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Utilization of Exogenous Inorganic Carbon Species in Photosynthesis by *Chlorella pyrenoidosa*[1]

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ABSTRACT

The nature of the inorganic carbon utilized during photosynthesis by *Chlorella pyrenoidosa* was investigated using three experimental techniques (open gas analysis system with "artificial leaf" or "aqueous" chambers and O_2 electrode system) to measure carbon assimilation. Photosynthesis was studied as a function of pH and CO_2 concentration. The CO_2 concentration was inadequate to meet the requirements of photosynthesis only when HCO_3^- was added at high pH. Under all other conditions, the low and constant K_m (CO_2), in contrast to the highly variable K_m (HCO_3^-), suggested that CO_2 was the major species utilized.

Higher rates of photosynthesis were observed under limiting CO_2 conditions above pH 7.5 but rates of hydration of CO_2 were not consistent with the view that the stimulation in photosynthesis was supported by HCO_3^-. In the same pH region lower rates of photosynthesis were observed under saturating CO_2 conditions. These conflicting changes seemed not to be related to pH but to some as yet undetermined effect of bicarbonate. No support was obtained for the view that the quantum efficiency was different under conditions where CO_2 was assimilated compared to conditions where CO_2 and HCO_3^- could be assimilated, although at saturating CO_2, lower maximal rates of photosynthesis were observed in the latter conditions.

Several workers have reported that freshwater algae grown at air-levels of CO_2 exhibit a low CO_2 compensation point (8, 22, 38), little CO_2 evolution in the light (22) and little O_2 sensitivity of photosynthesis (6, 16, 22). Since air-adapted *Chlorella* (3, 17) and *Chlamydomonas* cells (7) have been shown to fix carbon via the Calvin cycle, it has been suggested that this apparent lack of photorespiration is achieved by a CO_2 concentrating mechanism, not based on the dicarboxylic acid transport prevalent in C_4 plants, but rather on the utilization of HCO_3^- (1, 2, 4, 7, 15). In this explanation HCO_3^- ions are actively transported into the cell, thus keeping the internal CO_2 and HCO_3^- concentrations higher than those that would be in equilibrium with the ambient CO_2 concentration.

Bicarbonate utilization has been conclusively demonstrated in algae (see refs. cited by Raven [30]) such as *Scenedesmus* (13), *Hydrodictyon* (29), and *Chara* (24), and further indicated in studies with *Chlamydomonas* (1, 2, 4) and *Anabaena* (2). With *Chlorella*, early work summarized by Rabinowitch (28) suggested that it was a CO_2 utilizer; more recent evidence by Steeman-Nielson and Jensen (35), Findenegg (14), and Bidwell (5) suggested that HCO_3^- could also be used.

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As air-grown *Chlorella* do not exhibit any characteristics of photorespiration it is important to determine whether it can utilize HCO_3^- and whether this HCO_3^- uptake is sufficient to suppress photorespiration. Here, we report on the nature of the inorganic carbon sources used by *Chlorella*, using three experimental systems to measure carbon assimilation.

MATERIALS AND METHODS

Plant Material. Axenic cultures of *Chlorella pyrenoidosa* Chick (strain 252), obtained from the Culture Collection of Algae at the University of Texas (Austin) were grown in a semicontinuous system (36) in 6-liter Erlenmeyer flasks containing 3 liters Gorham's culture medium No. 11 (19). Cultures were maintained in a growth cabinet (model 16S, Controlled Environments Ltd., Winnipeg, Manitoba, Canada) at a temperature of 25 C, illuminated continuously with a quantum flux density of 185–235 μE m^{-2} s^{-1}. An air stream was bubbled through the continuously stirred culture at a rate of 790 ml min^{-1}. Cultures were harvested during exponential growth, centrifuged at 600*g* for 10 min, washed with deionized H_2O, resuspended in 25 ml of the appropriate medium (except where stated otherwise), and stored at room temperature.

Gas Exchange Techniques. Photosynthetic CO_2 fixation was determined in the open gas analysis system described by Canvin (10) using both the artificial leaf technique of Lloyd *et al.* (22) and an aqueous method which allowed measurements to be obtained from algal cells suspended in liquid medium. For the artificial leaf, cells equivalent to 100 μg Chl were suspended on 16 cm^2 of filter paper (Whatman No. 3MM) and the rate of gas flow over the leaf was 235 ml min^{-1}.

The photosynthesis chamber (Fig. 1) used for the aqueous method was made of Plexiglas and consisted of: (*a*) the main section housing a thin inner sample chamber (6 mm thick × 116 mm wide × 112 mm high to give a total volume of 78 ml) with outlet and thermocouple ports (brass machined); and (*b*) an end section which when attached to the main section by 10 inset screws (separated by a rubber gasket prepared from an inner tube), covered the end of the sample chamber. The chamber had water jackets on both sides to assist in temperature control. The gas mixture was passed at a flow rate of 1.08 liters min^{-1} through the end into the sample chamber via a course sintered glass tubular filter (15.8 mm dia × 102 mm length of fritted section (171 mm total length), Corning Glass Works, Corning, New York) which fitted securely into the base of the inner chamber (prepared specifically to fit the filter tightly against a piece of rubber). The filter was changed after every set of measurements on a single sample of algae. The assembled chamber was supported with retort stands and the algal suspension (360 μg Chl in 25 ml) was illuminated from one side.

