic rates of a number of macrophytes were a function of the total inorganic carbon present in seawater. The culture chamber used in these studies was well mixed with recirculating seawater and, thus, problems of mass transport of CO_2 at the leaf surface may have been eliminated. The problem of mass transport limitation of CO_2 at the plant surface is probably very important in seaweed mass cultures and virtually no research has been focussed on this important research topic. The mixing requirements in seaweed mass cultures are completely unknown and, as shown by Lapointe *et al.* (1976), even in well-flushed and aerated (with air) seaweed cultures pH values increased substantially (>9) indicating a drain on the HCO_3^- alcalinity.

Interpretation of the divergent results reviewed in this section is almost impossible. Although an optimum pH for algal growth has been demonstrated in a number of studies, few have been able to show that pH was the only factor affecting the observed growth. The difficulty in separating pH effects on growth from those due to changes in the $H_2CO_3^* - HCO_3^- - CO_3^=$ system, from the precipitation of other essential nutrients and from mixing effects has plagued many researchers.

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APPENDIX B

INORGANIC CARBON LIMITATION AND THE CHEMICAL COMPOSITION OF TWO FRESHWATER GREEN MICROALGAE[+]

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ABSTRACT

Two freshwater chlorophytes, Chorella vulgaris and Scenedesmus obliquus were grown in inorganic carbon-limited continuous cultures in which HCO_3^- was the sole source of inorganic carbon. The response of steady-state growth rate (μ) to external total inorganic carbon concentration was well described by the Monod equation; however, the response to internal nutrient concentration was only moderately well represented by the Droop equation when the internal carbon concentration was defined on a cellular basis, and totally inapplicable when total biomass (dry weight) was used to define internal carbon because the carbon:dry weight ratio did not vary over the entire growth rate spectrum. In batch cultures maximum growth rates were achieved at P_{CO_2} levels present in atmospheric air and HCO_3^- concentrations of 3 mM. No growth was observed at 100% CO_2. Both nitrogen uptake and chlorophyll synthesis were tightly coupled to carbon assimilation, as indicated by the constant C:N and C:Chl ratios found at all growth rates. The main influence of inorganic carbon limitation is not on chemical structure of the biomass, but rather on cell size:higher values of μ lead to bigger cells.

Variations in the chemical composition of phytoplankton are tightly coupled to changes in growth rate, (15,20,40). This growth rate-dependence, to a large degree, provides a good description of the nutritional state of a cell population in response to different degrees of nutrient limitation (15,37). For example, significant variations in the cell quota (cellular concentration of limiting nutrient) for either phosphorus or nitrogen occur when the respective nutrient is limiting in continuous culture and the dilution rate (≃ growth rate) is varied (7,11,17,37). Droop (6) has demonstrated that the cell quota is related to growth rate by a rectangular hyperbolic equation of the form $\mu = \bar{\mu}[1-k_Q Q^{-1}]$ (equation 1), in which μ is the specific growth rate (T^{-1}), $\bar{\mu}$ is the specific growth rate for which Q is infinite, Q is the cell quota (mass·[cell]$^{-1}$), defined above, and k_Q is the minimum concentration of limiting nutrient per cell required before growth can proceed. The equation is empirical and its utility is related to which limiting nutrient is being considered (16). For nutrients that constitute a small fraction of total cellular material such as PO_4^{-3} and vitamin B_{12} the ratio $k_Q:Q_M$ (Q_M is the upper bound in cell quota associated with the true maximum growth rate μ) is very small (e.g. < 0.1), indicting a large variation in Q in the range $0 < \mu \leq \hat{\mu}$ (16). Then, according to equation 1, $\hat{\mu} \simeq \bar{\mu}$. When nitrogen, which constitutes ~5-10 percent of total cellular biomass, is the limiting nutrient the variability in Q is more restricted and $k_Q:Q_M$ is ~0.2 so that $\hat{\mu} \simeq 0.8\ \bar{\mu}$ (16,17). Under these conditions the applicability of equation 1 is restricted and there is no substitute for determining $\bar{\mu}$ and Q_M independently .

To date, virtually no information is available on the degree of cellular carbon variations in phytoplankton when inorganic carbon is the limiting nutrient. Goldman et al. (18) and Pipes (34) did observe, however, that the yield coefficient on a weight basis (= cellular dry weight/cellular organic carbon) for several freshwater green algae was invarient over the entire growth rate spectrum in inorganic carbon-limited continuous cultures. However, cell numbers were not measured, and thus cell quotas for carbon were unavailable.

Goldman et al (18), moreover, demonstrated that at steady state under inorganic carbon-limitation μ was related to the residual total inorganic carbon concentration $C_{T_1} = (CO_2 + H_2CO_3 + HCO_3 + CO_3^{=})$ according to the Monod equation $\mu = \hat{\mu} C_{T_1} (K_s + C_{T_1})^{-1}$ (equation 2) in which K_s is the half saturation coefficient (ML^{-3}), or the concentration of limiting nutrient for which $\mu = \hat{\mu}/2$. In addition, K_s was found to be a function of culture pH. For microalgae it has been virtually impossible to demonstrate relationships between μ and residual (external) limiting nutrients because K_s values for the common nutrients studied (e.g. nitrogen, phosphorus) have been below levels of detectability even though the Droop (internal nutrient) equation and the Monod (external nutrient) equation are compatible at steady state (3,11).

In the current study, we expanded on the earlier study of Goldman et al. (18) and examined the utility of the Droop and Monod equations for inorganic carbon-limited growth of two freshwater green microalgae,

Chlorella vulgaris and Scenedesmus obliquus, in continuous culture. In addition, we studied how the chemical composition of the above algae varied with growth rate under inorganic carbon limitation.

MATERIALS AND METHODS

The continuous-culture apparatus (a bank of eight 0.5-liter cultures), the culturing protocols, and the experimental analyses were virtually identical to those described previously (16,17). Continuous lighting (2,093 J $m^{-2}min^{-1}$ visible), temperature control (20°C), and mixing with magnetic bar stirring were employed in the continuous culture experiments. Aeration with mixtures of 100% CO_2 and laboatory air at several bubble rates were used only in some of the batch experiments to determine $\hat{\mu}$. CO_2 from a gas cylinder and laboratory air were first mixed in the desired proportion in a 2-gas proportioner. The specific gas bubbling rate $G(T^{-1})$ (= gas bubbling rate/culture volume) was set by passing the gas mixture through a flowmeter-regulator before it entered the culture bottom in these latter experiments. In all other experiments gas bubbling was not employed and HCO_3^- was the sole source of inorganic carbon. The freshwater chlorophytes Chlorella vulgaris and Scenedesmus obliquus were obtained from the laboratory of M. Gibbs at Brandeis University.

The freshwater medium was similar to that used previously (18) and consisted of 2.0 mM NH_4Cl, 0.4 mM $MgCl_2$, 0.4 mM $MgSO_4 \cdot 7H_2O$, 0.2 mM $CaCl_2 \cdot 2H_2O$, 0.04 mM H_3BO_4, and trace metals in a twofold dilution of f medium (23). The medium for the continuous culture experiments was

buffered with 10 mM phosphate consisting of equi-molar concentrations of K_2HPO_4 and KH_2PO_4, resulting in a pH of 7.1-7.2. The concentration of total inorganic carbon in the medium (C_{T_o}) was 10 mg C· liter^{-1} supplied from a mixture of $NaHCO_3$ and $NaCO_3$. For the batch studies the ratio of di- to mono-PO_4^{-3} in 25 mM buffer was varied depending on the partial pressure of CO_2 in the gas mixture. Up to 10 mM HCO_3^- was added in some of these batch experiments. Medium was dispensed to the continuous cultures via a multichannel peristaltic pump (Harvard no. 1203). All tubing was glass, except for small sections of silicone inserted through the pumps.

