Effect of Nitrogen-Mediated Changes in Alkalinity on pH Control and CO_2 Supply in Intensive Microalgal Cultures

JOEL C. GOLDMAN, MARK R. DENNETT, and CAROL B. RILEY,
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Summary

The freshwater alga *Scenedesmus obliquus* was grown in continuous culture at a fixed dilution rate of 0.5/day, but at varying pH in the range 4.17–10.67. The pH was regulated in the range 4.17–7.67 by continuously bubbling 1% CO_2-enriched air into the cultures and by varying the source of nitrogen (NO_3^-, NH_4^+, or urea) in the growth medium, which, in turn, led to changes in culture alkalinity. Culture alkalinity and P_{CO_2} were the sole determinants of pH. A pH-stat system, together with NO_3^- in the medium, was used to regulate the pH in the range 7.92–10.67. Maximum productivity, which occurred at pH 6.6, was dependent on N source only to the extent that culture alkalinity was a function of nitrogen uptake. The results demonstrate that the choice of N is a critical factor in controlling the pH of large-scale algal cultures. NH_4^+ is a poor source of N because it leads to destruction of culture alkalinity and concomitant growth-inhibiting reductions in pH, whereas NO_3^- has an opposite effect, although pH is not so severely affected in this case. Urea is, by far, the most suitable N source for maximizing algal yield when it is supplied in combination with the proper amounts of HCO_3^- alkalinity in the growth medium and percent CO_2 in the bubbled gas that will lead to an equilibrium pH near the optimum pH.

INTRODUCTION

In a recent study[1] the authors demonstrated that the most effective way to supply inorganic carbon in excess to intensive and continuous microalgal cultures was to bubble air enriched with CO_2 into the growth chamber. Bicarbonate alkalinity in the inflowing medium, although a very efficient source of inorganic carbon for growth, could not be provided in the quantities required to optimize biomass yields due to problems associated with the formation of chemical precipitates.[1] In addition, it was found[2,3] that pH control was necessary not only to optimize yields of certain algal species that were sensitive to alkaline pH, but also to avoid displacement of these algae by hardy and undesired contaminant species.

Normally, continuous bubbling of CO_2-enriched air is provided in laboratory cultures both to control pH and to ensure that inorganic carbon is not yield limiting.[4] As was shown,[1] however, only when light and other nutrients are nonlimiting is algal yield directly proportional to the flux of inorganic carbon into the culture (product of bubbling rate and percentage of CO_2

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enrichment in the air supply). Thus, using indiscriminately large CO_2 enrichments and/or bubbling rates to ensure light limitation can lead to tremendous wastage of CO_2.[1] Moreover, without a strong chemical buffering system, acidic and potentially toxic pH conditions can develop also when excess CO_2 is bubbled continuously into the culture.

From both a technical and economic standpoint the major goal in algal biotechnology is to establish conditions for yield optimization (forcing sunlight to become the major yield determinant) with a minimal input of materials and energy.[5] As recently discussed,[6] the cost of supplying adequate CO_2 to large-scale outdoor cultures of microalgae can be a major economic constraint to many of the potential applications of the process. Therefore, a full understanding of the complex interactions between reactions in the aqueous CO_2—HCO_3^-—CO_3^{2-} system and photosynthetic assimilation of inorganic carbon is necessary to avoid undue wastage of CO_2. In this study we shall expand on our earlier experiments dealing with the biotechnology of inorganic carbon supply to intensive microalgal cultures[1-3] and investigate how the source or inorganic nitrogen influences the buffering capacity, and concomitantly the pH and rate of CO_2 bubbling during continuous culture growth of the freshwater chlorophyte *Scenedesmus obliquus*.