The algae were permitted to equilibrate in the open systems at a temperature of 25 C, a quantum flux density of 400 μE m^{-2} s^{-1}, a RH in excess of 90%, and an inlet CO_2 concentration of 350 μl l^{-1} (11.7 μM dissolved CO_2). Measurements were started when the

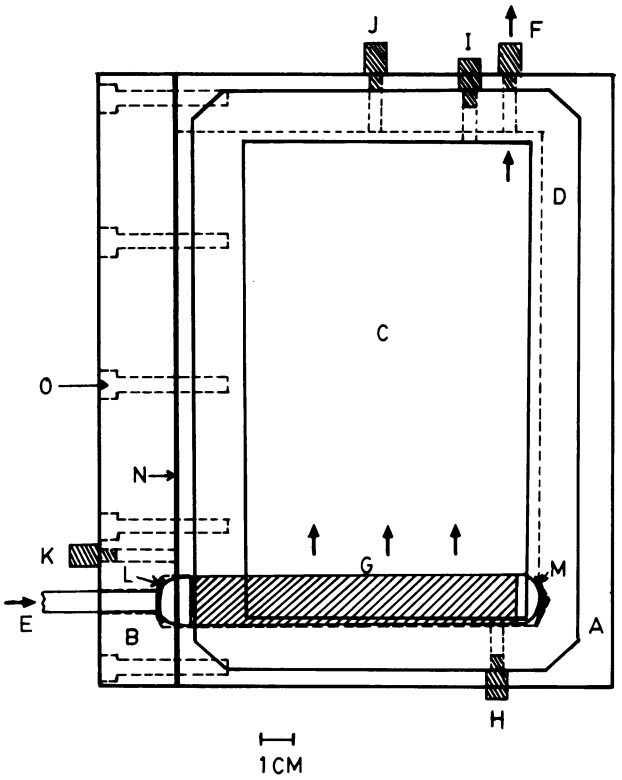

FIG. 1. Side view of chamber for determining photosynthesis of algae in liquid suspensions using the open gas analysis system. A: main section; B: end section; C: inner sample chamber (volume 6 mm × 116 mm × 112 mm = 78 cm^3); D: water jacket (only front water jacket is shown); E: gas entrance; F: gas exit; G: sintered glass tubular filter; H: water inlet port; I: water outlet port; J: thermocouple port; K: sample removal port (normally clamped off); L: rubber "O" ring seals; M: rubber piece; N: rubber gasket; O: inset screws; (→) shows direction of gas flow.

CO_2 depletion on the trace remained constant, which was about 1 h after the algae had been placed in the leaf chamber, and continued for a period of 2 h. All experiments were conducted at 21% O_2. pH on the artificial leaf was determined with a combination pH microelectrode (Bio-Rad).

Photosynthesis was also measured as O_2 evolution in an O_2 electrode chamber (Yellow Springs Instruments, model 5331) using a light intensity of 600 $\mu E\ m^{-2}\ s^{-1}$ provided by a projector (Hanimex 500). A constant temperature circulator bath maintained the cuvette temperature at 25 C. Assays were performed in a total volume of 2.0 ml which included 100 μl *Chlorella* (20 μg Chl) in the appropriate 50 mM buffer and pH. These buffers were prepared CO_2-free as described by Lorimer *et al.* (23). After the algae depleted the endogenous CO_2 (contained in the medium or as a pool in the cells [4]) the background rate of O_2 evolution, which remained constant for periods of at least 1 h, was typically 4–5 μmol $O_2\ mg^{-1}$ Chl h^{-1}. Since similar rates of HCO_3^--dependent O_2 evolution were obtained at any time during this period, HCO_3^- was routinely added 5–10 min after the CO_2 was depleted.

The concentrated algal cells (1 μg Chl/5 μl) were stored at room temperature in deionized H_2O and bubbled with air. They maintained their photosynthetic rate over an 8-h period.

HCO_3^- and CO_2 concentrations in the open and O_2 electrode systems were calculated according to Buch (9). Kinetic constants were calculated using a nonlinear regression computer program following the methods of Wilkinson (39). Chl was extracted with boiling 100% methanol and $MgCO_3$ and the concentration determined using the extinction coefficients for Chl *a* and *b* (18).

RESULTS

A concentration of Mes buffer higher than 25 mM was necessary to maintain pH 5 in the aqueous system but no difference in photosynthetic rates were observed between cells suspended in 0.05 and 0.1 M Tricine at pH 8.5. Subsequently, cells were resuspended in 0.1 M buffer at the appropriate pH. Of the buffers tested (Mes, citrate, Tes, Mops[2], Hepes, Taps, Tricine, Caps, glycine), only citrate and Caps substantially reduced the rate of CO_2 fixation. The presence of growth medium with the buffer and the order of exposure to CO_2 concentrations had no effect on the CO_2 response curves for photosynthesis.