Chemical analyses for C_{T_o} and C_{T_1}, were carried out on a Dohrmann DC-54 Ultra-Low Total Carbon Analyzer, modified for inorganic carbon analyses according to Goldman (12). The instrument has a precision of $\pm$10 µg C· liter^{-1} (or $\pm$ 2%) and an accuracy down to ~ 50 µg C· liter^{-1}. Particulate carbon and nitrogen were measured on a Perkin-Elmer 240 elemental analyzer . Cells were counted in a Spencer Bright-line hemacytometer. Dry weights were made on 100 ml samples retained on pre-combusted glass-fiber filters and combusted at 500-550°C for >4 hours. Chlorophyll *a* was measured on acetone-extracted samples by fluorometry according to Strickland and Parsons (48). Culture and medium pH was measured with a combination probe mounted on a Corning 110 meter. All measurements were made directly on culture samples at the steady state, defined as the time when culture absorbance, measured on a Bausch and Lomb Spectronic 88 at 600 nm, did not vary more than $\pm$ 10 percent for at least 2 consecutive days. The cultures were not axenic for the reasons cited earlier (11).

The maximum growth rate was estimated both by the cell washout technique (11) and the enriched-culture batch technique (17). Batch experiments were carried out using either bubbled gas or HCO_3^- as the source of inorganic carbon. Three concentrations of HCO_3^- were used: 3, 6, 10 mM. Gas mixtures included air (P_{CO_2} = 0.036%) at 3 bubbling rates (G = 25, 50, 75 hr^{-1}), and 1%, 5%, and 100% P_{CO_2} in air at a constant G of 50 hr^{-1}. The maximum growth rate was determined from each experiment by linear regression analysis of the plot of the natural log of the cell count versus time. In each experiment 3 measurements were made during exponential growth for cellular carbon (Q_{cM}) and nitrogen (Q_{nM}). Inocula for the batch cultures were taken from continuous cultures at steady state to give initial cell numbers of 0.1×10^5 to 0.3×10^5 cells $\cdot ml^{-1}$.

The kinetic coefficients $\bar{\mu}$ and k_Q were determined from regression analyses of the linearized version of equation 1 as follows: $Y = Y_Q(1-\mu\bar{\mu}^{-1})$ (equation 3), in which Y is the cellular yield coefficient (Q^{-1}), and Y_Q is the maximum cellular yield coefficient (k_Q^{-1})(7); K_s and $\hat{\mu}$ were determined from regression analyses of the linearized version of equation 2 as follows: $C_{T_1} = (C_{T_1}\mu^{-1})\hat{\mu} - K_s$ (equation 4) (18). Forty six steady state measurements were made for C. vulgaris in the growth rate range 0.17-2.05 day^{-1}, and 33 measurements for S. obliquus in the range 0.17-1.56 day^{-1}.

RESULTS

Maximum growth rate. Estimates of $\hat{\mu}$ by the washout technique were 1.59 day^{-1} for S. obliquus and 2.11 day^{-1} for C. vulgaris. For C. vulgaris the magnitude of $\hat{\mu}$ determined by the batch technique, regardless of whether HCO_3^- (in the range 3-10 mM) or bubbled CO_2 (in the P_{CO_2} range 0.036 - 1%) was the inorganic carbon source, was comparable to the washout technique, ranging from 1.94 to 2.08 day^{-1} and averaging 2.02 day^{-1} (Table 1). With 5% P_{CO_2} $\hat{\mu}$ was diminished considerably (0.97 day^{-1}) and with 100% CO_2 no growth was observed. For S. obliquus, on the other hand, $\hat{\mu}$ by the batch technique was comparable to the washout method when bubbled CO_2 was the inorganic carbon source at any P_{CO_2} level (except for 100% CO_2 for which no growth occurred): $\hat{\mu}$ ranged from 1.47- to 1.77 day^{-1} and averaged 1.57 day^{-1}. Moreover, increasing concentrations of HCO_3^- beyond 3 mM ($\hat{\mu}$ = 1.67 day^{-1}) led to significant reductions in $\hat{\mu}$ (down to 1.16 day^{-1} with 10 mM HCO_3^-) (Table 2). There was no effect of bubble rate on $\hat{\mu}$ for either species when air was the inorganic carbon source. Culture pH values varied between 6.8 and 7.7, the highest values occurring in the 10 mM HCO_3^- experiments (Tables 1 and 2).

Half saturation coefficient. The response of μ to external (residual) inorganic carbon concentration (C_{T_1}) was well described by equation 4, leading to K_s values of 0.20 mg C $\cdot liter^{-1}$ for C. vulgaris (Fig. 1A) and 0.16 mg C $\cdot liter^{-1}$ for S. obliquus (Fig. 1B).

Cellular carbon variations. The cellular carbon: dry weight ratio (Q_c') was invarient with μ for both species: 0.46 ± 0.07 S.D. for C. vulgaris (Fig. 2A) and 0.48 ± 0.08 S.D. for S. obliquus (Fig. 3A). In contrast the carbon cell quota (Q_c) increased with μ for both species (Figs. 2B and 3B). The kinetic coefficients k_Q and $\bar{\mu}$, derived from equation 3, were respectively 2.2 pg C ·cell^{-1} and 2.16 day^{-1} for C. vulgaris and 7.3 pg C ·cell^{-1} and 2.44 day^{-1} for S. obliquus (Table 3).

There was excellent agreement between estimates of Q_{cM} by the batch technique and from the experimental data in Fig. 2B and 3B for both species: Q_{cM}=13.5 pg C ·cell^{-1} (Table 1) to 15 pg C ·cell^{-1} (Fig. 2B) for C. vulgaris and 29.3 pg C ·cell^{-1} (Table 2) - 28 pg C ·cell^{-1} (Fig. 3B) for S. obliquus. The resulting ratios of k_Q:Q_{cM} were 0.16 for C. vulgaris and 0.25 for S. obliquus (Table 3). The ratio $\hat{\mu}$:$\bar{\mu}$ determined from summarized experimental data for $\hat{\mu}$ (Table 3) and $\bar{\mu}$ from equation 3 did not compare well with the values derived from equation 1 with the ratio k_Q:Q_{cM} inserted (Table 3): respectively 0.94-0.98 versus 0.85 for C. vulgaris and 0.65 versus 0.75 for S. obliquus (Table 3).

Cellular nitrogen variations. There appeared to be tight coupling between nitrogen and carbon assimilation at all growth rates. The cellular C:N ratio (by weight) was virtually invarient with varying μ for both C. vulgaris (Fig. 4A) and S. obliquus (Fig. 4B), ranging between 5 and 6. The maximum nitrogen cellular content (Q_{nM}), averaged from the batch culture data in Tables 1 and 2, was 2.8 pg N ·cell^{-1} for C. vulgaris and 4.8 pg N ·cell^{-1} for S. obliquus.