THEORETICAL CONSIDERATIONS

HCO_3^- Alkalinity and pH Control

The pH of poorly buffered algal cultures is regulated primarily by photoautotrophic growth that involves biological transformations of cationic and anionic chemical constituents under conditions of electrical charge balance.[7,8] Because carbon and nitrogen are the two major cellular components of algae (ca. 50% C and ca. 8% N when nutrients are nonlimiting), the major determinants of culture pH are the inorganic ions of C and N in the aqueous medium. For example, when HCO_3^- alkalinity is the main source of inorganic carbon, photoassimilation of carbon by way of the reaction:

$$HCO_3^- \rightarrow CO_2 + OH^- \quad (1)$$

leads to an increase in culture pH, but no change in alkalinity, whereas when photosynthetic CO_2 is derived from external sources, such as the atmosphere or bubbled gas, both pH and alkalinity remain unaltered.[7]

Similarly, uptake of NO_3^- leads to a rise in pH and concomitant increase in alkalinity[9] by way of the reaction:

$$NO_3^- \rightarrow \text{org N} + OH^- \quad (2)$$

Thus the combination of HCO_3^- and NO_3^- in the growth medium will lead to the maximum production of OH^- and resulting pH increase. In contrast, NH_4^+ uptake by way of the reaction:

$$NH_4^+ \rightarrow \text{org N} + H^+ \quad (3)$$

has an opposite effect on pH and alkalinity.[9] However, when HCO_3^- alkalinity is the major carbon source the impact of NH_4^+ uptake on culture pH will be small because the stoichiometric requirements for C and N by an algal cell are in the ratio of ca. 5:1 when nutrients are not limiting.[10] so that only about 20% of OH^- produced [eq. (1)] will be neutralized by H^+ formed [eq. (3)]. Thus in each of the above cases the resultant pH is controlled primarily by the magnitude of algal growth and the initial concentrations of HCO_3^- and the N source, NH_4^+ or NO_3^-. For urea, which is an uncharged chemical species, uptake of N has no effect on either pH or alkalinity.[11]

Bubbled CO_2 and HCO_3^- Alkalinity

An effective way to control the pH of an intensive algal culture is with a pH-stat system. The system consists of a pH controller that regulates the opening and closing of a solenoid value on a CO_2-enriched gas line connected to the culture. In this way bubbled CO_2 is provided whenever the pH rises above a designated level so that not only is the pH regulated, but adequate inorganic carbon is supplied on demand. An important feature of this system, often not considered, is that the aqueous medium must contain some HCO_3^- alkalinity in order that a potential driving force exists to elevate the pH to the desired level when there is a photosynthetic demand for inorganic carbon [eq. (1)]. When the desired pH is exceeded gaseous CO_2 is added to neutralize any additional OH^- formed [the reverse reaction in eq. (1)]. At that point the photosynthetic demand for inorganic carbon is balanced by the CO_2 bubbled into the culture.

Another feature of this system is that chemical and not biological constraints determine the lowest pH that can be maintained within the CO_2—HCO_3^-—CO_3^{2-} equilibrium system. Principally, the lowest pH is established when the concentration of bubbled CO_2 is in equilibrium with dissolved CO_2. The concentration of dissolved CO_2 for a particular pH is a function of the HCO_3^- alkalinity in solution. Hence, both the P_{CO_2} in the bubbled gas and the alkalinity of the culture determine the equilibrium pH which is defined as[7]:

$$pH_E = -\log_{10}\left[\frac{K_w + CP_{CO_2} + [(K_w + CP_{CO_2})^2 + 8A_F CK_2 P_{CO_2}]^{1/2}}{2A_F}\right] \tag{4}$$

in which pH_E is the equilibrium pH; K_w, K_2, and C, respectively, are the ionization constant for water, the second ionization constant in the CO_2—HCO_3^-—CO_3^{2-} system, and the product of k_{CO_2}—the Henry's constant for CO_2 in water—and K_1'—the apparent first ionization constant in the CO_2—HCO_3^-—CO_3^{2-} system—all corrected for temperature and ionic strength; and the variables P_{CO_2} and A_F, respectively, are the partial pressure of CO_2 in the bubbled gas and the HCO_3^- alkalinity of the culture.

As demonstrated in eqs. (2) and (3), both the source of N and the photo-

synthetic demand for this nutrient will determine the final alkalinity in solution. Therefore, bubbling of CO_2-enriched air will be continuous for any pH controller setting $\leq pH_E$ and the pH will remain equal to pH_E. The only additional constraint on establishing pH_E is that the supply of inorganic carbon must exceed the photosynthetic demand for carbon so that P_{CO_2} in the bubbled gas and in solution essentially are equal.