Since the ratio of CO_2 to HCO_3^- present in solution is altered by changing pH, the measurement of photosynthesis as a function of inorganic carbon concentration and pH allows one to infer which form (CO_2 or HCO_3^-) is utilized in photosynthesis (4, 30). Rates of photosynthesis of *Chlorella* were measured as a function of pH at different CO_2 or added HCO_3^- (total inorganic carbon) concentrations (Figs. 2–4). In all experimental methodologies, the photosynthetic rates at saturating or near saturating CO_2 concentrations were higher at pH 5.0 than at pH 8.5. At CO_2 concentrations equal or below atmospheric levels (10 μM) CO_2 fixation was greatest at pH 8.5.

CO_2 compensation points, determined by interpolation to the point of zero net photosynthesis (22), were about 0.3 μM (Table I) regardless of the pH, except where CA was added. When CA was added the compensation point declined to about 0.11 μM CO_2, though the variation was large in the case of the aqueous system (Table I). In the O_2 electrode system, the photosynthetic rate extrapolated to zero CO_2. CO_2 evolution into CO_2-free air in the light (22) was low, amounting in most cases to less than 10% of the photosynthetic rate under normal atmospheric conditions (Table I).

Assuming full equilibration of CO_2 and HCO_3^-, the Michaelis-Menten kinetic parameters for photosynthesis (V_{max}, the maximum rate of photosynthesis; K_m, the concentration of CO_2 at which the rate of photosynthesis attains one-half of its maximum value) were calculated using nonlinear regression analysis according to Wilkinson (39) (Table II). In the open systems, the K_m (CO_2) remained fairly constant over the pH range 5.0–8.5, al-

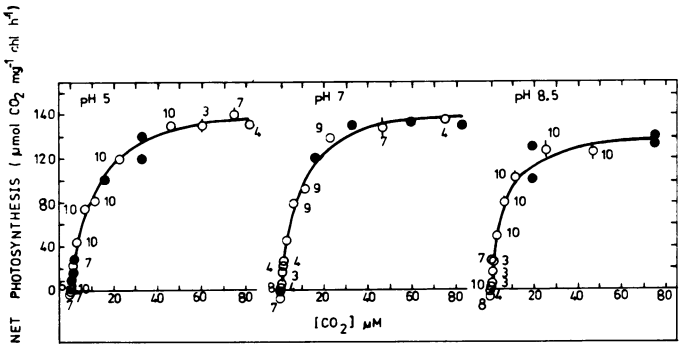

FIG. 2. CO_2 response curves for *C. pyrenoidosa* photosynthesis as a function of pH using artificial leaf technique in the open gas analysis system. Measurements were conducted at 25 C, a quantum flux density of 400 $\mu E\ m^{-2}\ s^{-1}$ and 21% O_2. Mes, Hepes, and Taps buffers (0.1 M) were used at pH 5.0, 7.0, and 8.5, respectively. [CO_2] represents the average dissolved CO_2 concentration. Bars are SE of mean with the number of measurements shown beside each point. (●): measurements where sample number was less than 3. (——): fitted according to Wilkinson (39).

[2] Abbreviations: Mops: morpholinopropane sulfonic acid; Taps: tris(hydroxymethyl)methylaminopropanesulfonic acid: Caps: cyclohexylaminopropanesulfonic acid; CA: carbonic anhydrase.

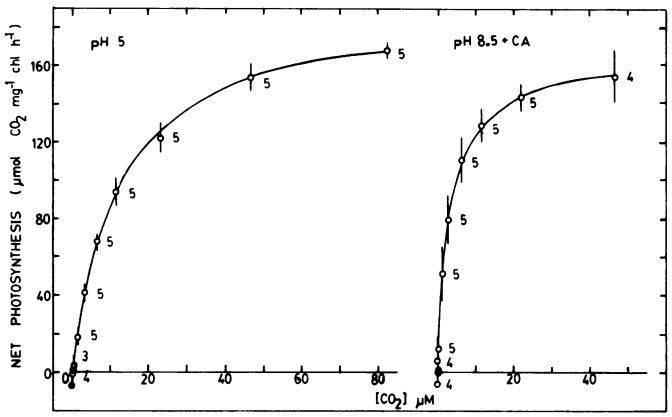

FIG. 3. CO_2 response curves for *C. pyrenoidosa* photosynthesis as a function of pH using the aqueous technique in the open gas analysis system. Measuring conditions and explanations as for Figure 2. CA (260 Wilbur-Anderson units) from bovine erythrocytes (Sigma) were added to experiments at pH 8.5.