Cellular chlorphyll variations. Cellular chlorophyll like carbon and nitrogen, increased with increasing μ, ranging from ~0.04pg Chl $\cdot cell^{-1}$ to ~ 0.25 pg Chl $\cdot cell^{-1}$ for C. vulgarus (Fig. 5B) and ~ 0.1 pg Chl $\cdot cell^{-1}$ to 0.25 pg Chl $\cdot cell^{-1}$ for S. obliquus (Fig. 6B) in the range $0 < \mu \leq \hat{\mu}$. The carbon:chlorophyll ratio decreased slightly from ~ 75 to 50 between $0 < \mu \leq \hat{\mu}$ for C. vulgaris (Fig. 5A), but generally was invarient at ~100 with increasing μ for S. obliquus (Fig. 6A), although there was substantial scatter in the data at $\mu < 0.3$

DISCUSSION

Inorganic carbon-limited growth kinetics. Interpretation of inorganic carbon kinetic data is premised on the knowledge that the actual substrate for assimilation is known or that the rate reactions within the CO_2 - HCO_3^- - $CO_3^=$ chemical system are all fast enough so that the total flux of inorganic carbon into biomass via photosynthesis is the rate-limiting step (11). Of the several rate reactions in the CO_2 - HCO_3^- - $CO_3^=$ system, only the reactions $H_2CO_3 \rightarrow CO_2 + H_2O$ (equation 5) at pH < 8 and $HCO_3^- \rightarrow CO_2 + OH^-$ (equation 6) at pH > 10, or both in the pH range 8-10, are relatively slow (25). Thus, regardless of whether an alga is an obligate CO_2 user or can assimilate HCO_3^- directly, the uptake of any carbon species will be indistinguishable from that of the total inorganic carbon pool when the above reactions are not rate limiting. For example, Goldman et al (18) demonstrated that for a range of growth rates, algal biomass, and total inorganic carbon concentrations similar to those used

in the current study, and for most natural water situations in which the amount of total inorganic carbon present is in excess relative to the demand of phytoplankton, reactions 5 and 6 generally are not rate limiting; hence, under these conditions it is valid to use C_{T_1} as the substrate in equation 2.

Moreover, when the pH is varied but the chemical reactions in the CO_2 - HCO_3^- - $CO_3^=$ system remain non-limiting, it is impossible to determine the form of inorganic carbon used in photosynetheis by comparing K_s values that are based on relative CO_2 versus HCO_3^- concentrations. The two K_s values under these conditons will always be related by the equilibrium constants defining the chemical system (19). Lehman (26) carried these arguments further by showing than even when CO_2 was the source of inorganic carbon for photosynthesis, facilitated transport of HCO_3^- across cell membranes supplemented CO_2 transport to maintain high total fluxes of inorganic carbon to the sites of photosynthesis.

The values of K_s (based on C_{T_1}) found in the current study for C. vulgaris (0.20 mg C $\cdot$liter^{-1}) and S. obliquus (0.16 mg C $\cdot$liter^{-1}) are virtually identical to those determined for two other chlorophytes, Selenastrum capricornutum (0.40 mg C $\cdot$liter^{-1}) and Scenedesmus quadricauda (0.22 mg C$\cdot$liter^{-1}) grown in the pH range 7.1-7.2 (18). These K_s values are considerably lower than those found for cultured and natural populations of estuarine and marine phytoplankton measured during short-term ^{14}C-incubation studies (4,27). However, the K_s values determined in these latter experiments are not comparable with steady state continuous culture experiments because in the former case they were

based on total inorganic carbon uptake per unit time over 1-2 hours incubations, whereas in the latter studies they were determined as a function of the steady state growth rate. Photosynthetic rates, particularly when measured over short intervals, are not necessarily coupled to growth rates (30).

Markl (28) found that the K_s for photosynthesis, based on CO_2 levels at the cell surface, was ≤ 1 µg C $\cdot$liter^{-1} for steady state turbidostatic growth of C. vulgaris at various light intensities and influent P_{CO_2} levels in bubbled gas, which is over 2 orders of magnitude lower than the K_s values reported in this study. It would thus appear that when inorganic carbon is supplied primarily in the gaseous form the true affinity for inorganic carbon at the cell surface is so high that the main mass transport bottleneck occurs at gas-liquid interfaces. When HCO_3^- is the major source of inorganic carbon, chemical conversion rates of HCO_3^- to CO_2 for obligate CO_2 users or the efficiency of HCO_3^- transport across cell membranes for species capable of facilitated HCO_3^- transport (26) are, in principal, the major potential rate bottlenecks. However, it has been repeatedly demonstrated that the enzyme carbonic anhydrase, which catalyses reactions 5 and 6, is produced when cells are grown in low P_{CO_2} environments (1,10,21,22,24,31), thus providing additional, albeit indirect, evidence that microalgae have very high affinities for inorganic carbon. Therefore, it is virtually impossible to distinquish between uptake of a particular form of inorganic carbon and the response to the entire carbon pool C_T without rapid kinetic experiments such as used by Lehman (26) and Sikes et al. (42).

Sources of inorganic carbon and $\hat{\mu}$. The ability of C. vulgaris and S. obliquus to grow at maximum rates in batch culture at inorganic carbon concentrations as low as 3 mM HCO_3^- or 0.036% P_{CO_2} (Tables 1 and 2) appears to be a common characteristic of many freshwater and marine algae (1,36,43,49), and is another indication of the remarkable affinity these organisms have for inorganic carbon. There also is general agreement that maximum photosynthetic rates of species such as Chlorella and Scenedesmus can be sustained on similiar and even lower CO_2 concentrations (2,9,45,46).

The percentage of CO_2 in the air supplied to a culture, however, is a relatively meaningless term in trying to ascertain the amount of CO_2 required for maximum photosynthesis if no accounting is made for the concentration of CO_2 in solution which is really available to the algae (29). This concentration, as demonstrated by Markl (28), is a function of the sparging rate and degree of turbulence and their combined effect on the CO_2 tension at the cell surface where the demand for inorganic carbon occurs; for example, with optimum tubulence maximum phytosynthetic rates were attained when the P_{CO_2} concentration at the cell surface was 0.0005% (28). The lowest sparging rate (G=25 hr^{-1}) used in the current study clearly was high enough to prevent any mass transport limitations.

The narcotic effect of 100% CO_2 on both species has been observed previously (44), although no satisfactory explanation exists for the phenomenon. The decrease in $\hat{\mu}$ at 5% CO_2 in air observed for C.

vulgaris (Table 1) is not susbstantiated by similar data in the literature, as 5% CO_2 has been commonly employed to prevent carbon limitation in Chlorella and other algal cultures (24). Possibly a lack of conditioning at this CO_2 level led to the apparent reduction in $\hat{\mu}$ (47). The decrease in $\hat{\mu}$ for S. obliquus with increasing HCO_3^- concentrations greater than 3 mM is likewise difficult to explain. Osterlind (32) found a decrease in μ with increasing HCO_3^- concentration and concombitant increasing pH, which he attributed to $CO_3^=$ toxicity. In our cultures the pH rose only slightly from 6.8 at 3 mM HCO_3^- to 7.7 at 10 mM HCO_3^- (Table 2), so that $CO_3^=$ levels were always minimal. Pratt (35) observed deliterious effects of the sodium salts of $HCO_3^=$ and $CO_3^=$ on algal growth so that such an effect on our study cannot be ruled out.

Effect of growth rate on carbon cell quota. The invarience in Q_c' with changing μ, representing ~ 45-50 % carbon in the biomass (Figs. 2A and 3A), is identical to previous results (18,34), and conclusively demonstrates the inapplicability of equation 1 for describing the relationship between μ and internal carbon when inorganic carbon is limiting and Q is defined on a dry weight or total biomass basis. Droop (8) pointed out that his original formulation of equation 1 was based on the consideration that total biomass was the proper unit for calcuating Q, and that only when cell volume was invarient with changing μ was it acceptable to replace biomass with cell number in this term. Yet, the general convention in most phytoplankton studies, both experimental (40) and theoretical (5),

has been to use concentration of internal nutrient per cell number as a measure of Q. The choice of biomass units actually is academic because equation 1 is purely empirical without any fundamental theoretical basis.