MATERIALS AND METHODS

Algal Culture and Growth Medium

The freshwater chlorophyte *Scenedesmus obliquus* was obtained from the laboratory of M. Gibbs at Brandeis University. The growth medium contained 0.4m*M* $MgCl_2$, 0.4m*M* $MgSO_4$, 0.2m*M* $CaCl_2$, 2m*M* $NaHCO_3$, 1m*M* KH_2PO_4, trace metals plus the sodium salt of ethylenediaminetetraacetic acid (EDTA) in a twofold dilution of the amount in f medium,[12] and 12m*M* inorganic N in the form of either $NaNO_3$, NH_4Cl, urea, or varying mixtures of two of the three N sources. In some experiments $NaHCO_3$ was eliminated from the medium.

Culture Methods and pH Control

The continuous-culture apparatus (a bank of eight 0.5-L cultures), the culturing protocols, the pH control system, and the experimental analyses were virtually identical to those described previously.[1,2] Continuous lighting (0.06–0.07 cal cm^{-2} min^{-1}), temperature control (20°C), and mixing with Teflon-coated stirring bars were employed.

The culture pH was maintained over the range 3.60–7.67 by employing different combinations of N sources and either 0 or 2m*M* HCO_3^- in the medium, and over the range 7.90–10.67 by use of a pH-stat system together with NO_3^- as the sole N source. Results from the latter experiments were part of a larger study on pH effects in microalgae which were reported elsewhere.[2,3] Carbon-dioxide-enriched air (1%) was bubbled continuously into the cultures maintained in the lower pH range and intermittently with the higher pH cultures, as regulated by the pH-stat system.[2] The pH-stat system, as described previously,[2] consisted of a pH controller that activated a solenoid valve on a pressurized CO_2-enriched line when a desired pH was exceeded; bubbled gas then entered the culture through a port at the base of the culture, lowering the pH to the designated level. Fluctuations in pH did not exceed ± 0.1 units from the set value. At the start of an experiment when algal biomass was increasing to a steady-state level, the pH rose to the designated value because inorganic carbon was provided solely from bicarbonate alkalinity [eq. (1)]. Once the designated pH was attained, any additional inorganic carbon requirements were met by the flow of bubbled CO_2, as regulated by the pH controller. In this manner, it was ensured that inorganic carbon was not limiting at any pH level tested because there was no restriction on the amount

of inorganic carbon supplied—as long as there was a photosynthetic requirement for inorganic carbon this demand was always met by the introduction of gaseous CO_2.

A mixture of 1% CO_2 in air was obtained by blending 100% CO_2 from a pressurized cylinder with laboratory air in a two-gas proportioner. The flow rate of gas to each culture was held constant at 0.7 L/min with a rotometer on the gas inlet. The total amount of CO_2 supplied to each culture was monitored by wiring a clock to the solenoid valve and recording the time the valve was open. Bubbled air (0.036% CO_2) was added continuously at a rate of 0.7 L/min to several cultures that were grown solely on NO_3^-, NH_4^+, or urea. In this fashion the final culture pH was determined by the quantity of algae produced and its concomitant effect on the CO_2—HCO_3^-—CO_3^{2-} chemical system.[7]

Culture Operation and Analyses

Each experiment consisted of establishing steady state levels of biomass at a fixed dilution rate (medium flow rate/culture volume) of 0.5/day and at designated pH levels. When steady state was attained culture samples were analyzed for particulate carbon (*PC*), particulate nitrogen (*PN*), and total dissolved inorganic carbon (C_T) according to methods described previously.[1] The actual C_T measurements were compared with estimates of C_T from the pH-stat experiments by first estimating the steady-state alkalinity from the equation

$$A_F = A_M + PN \tag{5}$$

in which A_F is the steady-state alkalinity (meq), A_M is the initial HCO_3^- alkalinity in the growth medium (meq), and *PN* is the steady-state particulate (algal) nitrogen produced (m*M*) when NO_3^- is the sole N source. The C_T was calculated from the equation[13]

$$C_T = (A_F - [OH^-] + [H^+])/(\alpha_1 + 2\alpha_2) \tag{6}$$

in which α_1 and α_2, respectively, are the pH-dependent ionization coefficients for HCO_3^- and CO_3^{2-} in the CO_2—HCO_3^-—CO_3^{2-} equilibrium system and $[OH^-]$ and $[H^+]$, respectively, are the hydroxl and proton concentrations, likewise dependent on pH. The equilibrium constants K_1' and K_2, used to determine α_1 and α_2, were corrected for temperature and salinity.[13]