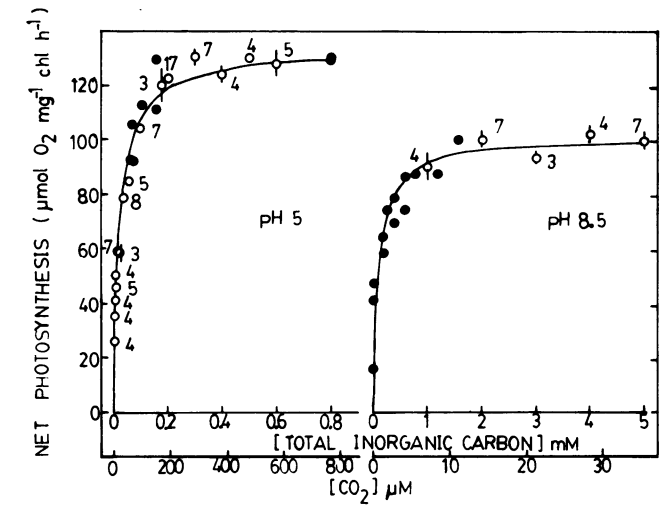

FIG. 4. Bicarbonate-dependent O_2 evolution for *C. pyrenoidosa* as a function of pH. Measurements were performed at 25 C, 600 μE m^{-2} s^{-1} and an O_2 concentration of 18–21% using a sealed O_2 electrode system. The background rate was subtracted from the HCO_3^--dependent rate of O_2 evolution. [Total inorganic carbon] represents the concentration of added HCO_3^-; the [CO_2] in equilibrium with the HCO_3^- is shown for comparative purposes. Remainder of explanation as for Figure 2.

though when CA was added in the aqueous technique, the K_m (CO_2) decreased to a value of 3.2 μM as compared to 11.9 μM at pH 5.0. In contrast, the K_m (HCO_3^-) changed by several hundred-fold when the pH was changed from 5 to 8.5. When O_2 evolution as a function of pH was examined in the closed system, the K_m (CO_2) decreased and the K_m (HCO_3^-) increased with increasing pH, but the change in K_m (CO_2) was much less than the change in K_m (HCO_3^-). All of these results suggest that CO_2 was the carbon species utilized in photosynthesis (4, 30) although there may be some contribution from HCO_3^- at the high pH, especially in the O_2 electrode system, where the inorganic carbon was added as HCO_3^-.

The addition of CA, which facilitates the establishment of the equilibrium between CO_2 and HCO_3^-, appeared to cause a reduction in the K_m (CO_2) (Table II). The effect of CA addition was investigated further using the artificial leaf with the cells suspended in buffer and in fresh medium plus buffer. (Table III). Without CA, the K_m (CO_2) in each resuspension condition was

Table I. *Compensation Points and CO_2 Evolution Into CO_2-Free Air in the Light by C. pyrenoidosa*

Values were determined from results of Figures 2 and 3.

pH	CO_2 Evolution	Compensation Point
	μmol mg^{-1}Chl h^{-1}	μM
Artificial leaf		
5.0	6.4 ± 1.4[b] (7)[c]	0.36[d] ± 0.14 (10)
7.0	7.8 ± 1.7 (7)	0.32 ± 0.08 (9)
8.5	7.2 ± 1.9 (8)	0.29 ± 0.07 (10)
8.5 + CA[a]	5.1 ± 1.1 (4)	0.12 ± 0.03 (4)
Aqueous technique		
5.0	7.1 (1)	0.29 ± 0.19 (4)
8.5 + CA[a]	6.1 ± 1.9 (4)	0.11 ± 0.08 (5)

[a] Wilbur-Anderson units (260) of CA from bovine erythrocytes were added to the resuspension medium.
[b] Mean ± SD.
[c] Numbers in parentheses represent the sample number.
[d] Equivalent to 10.7 μl l^{-1} CO_2 in the gas phase.

Table II. *Influence of pH upon Kinetic Parameters of Photosynthetic Carbon Dioxide Fixation and Oxygen Evolution by C. pyrenoidosa*

pH	K_m[a,f] (Total)	K_m[a,f] (HCO_3^-)	K_m[a,f] (CO_2)	V_{max}[a]
	μM			μmol mg^{-1}Chl h^{-1}
Artificial leaf				
5.0	10.7	0.5	10.2 ± 0.9[e]	177.3 ± 3.6[b]
7.0	48.1	39.3	8.8 ± 0.9	181.5 ± 5.9[b]
8.5	765.3	743.1	5.4 ± 0.6	148.2 ± 4.9[b]
Aqueous technique				
5.0	12.4	0.5	11.9 ± 1.2	192.0 ± 6.4[b]
8.5 + CA[d]	439.3	426.6	3.2 ± 0.7	164.0 ± 9.7[b]
Oxygen electrode				
5.0	22.5 ± 1.6	0.8	21.8	132.6 ± 1.9[c]
8.5	97.7 ± 0.0	94.9	0.7	97.5 ± 2.2[c]