On a cellular basis there is significant variation in Q_c for both species. The degee of variation of Q_c, as indicated by the ratio $k_Q:Q_{cM}$ (Table 3) is much more pronounced for C. vulgaris ($k_Q:Q_{cM}$ = 0.15) than for S. obliquus ($k_Q:Q_{cM}$ = 0.26). C. vulgaris, which is considerably smaller than S. obliquus, must be capable of larger relative increases in cell size with increasing μ than S. obliquus. Cell size may thus be as important a parameter in dictating the potential range in Q for a particular limiting nutrient, as it is influencing the absolute value of k_Q (41).

The differences between estimates of $k_Q:Q_{cM}$ from experimental data for Q_{cM} and from regression analyses of kinetic data using equation 3, point out the dangers in indiscriminately using the latter approach. The values of Q_{cM} from batch data and k_Q from the curves in Figs. 2B and 2C are far more precise than determinations of the slope of equation 3, which establishes $\bar{\mu}$ (17). Thus the utility of equation 1 for describing inorganic carbon-limitation in algae, is restricted and $\bar{\mu}$ and Q_{cM} must be determined experimentally, even when the cell quota is defined as cellular carbon.

Cellular chemical ratios. The tight coupling between carbon assimilation on the one hand and nitrogen uptake (Fig. 4) and chlorophyll synthesis (Figs. 5 and 6) on the other hand is best represented by the invarience

in the C:N and C:Chl ratios with changing μ. The magnitude of the C:N ratio of 5-6 for both species represents the lower limit possible with microbes and indicates a cell population in a well-balanced nutritional state, i.e. ~ 50% protein in total biomass (15). Similarly, C:Chl ratios in the range 50-100 are indicative of well-nourished cells (15).

The effect of inorganic carbon-limitation on cellular chemical composition is quite different then when nitrogen or phosphorus is limiting. Under nitrogen limitation, the nitrogen cell quota increases with increasing μ, but generally the carbon and phosphorus cellular contents either remain constant (33,38,39), or increase in a theshold fashion only close to $\hat{\mu}$ (17). For phosphorus limitation both carbon and nitrogen cellular contents typically are independent of μ (13,33,38). In contrast, the cellular chlorophyll content seems to increase with increasing μ regardless of which nutient is limiting (40).

It would appear that the major effect of inorganic carbon-limitation on cell physiology is not so much an effect on the chemical structure of the cell, but rather its influence on cell size: decreases in cell size are related to decreasing μ, which, in turn, represents an increasing degree of inorganic carbon-limitation.

Algal productivity. An important consequence of the very low K_s values established for inorganic carbon-limited growth is that the steady state level of algal carbon virtually is equal to C_{T_o} at all growth rates until just before $\hat{\mu}$ because $C_{T_o} >> C_{T_1}$ (Fig. 1). Then algal productivity P ($ML^{-3}T^{-1}$) increases linearly with increasing μ and is a maximum just

before $\hat{\mu}$, followed by a rapid decrease to zero at $\hat{\mu}$ (Fig. 7). Under these conditions peak productivity is synonymous with high μ. This situation is true, however, only when HCO_3^- is the source of limiting nutrient and is supplied to the culture as part of the influent liquid medium. When bubbled CO_2, which is supplied independent of the medium, is the source of inorganic carbon a decrease in algal biomass occurs with increasing μ and peak productivity will occur when μ is considerably less than $\hat{\mu}$ (34). In attempting to optimize productivity in algal mass cultures consideration must be given to these bioengineering constraints (14).

Conclusions. In the current study the relationship between growth rate and inorganic carbon limitation was best described by the Monod equation. The Droop equation was inapplicable when total biomass was used in place of cell number in defining Q and of restricted use when μ was related to cellular carbon. Both equations are empirical and must be used with caution in descriptions of algal growth response to nutrient limitation. Rather interestingly, the applicability of the Monod and Droop equations respectively increase (i.e. measurable K_s values) and decrease (i.e. large k_Q:Q_M ratios) when the limiting nutrient comprises a larger fraction of cellular biomass. Phosphorus and carbon represent the extreme examples of this concept.

The affinity that algae have for inorganic carbon is high enough to prevent distinguishing between CO_2 and HCO_3^- uptake on the basis of

chemical equilibrium considerations. The major factors controlling inorganic carbon uptake are physical mass transport bottlenecks at gas-liquid interfaces and the photosynthetic process itself. The mass flux of CO_2 and/or HCO_3^- across cell membranes does not appear to be a rate limiting step.

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TABLE 1. GROWTH AND CELLULAR COEFFICIENTS FOR CHLORELLA VULGARIS AT MAXIMUM GROWTH RATE IN BATCH AND CONTINUOUS CULTURE.

Growth Mode	Carbon Source (conc.)	G (hr^{-1})	$\hat{\mu}$ (day^{-1})	Q_{cM} (pg C $cell^{-1}$)	Q_c' (mg C:mg wt)	Q_{nM} (pg N $cell^{-1}$)	K_s (mg C $liter^{-1}$)	Culture pH
Continuous	HCO_3^-=1 mM		2.11	~15[a]	0.46	~2.7	0.20	7.1-7.2
Batch	=3		1.94	12.2		2.7		6.9
	=6		2.07	14.2		2.8		7.5
	=10		2.02	14.7		3.1		7.7
	Bubbled Gas							
	P_{CO_2} = 0.035%	25	1.97	12.8		2.5		6.8
		50	2.06	14.0		2.3		6.8
		75	2.08	11.5		2.6		6.8
	=1%	50	1.97	16.0		3.3		6.8
	=5%	50	0.97					6.8
	=100%	50	0					6.0

[a] estimated by eye.

TABLE 2. GROWTH AND CELLULAR COEFFICIENTS FOR SCENEDESMUS OBLIQUUS AT MAXIMUM GROWTH RATE IN BATCH AND CONTINUOUS CULTURE.

Growth Mode	Carbon Source (conc.)	G (hr^{-1})	μ (day^{-1})	Q_{cM} (pg C $cell^{-1}$)	Q'_c (mg C:mg wt)	Q_{nM} (pg N $cell^{-1}$)	K_s (mg C $liter^{-1}$)	Culture pH
Continuous	HCO_3^-=1 mM		1.59	~28[a]	0.48	~4.7	0.16	7.1-7.2
Batch	=3			6		5.4		6.8
	=6		1.31	22.6		3.4		7.5
	=10		1.16	20.2		3.0		7.7
	Bubbled Gas							
	P_{CO_2} = 0.036%	25	1.30	25.2		4.5		6.8
		50	1.33	29.5		4.4		7.0
		75	1.37	27.6		4.8		6.8
	=1%	50	1.27	33.8		5.3		6.8
	=5%	50	1.38	30.2		4.2		6.8
	=100%	50	0					6.0

[a] estimated by eye.

TABLE 3. SUMMARY OF KINETIC DATA FOR THE TWO FRESHWATER GREEN ALGAE GROWN IN INORGANIC CARBON-LIMITED CONTINUOUS CULTURES

Species (Growth Mode)	Datum Points	$\hat{\mu}^a$ (day^{-1})	$\bar{\mu}^b$ (day^{-1})	k_Q^b (pg C·cell^{-1})	Q_{cM} (pg C·Cell^{-1})	Q_{nM} (pg N·cell^{-1})	$k_Q:Q_{cM}$	$\hat{\mu}:\bar{\mu}$
C. Vulgaris								
Continuous	45	2.11	2.16	2.2	15[c]	2.8[c]	0.15	0.98
Batch	12[d]	2.02			13.5[a]		0.16	0.94
S. Obliquus								
Continuous	33	1.59	2.44	7.3	28[c]	4.8[c]	0.26	0.65
Batch	17[d]	1.59			29.3[a]		0.25	0.65

[a] data averaged from data in Tables 1 and 2.