RESULTS

Nitrogen Source and Culture pH

The main determinant of final culture pH was the resultant alkalinity A_F together with the percentage CO_2 in the bubbled gas (Table I). By manipulating the three N sources, NO_3^-, NH_4^+, and urea, either singularly or in combination, together with up to 2 meq of HCO_3^- alkalinity added to the

Table I

Effect of Nitrogen Source and Concentration of HCO_3^- Alkalinity in Medium on Final Alkalinity (A_F) and Equilibrium pH (pH_E) of Continuous Cultures of *Scenedesmus obliquus* at a Dilution Rate of 0.5/day and with 1% CO_2 in Air Bubbled continuously into the Culture

Medium N (meq)			A_M	Total *PN*	NO_3—*PN*[a]	NH_4—*PN*[b]	A_F	Culture
NO_3^-N	Urea-N	NH_4^+—N	(meq)	(m*M*)	(m*M*)	(m*M*)	(meq)	(pH_E)
12.0	0	0	2.0	8.4	8.4		10.4	7.92[d]
			2.0	9.3	9.3		11.3	7.92[d]
8.0	4.0	0	2.0	9.2	5.2		7.2	7.68
			2.0	7.8	3.8		5.8	7.41
			0	7.8	3.8		3.8	7.12
4.0	8.0	0	2.0	10.7	2.7		4.7	7.15
			0	11.0	3.0		3.0	7.07
0	12.0	0	2.0	10.9			2.0	7.18
			0	12.8			0	6.84
0	10.0	2.0	2.0	13.1		2.0	0	6.96
			2.0	12.5		2.0	0	6.60
			0	13.1		2.0	0[c]	6.66
0	6.0	6.0	2.0	12.3		6.0	0[c]	6.35
			0	1.5		1.5	0[c]	4.17
			0	2.2		2.2	0[c]	5.10
0	0	12.0	2.0	3.0		3.0	0[c]	5.00
0	0	12.0	2.0	1.9		1.9	0.1	4.80
0	0	12.0	0	0		0	0	3.60[e]

[a] Represents *PN* derived from NO_3^-, assuming that urea is assimilated first.

[b] Represents *PN* derived from NH_4^+, assuming that NH_4^+ is assimilated first.

[c] Excess acidity produced.

[d] pH controller set a 7.5, which was lower than pH_E.

[e] *PN* initially produced to drive pH down, followed by cell washout.

medium, a wide range of A_F and resultant pH values was established that bracketed the physiological limits for *S. obliquus* viability. The maximum tolerable pH was 10.67, which was established using NO_3^- as the N source together with a pH-controller setting of 11[2], whereas the minimum pH for which steady state could be sustained was 4.17, occurring when equal amounts of NH_4^+ and urea N, but not HCO_3^-, alkalinity were part of the growth medium. When NH_4^+ was the sole source of N, the pH varied between 3.60 and 5.00, depending on the alkalinity initially present.

When urea was added to the medium, either singularly or in varying combinations with NO_3, the pH stayed between 6.84 and 7.18 depending on the final alkalinity (Table I). With only NO_3^- added the controller could not be activated at pH settings below 8.0 because sufficient alkalinity was generated to sustain pH_E at 7.92. Hence, bubbling of 1% CO_2-enriched air was continuous at all pH values ≤ 7.92 ($=pH_E$). The experimental data for pH_E and A_F under these conditions was reasonably well described by eq. (4) (Fig. 1). Similarly, the measured C_T values from the pH-controller experiments with NO_3^- compare extremely well with the estimated C_T values determined from eqs. (5) and (6) (Fig. 2).