[a] Kinetic constants were calculated from data in Figures 2–4, according to Wilkinson (39); compensation point was subtracted from the CO_2 concentration before calculation.
[b] μmol CO_2 mg^{-1}Chl h^{-1}.
[c] μmol O_2 mg^{-1}Chl h^{-1}.
[d] CA (260 Wilbur-Anderson units) added to suspension.
[e] Numbers represent mean ± SD.
[f] Assuming equilibrium the CO_2, HCO_3^-, and total carbon concentration were calculated according to Buch (9).

the same at pH 7 and 8.5 whereas the K_m (HCO_3^-) changed over 20-fold. With CA addition, the change in the K_m (CO_2) between pH 7 and 8.5 was again much less than the change in K_m (HCO_3^-). These results are again consistent with CO_2 being the carbon species utilized in photosynthesis (4). Both the K_m (CO_2) and K_m (HCO_3^-) were reduced by the addition of CA (Table III). The stimulation of photosynthesis at low CO_2 concentrations by addition of CA to the suspension medium has been noticed before (5, 25) and in one case (5) it was suggested that the cells were using HCO_3^- rather than CO_2. With CA present, the HCO_3^- concentration may be higher and the utilization of HCO_3^- may be increased, thereby resulting in a decrease in the K_m (CO_2) and K_m (HCO_3^-). This possible explanation rests on the assumption, that without CA, the system was far out of equilibrium and the HCO_3^- concentration present was not that shown (Table III) but was less than that observed when CA was present. Although the system without CA may not be in equilibrium, the large change in K_m (HCO_3^-) between pH 7 and 8.5 suggests that any differences in HCO_3^- concentration brought about by the addition of CA

Table III. *Influence of Addition of CA to the Resuspension Medium upon Kinetic Parameters of Subsequent Photosynthetic Rates by C. pyrenoidosa Using the Artificial Leaf*

Measurements were performed at 25 C and 400 μE $m^{-2}s^{-1}$. Experiments on ± CA conditions were performed on the same day. Remainder of explanation as for Table II.

Resuspension Conditions	K_m[a] (Total)	K_m[a] (HCO_3^-)	K_m[a] (CO_2)	V_{max}[a]
		μM		$\mu mol\ CO_2\ mg^{-1}Chl\ h^{-1}$
Buffer				
Hepes (pH 7.0)				
− CA	29.0	23.7	5.3 ± 0.6	105.0 ± 31
+ CA	19.7	16.0	3.6 ± 0.4	118.0 ± 3.0
Taps (pH 8.5)				
− CA	609.9	592.2	4.3 ± 0.4	110.7 ± 3.1
+ CA	255.3	247.9	1.8 ± 0.2	105.8 ± 2.6
Fresh medium buffer				
Mops (pH 7.0)				
− CA	47.0	38.4	8.6 ± 1.5	162.6 ± 10.1
+ CA	33.3	27.2	6.1 ± 0.9	178.3 ± 7.9
Tricine (pH 8.5)				
− CA	1276.5	1239.5	9.0 ± 1.3	210.0 ± 10.5
+ CA	283.7	275.7	2.0 ± 0.1	178.0 ± 3.0

[a] Eight to 11 different CO_2 concentrations with a total sample number ranging from 11 to 22, were used to calculate the kinetic constants; compensation point was subtracted from the CO_2 concentration before calculation.

(especially at pH 8.5) are not likely to have much effect on photosynthesis. An alternative explanation for the effect of CA addition to the medium has been presented by Shiraiwa and Miyachi (34). They propose that in regions of CO_2 depletion CA can increase the CO_2 concentration by indirect supply of CO_2 from the HCO_3^- present. The net effect of the combination of direct and indirect CO_2 supply is that the apparent concentration of CO_2 required is decreased. This explanation would be consistent with our observations.

Another way of evaluating the relative importance of CO_2 or HCO_3^- to photosynthesis is to compare the rate of formation of the species not added to the rate of photosynthesis. For the data on photosynthesis at pH 8.5 in Figure 2, where CO_2 was added, the rate of formation of HCO_3^- can be compared to the photosynthetic rate. To calculate the production of HCO_3^-, the rate constant of Watt and Pasche (37) was used which included both water and OH^--catalyzed HCO_3^- formation (21). For the data on photosynthesis at pH 8.5 (Fig. 4) where HCO_3^- was added, the rate at which CO_2 could be produced was calculated using the equations derived by Lucas (24) using constants for freshwater after Buch (9). The calculated rates would be maximum rates as the solutions involved the assumption that all CO_2 and HCO_3^- produced would be consumed, thus keeping their concentrations at zero.

The rates of hydration and dehydration together with the photosynthetic rates are shown in Figure 5. In the artificial leaf, HCO_3^- production was clearly insufficient to maintain photosynthesis. In the O_2 electrode, CO_2 production was sufficient to maintain photosynthesis above 0.5 mM total carbon. Below that concentration CO_2 production was not sufficient, suggesting that HCO_3^- may also be utilized.