[b] data obtained from regress on analyses of equation 3.

[c] estimated by eye from Figs. 2B and 3B.

[d] datum points for measurement of cellular carbon and nitrogen.

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FIGURE LEGENDS

Figure 1. Relationship between specific growth rate and residual total inorganic carbon at culture at steady state in inorganic carbon-limited continuous culture. Curves determined by regression analyses of equation 4 and plot of equation 2: A) Chlorella vulgaris; B) Scenedesmus obliquus.

Figure 2. Relationship between specific growth rate and carbon cell quotas for Chlorella vulgaris in inorganic carbon-limited continuous culture: A) Q_C'-dry weight basis; B) Q_C-cellular basis. * is Q_{CM} from averaged batch culture data in Table 1.

Figure 3. Relationship between specific growth rate and carbon cell quotas for Scenedesmus obliquus grown in inorganic carbon-limited continuous culture: A) Q_C'-dry weight basis; B) Q_C-cellular basis. * is Q_{CM} from averaged batch culture data in Table 2.

Figure 4. Relationship between specific growth rate and cellular carbon:nitrogen ratio in inorganic carbon-limited continuous culture: A) Chlorella vulgaris; B) Scenedesmus obliquus.

Figure 5. Relationship between specific growth rate and cellular chlorophyll constitutuents in inorganic carbon-limited continuous cultures for Chlorella vulgaris: A) carbon:chlorophyll ratio; B) cellular chlorophyll content.

Figure 6. Relationship between specific growth rate and cellular chlorophyll constitutuents in inorganic carbon-limited continuous cultures for Scenedesmus obliquus: A) carbon:chlorophyll ratio; B) cellular chlorophyll content.

Figure 7. Relationship between specific growth rate and algal productivity in inorganic carbon-limited continuous culture: A) Chlorella vulgaris; B) Scenedesmus obliquus.

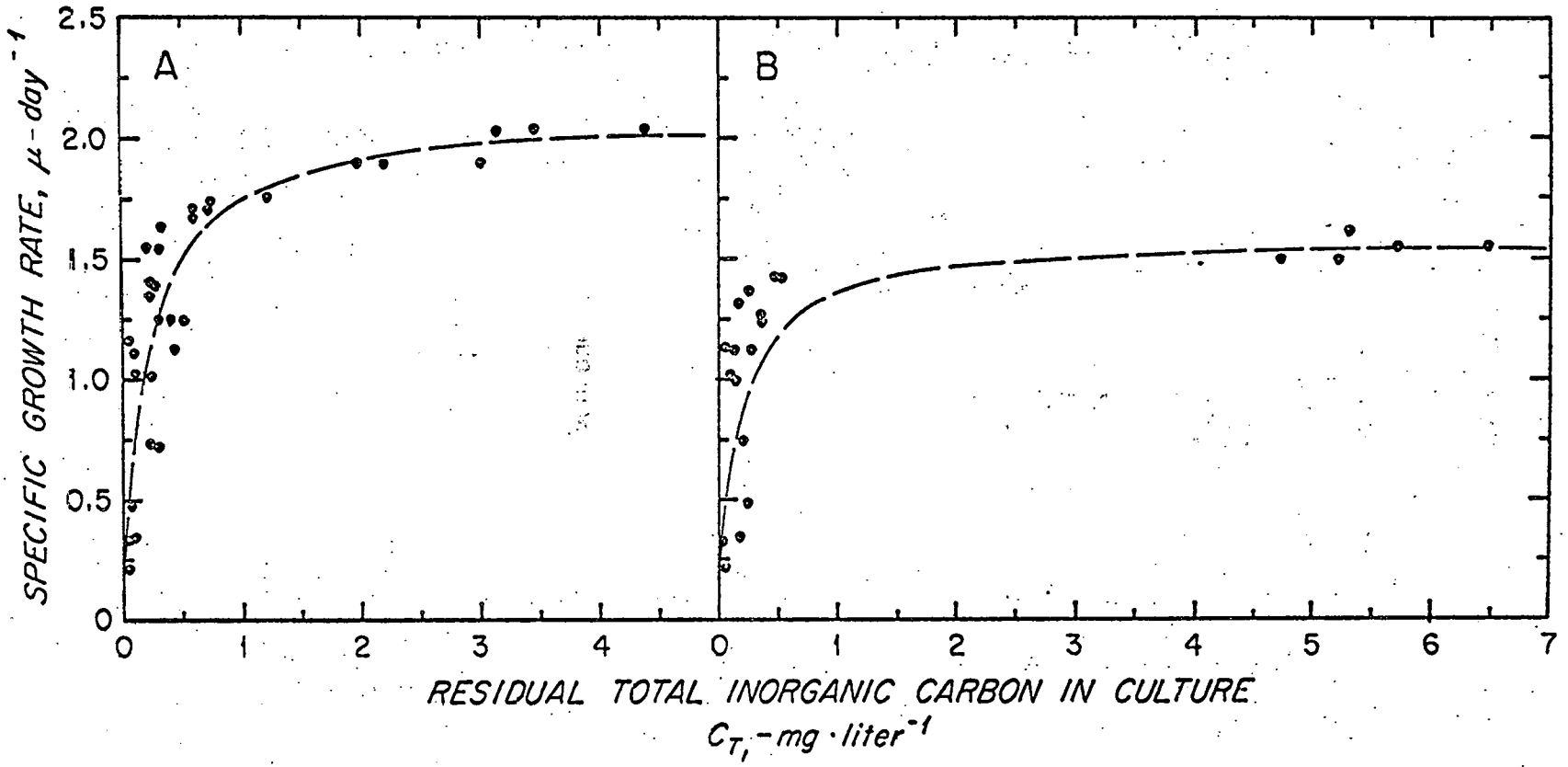
A
B
SPECIFIC GROWTH RATE, μ - day⁻¹
2.5
2.0
1.5
1.0
0.5
0
0
1
2
3
4
0
1
2
3
4
5
6
7
RESIDUAL TOTAL INORGANIC CARBON IN CULTURE
C_{T_i} - mg · liter⁻¹