pH Control of Productivity

Algal productivity for a CO_2 enrichment of 1% was maximum (ca. 225 mg C/day) at pH 6.6, but dropped precipitously at slightly lower pH and ceased at pH < 4.17 (Fig. 3). In the alkaline region (pH > 6.6) productivity also

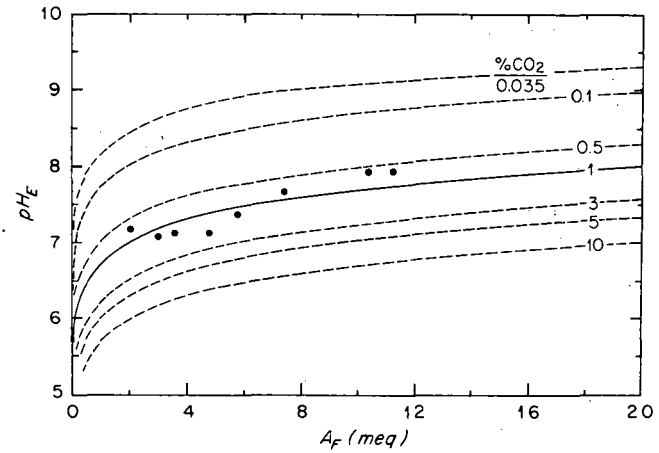

Fig. 1. Relationship between the equilibrium pH (pH_E) and final alkalinity (A_F) in an algal culture for varying percentages of CO_2-enriched gas. The symbols (●) represent experimental data from Table I in which pH_E was established with continuous bubbling of 1% CO_2-enriched air into continuous cultures of *S. obliquus* maintained at a dilution rate of 0.5/day. All curves were determined using eq. (4) with $K_1' = 5.75 \times 10^{-7}$ mol/L, $K_2 = 1.45 \times 10^{-10}$ mol/L, and $k_{CO_2} = 0.0387$ mol L^{-1} atm^{-1}, all corrected for temperature and ionic strength.

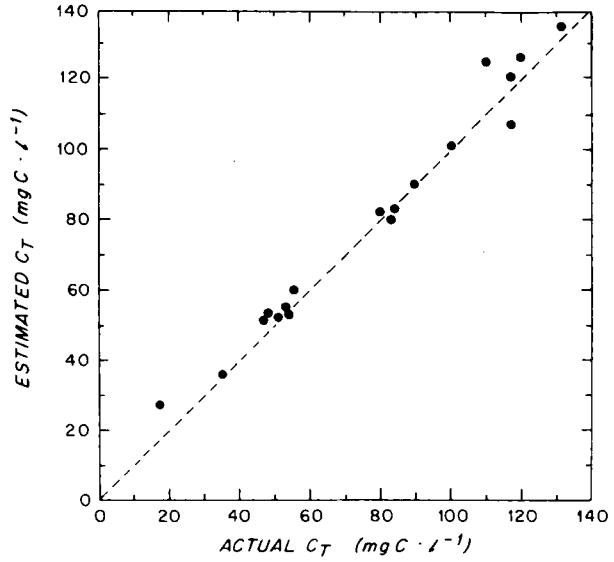

Fig. 2. Relationship between experimental and estimated total dissolved inorganic carbon (C_T) in continuous cultures of *S. obliquus* maintained at a dilution rate of 0.5/day and with pH-stat control. C_T was calculated from eq. (6) using estimates of A_F from eq. (5) and the same values of K_1 and K_2 used in Fig. 1 to determine the ionization coefficients α_1 and α_2.

diminished, but at a more gradual rate than under acid conditions. Above a threshold pH of 10.67, however, productivity dropped dramatically from ca. 100 mg C/day to zero (Fig. 3).