When photosynthetic rate and the hydration rate of CO_2 are determined over the pH range 5–10.5, it is clear that the rate of production of HCO_3^- is not sufficient to maintain photosynthesis (Fig. 6). In contrast the rate of production of CO_2 from HCO_3^- was sufficient to maintain photosynthesis at all pH values below pH 8.3 (Fig. 7). Above this pH photosynthesis would appear to depend on HCO_3^- utilization (Fig. 7). At saturating inorganic

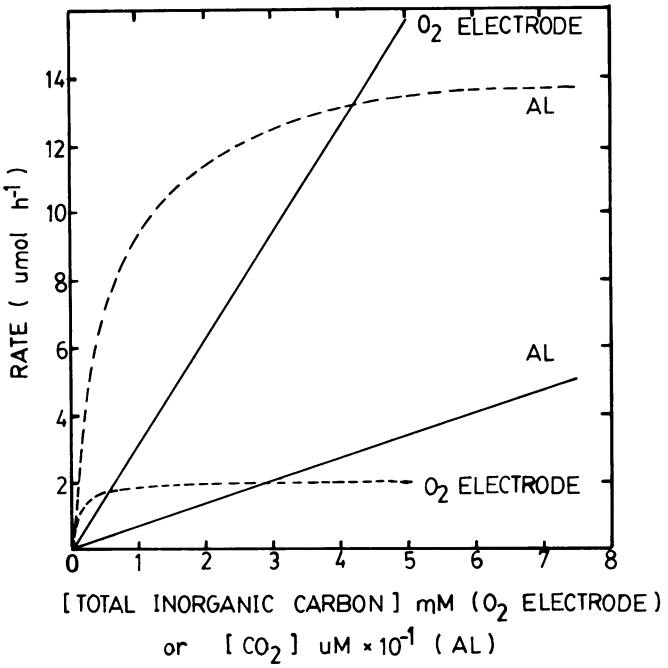

FIG. 5. Rates of CO_2 and HCO_3^- production (——) from the other carbon species and rates of photosynthesis for *C. pyrenoidosa* (---) at pH 8.5. In the artificial leaf (AL), Chl concentration was 100 μg and volume was 0.3 ml, and in the O_2 electrode, Chl concentration and volume were 20 μg and 2.0 ml, respectively. Photosynthesis data taken from Figures 2 and 4.

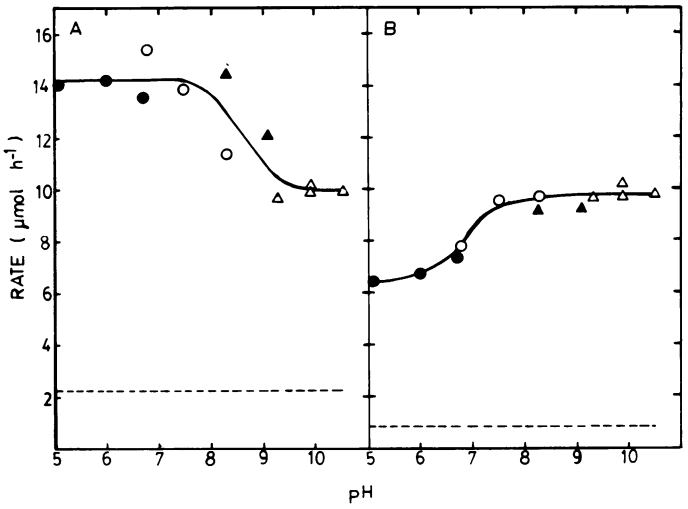

FIG. 6. Rates of CO_2 hydration (---) and photosynthesis of *C. pyrenoidosa* (——) as a function of pH at near saturating and limiting dissolved CO_2 concentrations. Measurements were performed at 25 C, 400 μE m^{-2} s^{-1}, and 21% O_2 using the artificial leaf; A: $[CO_2]$ = 46.8 μM; B: $[CO_2]$ = 11.7 μM. (●): Mes; (○): Hepes; (▲): Taps; (△): glycine. Volume and Chl concentration were 0.3 ml and 100 μg, respectively.

carbon concentrations, photosynthesis was depressed at the higher pH values (Figs. 6A and 7). It seems unlikely that this is a direct effect of pH on photosynthesis because at limiting CO_2 concentrations, a stimulation of photosynthesis at higher pH values was observed (Fig. 6B). It is tempting to ascribe the stimulation in the rate of photosynthesis observed above pH 7.5 (Fig. 6B) to the increased utilization of HCO_3^- in addition to CO_2, but HCO_3^- production was not sufficient to accomodate the increase in pho-