Fig. 1

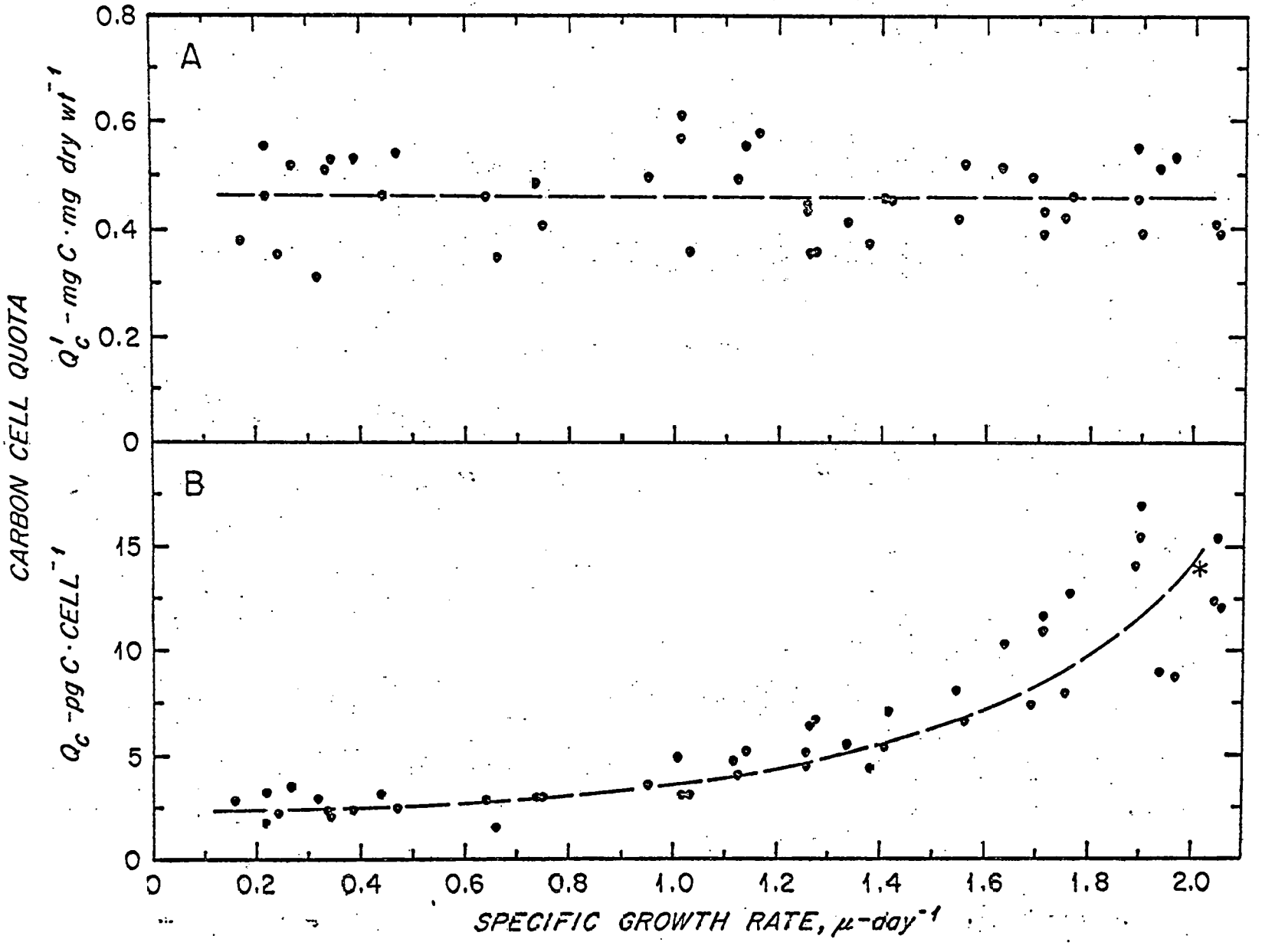
CARBON CELL QUOTA
A
Q_c' - mg C · mg dry wt^{-1}
0.8
0.6
0.4
0.2
0
B
Q_c -pg C · CELL^{-1}
15
10
5
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
SPECIFIC GROWTH RATE, μ-day^{-1}

Fig. 2

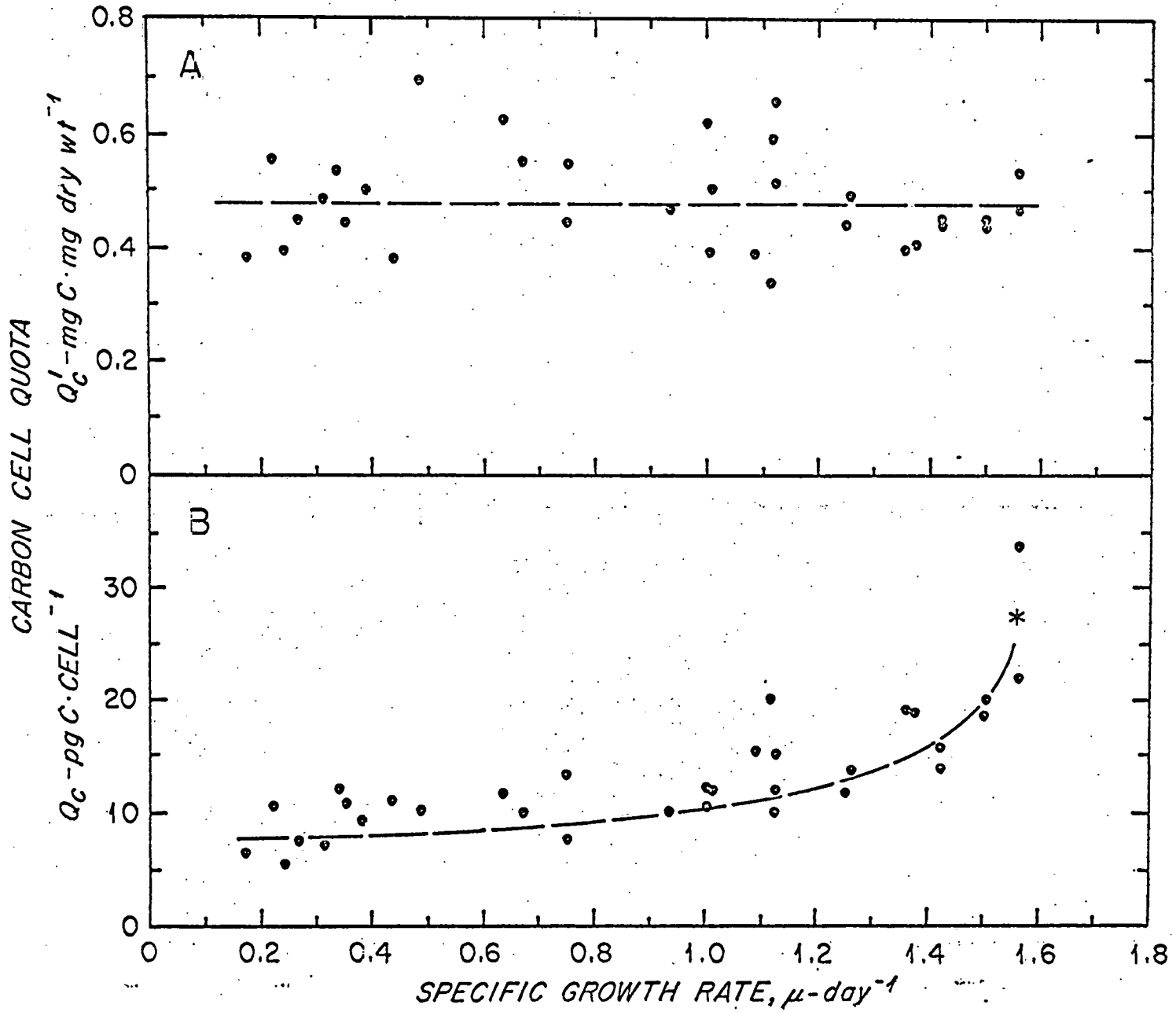
A
B
CARBON CELL QUOTA
Q_c'-mg C·mg dry wt^{-1}
Q_c-pg C·CELL^{-1}
SPECIFIC GROWTH RATE, μ-day^{-1}
0.8
0.6
0.4
0.2
0
30
20
10
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
*