An appreciable difference in productivity as a function of pH for the

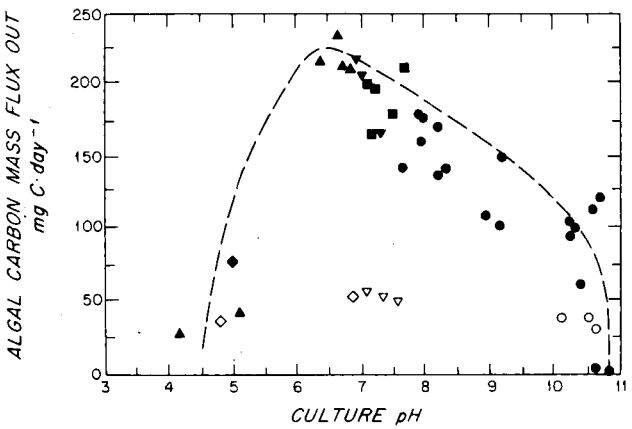

Fig. 3. Effect of culture pH on yield in continuous culture of *S. obliquus* maintained at a dilution rate of 0.5/day and grown on different N sources. Bubbling with 1% CO_2-enriched air (closed symbols) and with air (open symbols): (◆, ◇) NH_4^+, (▲) NH_4^+ + urea, (▼, ▽) urea, (■) urea + NO_3^-, (●, ○) NO_3^-.

cultures supplied bubbled air (0.036% CO_2) was not found; yields ranged from ca. 50 mg C/day at pH 7.0 to ca. 35 mg/day at both pH 5.0 and 10.5 (Fig. 3).

CO_2 Supply at High pH

The flux of inorganic carbon into the culture was in great excess relative to the amount of carbon photoassimilated whenever bubbling of 1% CO_2-enriched air was continuous. In contrast, pH regulation at a value only slightly higher than pH_E led to a dramatic reduction in the fraction of time CO_2 bubbling was necessary: down to 20% at pH 8.2 from 100% at pH 7.92 (Fig. 4). With further increases in pH above 8.2 there was a virtual linear and gradual decrease in the time the controller was activated, reaching zero just beyond the threshold pH of 10.67 (Fig. 4). Based on the above data plus the pH-dependent productivity data (Fig. 3), the fraction of CO_2 wasted decreased from $\geq 98\%$ when CO_2 bubbling was continuous ($pH \leq 7.92$) to ca. 50% at the threshold pH (Fig. 4).

DISCUSSION

The dramatic effect of pH on productivity of certain algal species in intensive culture is exemplified by the present results indicating that the optimal pH (pH_O) for maximum yield of the freshwater chlorophyte *S. obliquus* is restricted to a narrow band from ca. 6.5 to 7.0, and that even slightly acidic conditions can lead to drastic reductions in yield (Fig. 3). From previous studies[2] it is known that other chlorophytes such as the freshwater alga *Chlorella vulgaris* (which, interestingly, is able to tolerate acid pH better

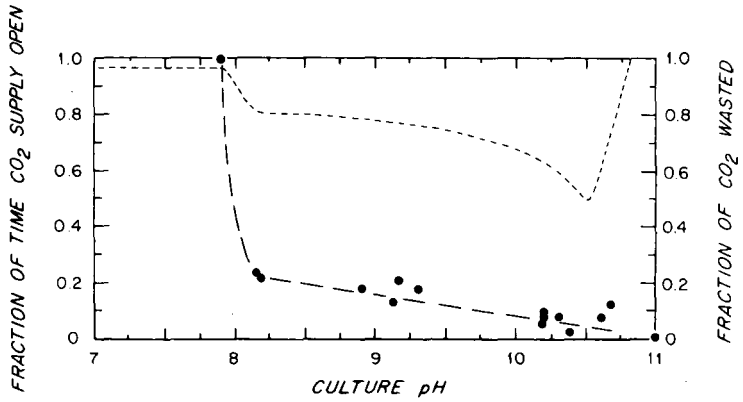

Fig. 4. Effect of culture pH on the fraction of time CO_2 was supplied to a continuous culture of *S. obliquus* at a dilution rate of 0.5/day when NO_3^- was the source of N and bubbling of 1% CO_2-enriched air was controlled by the pH-stat system (●). The dashed line represents the fraction of CO_2 wasted as a function of algal yield (from Fig. 3) and CO_2 supplied at varying pH.