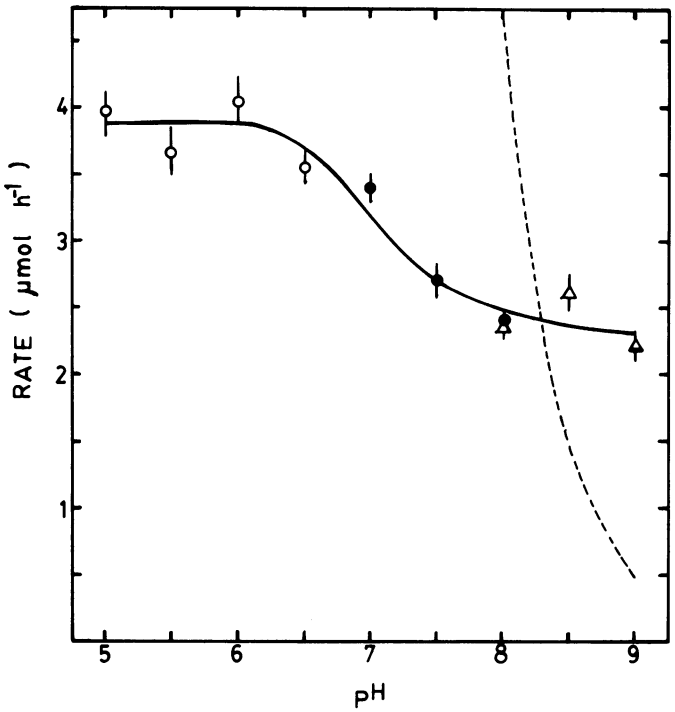

FIG. 7. Rates of dehydration (---) and photosynthesis of *C. pyrenoidosa* (——) as a function of pH. Measurements were performed at 25 C, 600 μE m^{-2} s^{-1}, and 21% O_2 by addition of 0.5 mM HCO_3^- to the O_2 electrode. The background rate of O_2 evolution was subtracted from the HCO_3^--dependent rate. (○): Mes; (●): Hepes; (△): Taps. Errors quoted are the SE of the mean. Sample number was 6–10 for each pH.

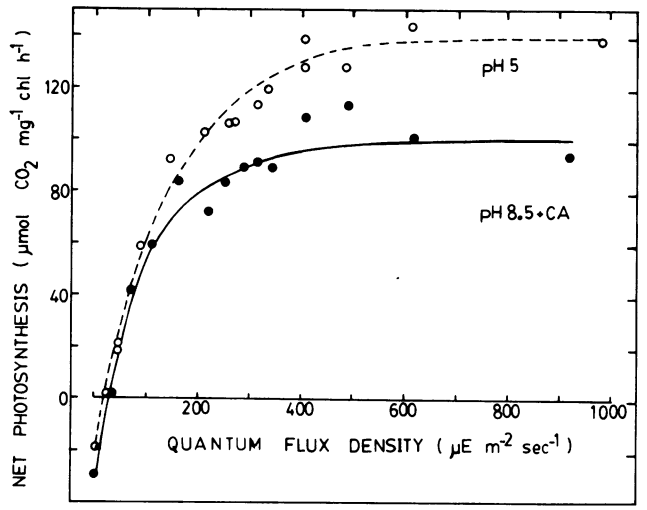

FIG. 8. Effect of light intensity on the rate of photosynthesis in *C. pyrenoidosa* using artificial leaf technique. Measurements were performed at 25 C, 21% O_2, and a dissolved CO_2 concentration of 60.2 μM at both pH 5.0 (0.1 M Mes) and pH 8.5 (+ 260 Wilbur-Anderson units of bovine CA were added to the resuspension medium) (0.1 M Taps).

tosynthetic rate.

At saturating CO_2 concentrations, photosynthesis was higher under conditions where only CO_2 was available than under conditions where both CO_2 and HCO_3^- was available (Figs. 6A and 7). Other authors, who have also observed a similar phenomenon, suggested from light intensity experiments, that the lower rate of photosynthesis in the presence of HCO_3^- indicates that more light was required to fix a mole of carbon supplied as HCO_3^- than was needed for a mole supplied as CO_2 (24, 29). For the conditions used in this study, it was found that especially at high light intensities, photosynthesis was greater when CO_2 was supplied (Fig. 8). However, photosynthesis was saturated at a light intensity of 400 μE m^{-2} s^{-1} at both pH values and in the region of limiting light similar quantum efficiencies were observed. The quantum efficiencies calculated on the basis of incident light (0.02 mol CO_2 E^{-1}) were low, probably because all of the light was not absorbed by the thin layer of algae.

DISCUSSION

Investigation of photosynthetic rates as a function of pH and several CO_2 concentrations in the open gas analysis system (Figs. 2 and 3 and Table II) show that comparable results were obtained using the artificial leaf and aqueous techniques. There is no evidence that results with the artificial leaf are unreliable due to drying, pH changes, or limitation in CO_2 diffusion during the course of the experiments. Measurements on photosynthesis in these two systems were comparable to those obtained with an O_2 electrode system where the inorganic carbon was supplied as HCO_3^-.