Fig. 3

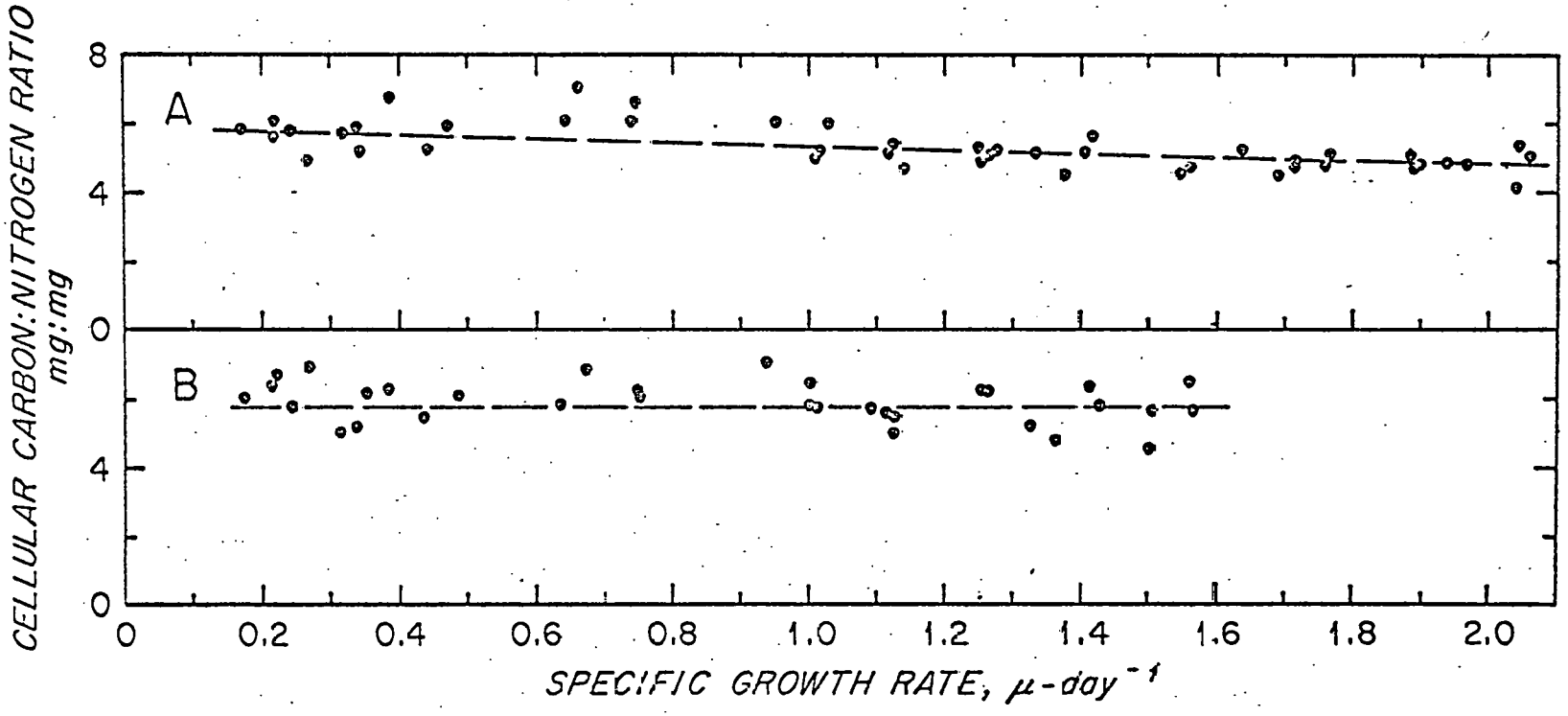
CELLULAR CARBON:NITROGEN RATIO
mg:mg
8
4
0
A
B
4
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
SPECIFIC GROWTH RATE, μ-day⁻¹

Fig. 4

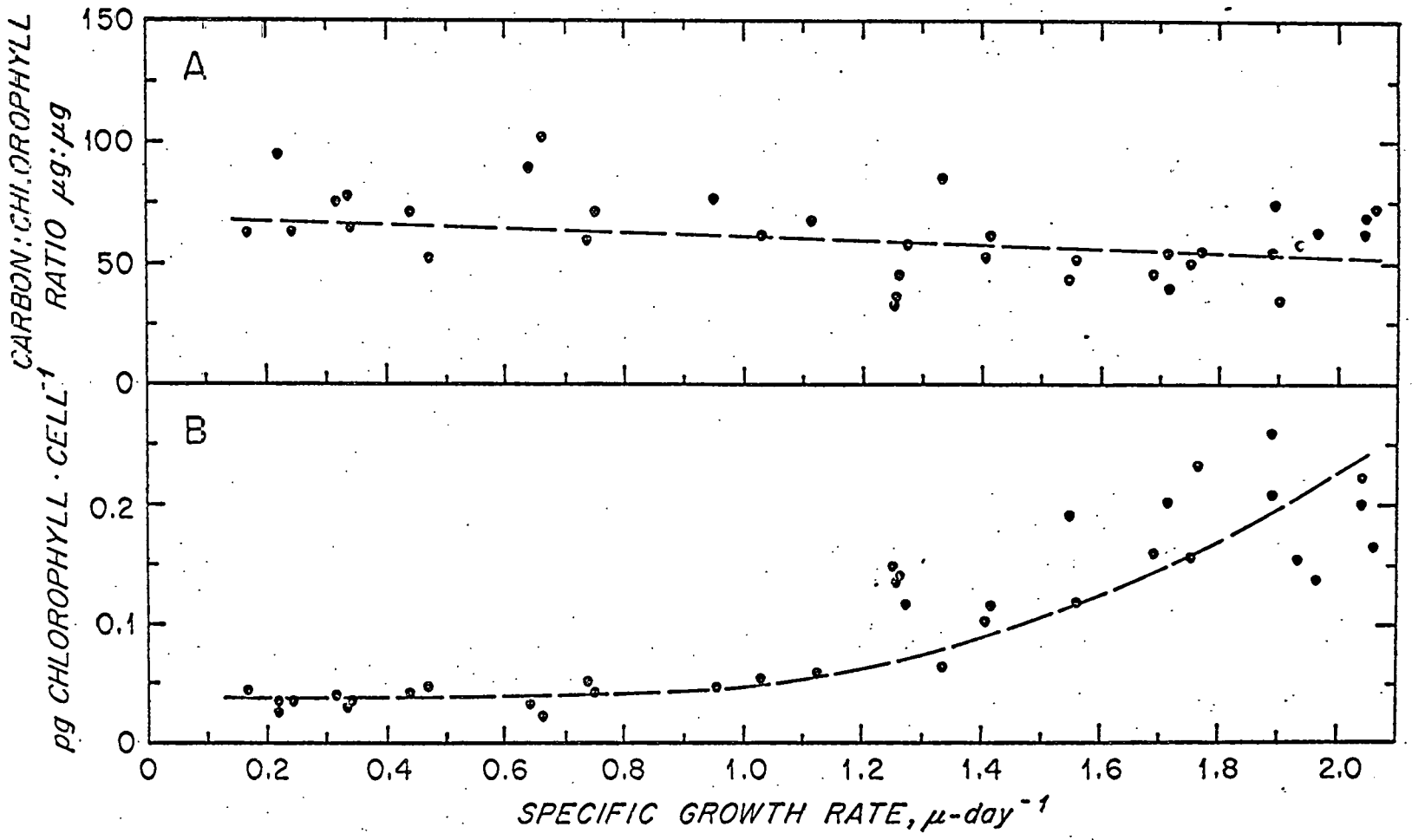
CARBON:CHLOROPHYLL RATIO μg:μg
pg CHLOROPHYLL · CELL⁻¹
A
B
150
100
50
0
0.2
0.1
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
SPECIFIC GROWTH RATE, μ-day⁻¹