than *S. obliquus*[1]), and the marine species *Dunaliella tertiolecta* generally are inhibited significantly at nonoptimal pH.[2] In contrast, the undesired marine diatom *Phaeodactylum tricornutum* is hardly affected by pH in the range 7.9-10.2.[2] The general conclusion, therefore, is that pH control is a necessary component of any mass culture application for which yield optimization and species control are major objectives.[3]

Control of pH in intensive cultures can be accomplished in either of three ways. First, a variety of buffer compounds, both organic and inorganic, are available; among these compounds are zwitterionic amino acids, primary aliphatic amines, and salts of inorganic phosphate.[13-15] The organic compounds, although extremely effective and, for the most part physiologically sound buffers, are exorbitantly expensive for all but the most limited laboratory applications involving small volume cultures. Inorganic phosphates similarly are of limited use, not only because of economic considerations, but also because they are sparingly soluble and in large enough quantities can lead to chemical precipitation, particularly in seawater.[13] Use of any of the above buffers, moreover, is limited to a narrow range of pH determined by the respective pK of the buffer employed.

The second technique for controlling pH is with combinations of HCO_3^- alkalinity and continuous bubbling of CO_2-enriched air.[16-18] Ideally, the goal of this technique is to force pH_E to equal pH_O, because, as shown in Figure 1, as long as the flux of inorganic carbon into the culture exceeds the uptake of carbon by the algae, a unique pH is established for a combination of alkalinity and P_{CO_2}. Thus, we could have maintained pH_O (6.6) in our cultures with numerous combinations of A_F and percent CO_2, e.g., 0.1 meq and 0.1%, 0.6 meq and 1%, 2 meq and 3%, or 3 meq and 5% (Fig. 1). The major limitation of this technique, unfortunately, is that it is difficult to control A_F when NO_3^- and NH_4^+ are the N sources (Fig. 1).

Even though it has been long recognized that culture pH is influenced by the source of nitrogen in the growth medium,[4,19-21] to date, little attention has been focused on the quantitative aspects of how alkalinity and buffer capacity are altered by physiological processes. Based on our results, it is apparent that the simple stoichiometry between ionic N uptake and alkalinity change is the major determinant of culture pH. However, when the N source is NO_3^- the situation is not critical because, as seen in Figure 1, large increases in alkalinity for a given P_{CO_2} in the bubbled gas result in relatively small increases in pH_E at least when $A_M > 2$ meq. For example, in the experiments with 1% CO_2 culture pH only rose from ca. 7-8 for an increase in A_F from 2-11 meq (Fig. 1). In addition, when NO_3^- was assimilated in the presence of bubbled CO_2, the buildup of alkalinity led to a large C_T reservoir in the culture in the form of HCO_3^- and CO_3^{2-} (Fig. 2). The buildup of excess C_T actually is advantageous in freshwater cultures because it provides a strong pH buffer capacity and reserve of inorganic carbon in case bubbling of CO_2-enriched gas is interrupted for short periods. Excess alkalinity in

marine cultures poses far more serious problems of culture instability due to the formation of precipitates at even slightly alkaline pH.[1,13]

In contrast, NH_4^+ assimilation will first lead to complete destruction of available alkalinity and then to the production of sufficient acidity to lower the pH to lethal levels unless sufficient HCO_3^- alkalinity is initially present in the medium (Table I). Of the three N sources used, urea clearly results in the most stable pH simply because it has no effect on alkalinity.[11] A desired culture pH_E is then established by initially choosing a combination of A_M and percent CO_2 from the curves in Figure 1.

Although there is a general nutritional preference among most freshwater and marine microalgae for NH_4^+, followed by urea, and then NO_3^-[22], all three sources of N are readily assimilated under steady-state conditions.[23] Hence, the pH effects on yield observed in Figure 3 are not related to preferential uptake of a particular N source. For example, in the current study[1] a peak yield of 225 mg C/day was achieved when a combination of 83% urea and 17% NH_4^+ was the N source and the pH was 6.6. Identical results were obtained in a previous study[1] when NH_4^+ replaced urea and the culture was buffered at pH 6.6 with phosphate salts. Similarly, peak productivity in the current study was hardly affected by replacing urea plus NH_4^+ with urea or urea plus NO_3^- as long as the pH stayed close to 6.5–7.0 (Fig. 3 and Table I).