With the three systems, the response of photosynthesis to inorganic carbon concentration as a function of pH (Figs. 2–4) indicated that in most circumstances CO_2 and not HCO_3^- was the species taken up by the cells to be used in photosynthesis. The K_m (CO_2) usually ranged from about 2 to 12 μM regardless of the pH, and this K_m was similar to that found for ribulose-1,5-bisP carboxylase activity in isolated chloroplasts (20) and purified enzyme preparations (23). Only in the O_2 electrode measurements where the inorganic carbon was added as HCO_3^- did the K_m (CO_2) fall outside those values. In contrast to the observed stability of the K_m (CO_2), the K_m (HCO_3^-) changed many hundredfold from pH 5 to 8.5 (Table II). Such changes in the observed affinity of the cells for the two inorganic carbon species are more consistent with CO_2 being the species utilized (4, 30). Earlier work (8, 12, 25–27, 38) also indicated a low K_m (CO_2) for *Chlorella* photosynthesis and a lack of dependence of photosynthesis on HCO_3^- concentration. These authors had also concluded that CO_2 was the carbon species utilized by *Chlorella*. This conclusion is further supported in the present work, because when CO_2 was supplied to the cells, the rate of formation of HCO_3^- in all conditions was not adequate to supply the carbon fixed in photosynthesis (Figs 5 and 6).

Conclusions that HCO_3^- is utilized in addition to CO_2 seem to be based either on measuring systems where the inorganic carbon was added as HCO_3^- (4, 14) or on the stimulation of photosynthesis at low CO_2 concentrations that is observed upon the addition of CA to the suspending medium (5). Findenegg (14) observed that the photosynthetic rate of *Chlorella fusca* as a function of pH did not follow the calculated rate of dehydration of carbonic acid. At total carbon concentrations below 0.5 mM, we confirm that CO_2 production from HCO_3^- was not adequate to sustain the rate of photosynthesis (Fig. 5) and under these conditions HCO_3^- absorption by the cells may be a source of CO_2 for photosynthesis. If HCO_3^- is utilized by the algae it cannot be used very effectively as the apparent affinity for HCO_3^- above pH 6.5 is low as the K_m (HCO_3^-) exceeds the K_m (CO_2) be severalfold (4; Tables II and III). If the inorganic carbon had been added as CO_2 to these systems an opposite conclusion may have been reached as, in that case, the hydration rate of CO_2 is much too slow to supply the HCO_3^- that would be required for photosynthesis (Figs. 5 and 6). We also confirm that photosynthesis of *Chlorella* at low concentrations of CO_2 and high pH is stimulated by the addition of CA to the suspension medium (Table III). The decrease in K_m values (CO_2 or HCO_3^-) suggests that, in these circumstances, HCO_3^- utilization supplements CO_2 utilization and the resultant higher CO_2 concentration in the cell gives rise to higher photosynthetic

rates. At limiting CO_2 concentrations photosynthesis increases above pH 7.5 in the absence of CA (Fig. 6B) and the rate of hydration of CO_2 was not adequate to supply enough HCO_3^- to sustain the increase in the photosynthetic rate. Although the role of CA in the external medium can not be explained with certainty, it appears likely that it could function to indirectly supply CO_2 to the cells (34).

The stimulation of photosynthesis at pH values above 7.5 and low CO_2 concentrations does not appear to be due to a direct effect of pH on photosynthesis because at saturating CO_2 concentrations and inhibition of photosynthesis was observed (Figs. 6 and 7). It seems that low HCO_3^- concentrations stimulate photosynthesis and high HCO_3^- concentrations inhibit photosynthesis but a logical explanation of the observations is not apparent.

This inhibition of photosynthesis at saturating CO_2 and high pH has been observed by Lucas (24) and Raven (30) who suggested that HCO_3^- uptake required more light energy than did CO_2 uptake. Raven (30) has suggested from inhibitor studies that a metabolic HCO_3^- pump related to a light dependent reaction may be involved; the reaction is dependent on PSII, but independent of ATP. However, these studies show that in the light limiting region there is no difference in the quantum efficiency of CO_2 fixation at pH 5 and 8.5 (Fig. 8). This result is again consistent with the conclusion that CO_2 is the major species of inorganic carbon being utilized at each pH and that HCO_3^- if used at all, plays only a minor role.

Chlorella is a C_3 plant based on the ratio of ribulose-1,5-bisP carboxylase to phosphoenolpyruvate carboxylase (11, 31, 32) or the analysis of products after short periods of $^{14}CO_2$ fixation (3, 17). Yet the low CO_2 compensation points, the apparent scarcity of CO_2 evolution into CO_2-free air (Table II and ref. 22) and the apparent lack of sensitivity of photosynthesis to O_2 discussed in an accompanying paper (33) indicate that photorespiration is low or absent. An elegant case, based on studies of low CO_2- and high CO_2-grown algae, has been presented that photorespiration in low CO_2-grown algae is suppressed as a result of HCO_3^- accumulation and a subsequent high internal CO_2 concentration (1, 2). If, as this work shows, *Chlorella* uses CO_2 and has only a low affinity for HCO_3^-, it will be necessary to examine closely this proposal as the explanation for the apparent lack of photorespiration in air-grown algae.

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