Fig. 5

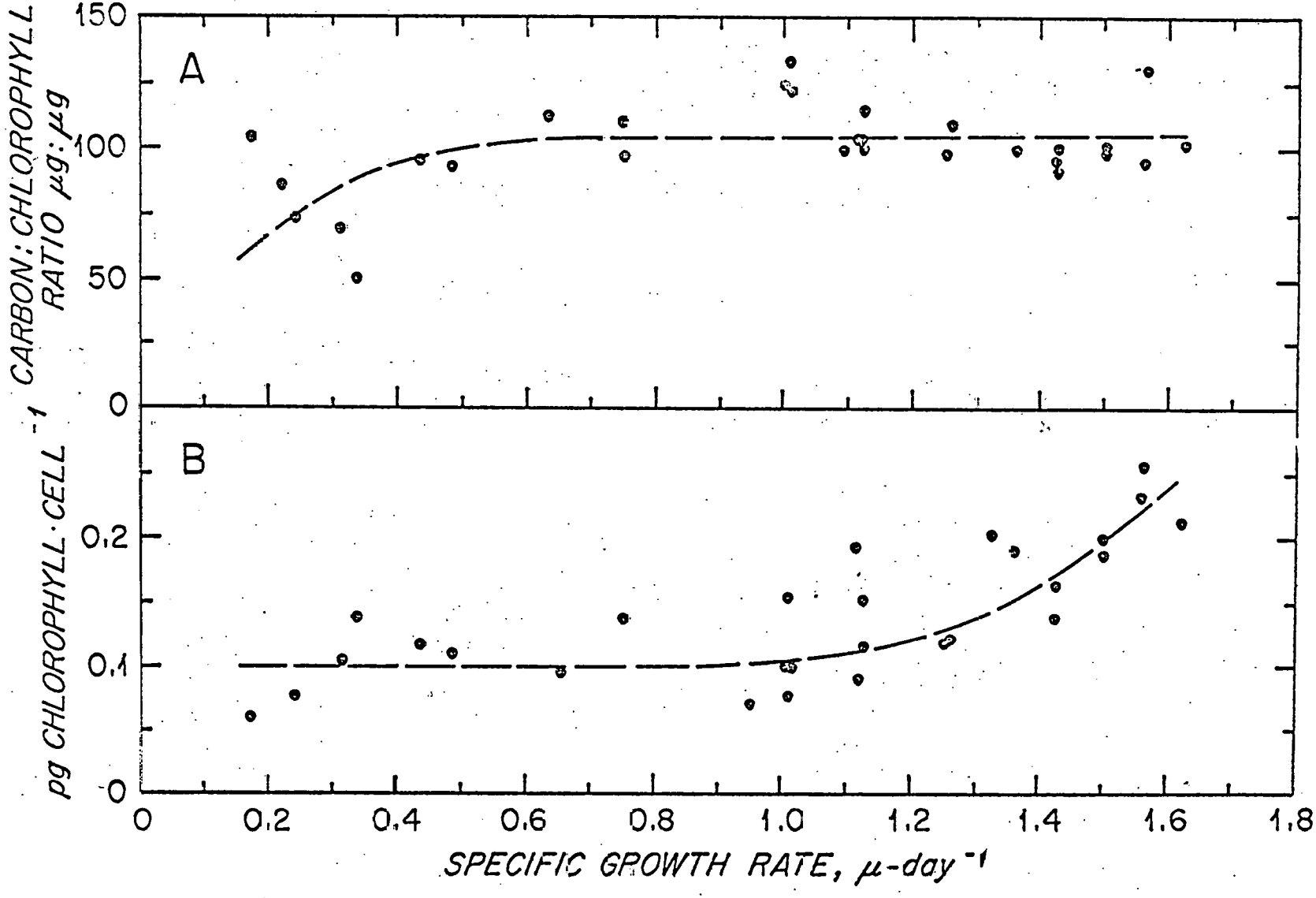
A
B
150
100
50
0
0.2
0.1
0
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
pg CHLOROPHYLL·CELL⁻¹
CARBON: CHLOROPHYLL RATIO μg:μg
SPECIFIC GROWTH RATE, μ-day⁻¹

Fig. 6

*U.S. GOVERNMENT PRINTING OFFICE: 1980-740-145/ 489 REGION NO. 4

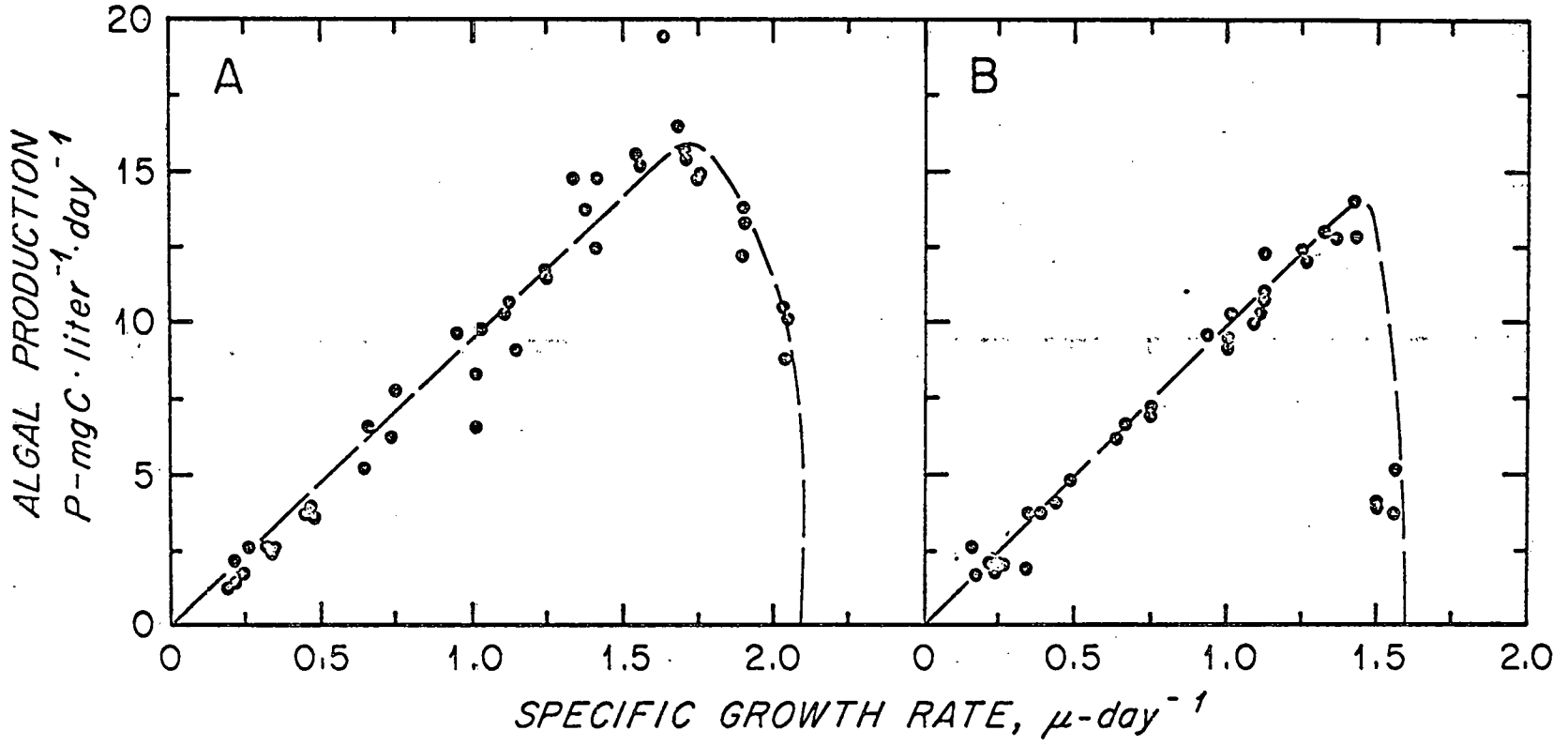

Fig. 7