In choosing a proper combination of A_M and percent CO_2 it must be recognized that the slopes of all the percent CO_2 curves in Figure 1 are very steep and tend to converge at low (< 1 meq) A_F. Theoretically then, pH control at values < 7 could be accomplished with very low CO_2 enrichment in the bubbling gas (approaching air); there is, however, a minimum percent CO_2 even at high bubbling rates that is necessary to prevent carbon limitation. For example, air bubbling (0.035% CO_2) at any pH in the current study (Fig. 3) and up to 0.17% CO_2 at pH 6.6 in the previous experiments[1] led to significant reductions in yield below the light-limiting level of 225 mg C/day. In addition, very low levels of alkalinity are to be avoided because even slight deviations from A_F caused, for example by phosphate assimilation,[13] could lead to large changes in pH_E (Fig. 1).

Still another problem in using continuous bubbling of CO_2-enriched air along with HCO_3^- alkalinity to control pH is that it is difficult to avoid wasting most of the CO_2 that is introduced into the culture. For example, even when productivity of *S. obliquus* was maximum at pH 6.6 over 98% of the introduced CO_2 was wasted (Fig. 4). In retrospect, it may have been possible to minimize these losses by reducing the bubble size to increase uptake efficiency and lowering the gas flow rate to reduce total influx of CO_2 without deviating from pH_O appreciably.[1] However, it is extremely difficult to maintain a tight balance between influx of CO_2 and organic carbon production without developing a diffusion gradient whereby P_{CO_2} in solution is less than P_{CO_2} in the bubbled gas, which, in turn, would lead to a rise in pH (Fig. 1).

The most effective way both to control culture pH and to minimize the

wastage of bubbled CO_2 is with the third method, a pH-stat system. The major advantage of this system is that bubbled CO_2 is supplied on demand to balance the production of OH^- [eq. (1)]. Yet, a disadvantage of this technique is that the pH controller is activated only when the desired pH is $> pH_E$. In the current experiments so much alkalinity was produced when NO_3^- was assimilated that >5% CO_2-enriched air would have been required to lower pH_E below the optimal pH of 6.6 in order that the pH controller could be activated (Fig. 1). Conversely, a combination of <1 meq HCO_3^- together with 1% CO_2 in air would have been necessary to reduce pH_E to the optimal level (Fig. 1). This latter condition could only have been achieved in the current experiments by replacing NO_3^- with urea and reducing HCO_3^- alkalinity in the medium.

Another important feature of these pH-stat experiments was that a tremendous savings in the amount of required CO_2 was realized with a minimal reduction in productivity by maintaining the pH only slightly above pH_E (Fig. 4). For situations in which it is possible to establish $pH_E < pH_O$ by choosing the proper combination of percent CO_2, N source, and A_M, the pH-stat system could be operated to maintain pH_O with minimal expenditure of CO_2.

CONCLUSIONS

The control of pH in intensive microalgal cultures primarily is a function of HCO_3^- alkalinity and P_{CO_2} in the culture. Although uptake of inorganic carbon by microalgae has no effect on alkalinity, large quantities of nutrients are consumed in intensive cultures so that the choice of nitrogen source can have a dramatic impact of A_F and the resulting pH. Clearly, NH_4^+ must be avoided as a source of N for intensive cultures because it is too impractical to add excess HCO_3^- alkalinity to control pH. NO_3^- has some advantages as an N source, but will result in the production of excessive alkalinity so that very high P_{CO_2} in the bubbled gas will be necessary if pH_O values less than 7 are required (Fig. 1). Accordingly, maximum productivity can be achieved most effectively by using urea as the N source and selecting a combination of HCO_3^- alkalinity in the growth medium and percent CO_2 in the bubbled gas to ensure that $pH_E \approx pH_O$. The choice of continuous or pH-stat control of gas bubbling to attain pH_O will be dictated by economic comparisons of the two systems. For very large mass cultures the cost of delivering large quantities of CO_2-enriched gas versus the cost of pH-stat control will be a major economic factor.

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