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Sources of CO_2 for Nuisance Blooms of Algae[1]

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Abstract. Bacterial production of CO_2 from sucrose substrate increased growth of seven species of algae in CO_2-limited laboratory cultures. Decomposition of organic matter in pond water also supplied enough CO_2 to support good algal growth in cultures deprived of other sources of CO_2. Estimates of CO_2 production from decay of dissolved organic matter in six pond waters ranged from 0.32 to 3.53 mg/L per 24 hr. The carbonate-bicarbonate equilibrium system is a major source of CO_2 for algal photosynthesis. However, in waters of low or extremely high alkalinity, this system will not support high rates of photosynthesis. In such waters CO_2 from decomposition will stimulate photosynthesis. Decomposable organic compounds must be considered with nitrogen and phosphorus as factors responsible for accelerated eutrophication and nuisance algal blooms.

INTRODUCTION

THERE is currently considerable interest in the importance of CO_2 from bacterial respiration in the development of nuisance growths of aquatic plants. Kuentzel (11, 12) concluded that massive blooms of blue-green algae were always associated with excessive amounts of decomposable organic matter and that the large amounts of CO_2 required for development of these blooms were derived from organic decomposition by bacteria. Lange (13, 14) reported that additions of sucrose to systems containing bacteria and blue-green algae resulted in abundant algal growth. Bacteria assimilated sucrose and produced CO_2 which accelerated algal growth in CO_2 limited cultures. King (10) presented evidence that the carbonate-bicarbonate equilibrium system was the most important factor governing algal activity in lakes and that this system was the only significant reserve photosynthetic carbon source. However, King maintained that CO_2 production by respiratory processes was important in replenishing CO_2 in the buffer system; and additions of biologically available organic matter to an aquatic system increased the amount of CO_2 available for photosynthesis. Carbon dioxide derived from the carbonate-bicarbonate equilibrium system was reported to be a major factor controlling growth of a rooted aquatic plant (*Najas* sp.) in Pickwick Reservoir on the Tennessee River (16).

Earlier research indicated that increased concentrations of nitrogen and, especially, phosphorus were responsible for eutrophication and associated nuisance growths of plants in lakes and streams (4, 6, 15, 21). However, proponents of the CO_2 hypothesis stress that eutrophication is strictly a phenomenon related to increases in available carbon for photosynthesis (10, 11, 12). These workers declare that most lakes contain adequate nitrogen and phosphorus to support nuisance growths of plants provided sufficient carbon is available. Kuentzel (12) advocates more emphasis on reduction of biochemical oxygen demand (BOD) in wastewater effluents than on removal of nitrogen and phosphorus. Some evidence is available to indicate that unpolluted natural lakes contain sufficiently high nitrogen and phosphorus levels to cause excessive plant production (7).

Boyd (4) argued against the unequivocal validity of the CO_2 hypothesis. Recent studies (3) on fish ponds revealed that new ponds located on highly inorganic soils and having waters of low alkalinity and organic matter contents developed massive blooms of blue-green algae during the first summer of fertilization with inorganic nitrogen and phosphorus or with phosphorus alone. However, CO_2 often limits photosynthesis in ponds (19), and abundant supplies of all essential resources are obviously required for high levels of photosynthesis. The present study was initiated to evaluate the effect of microbially produced CO_2 on growth of laboratory cultures of algae which were deprived of sufficient atmospheric CO_2 for maximum growth. Most of this research involved green algae since earlier work concentrated upon blue-green forms.

MATERIALS AND METHODS

Cultures. Pure cultures of algae were obtained from The Culture Collection of Algae at Indiana University (Table 1). These cultures were maintained on agar slants (18) at 26 C under 540 lux illumination (16 hr light and 8 hr dark) and transferred to fresh agar slants at monthly intervals. Stock liquid cultures were grown in Beyerinck's solution to which 25 ml of soil extract (2) and 3 drops of a solution of 1% Sequestrene 330 Fe iron chelate[3] were added per liter. Water which was distilled twice in a glass distillation apparatus was used for making solutions. Liquid stocks were maintained at 26 C under 4300 lux illumination (16 hr light and 8 hr dark) and transferred to fresh media at 7 to 10-day intervals. Aseptic techniques

Table 1. Stock cultures of algae from The Culture Collection of Algae, Indiana University, which were used as a source of organisms.

Species	Indiana Univ. Culture No.
Anabaena flos-aquae (Lyngbye) Breb.	1444
Ankistrodesmus falcatus (Corda) Ralfs	749
Chlamydomonas sp.	624
Chlorella pyrenoidosa Chick	26
Closterium microporum Naegeli	280
Scenedesmus dimorphus Kutz	746
S. quadricauda (Turp.) Breb.	76
Staurastrum sp.	173

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were used to prevent contamination of pure cultures by other organisms.

General. Nutrient concentrations listed in Table 2 were used in media for all experiments. Soil extract was not

Table 2. Nutrient composition of basic medium used in algal cultures.[a]

Reagent	Concentration
	(mg/L)
NH_4NO_3	250.00
K_2HPO_4	100.00
$MgSO_4 \cdot 7H_2O$	100.00
$CaCl_2 \cdot 2H_2O$	50.00
H_3BO_3	2.50
$MnCl_2 \cdot H_2O$	1.50
$ZnCl_2$	0.10
$CuCl_2 \cdot 2H_2O$	0.05
MoO_3	0.05

[a]Three drops of a 1% solution of iron chelate were added to each liter of solution.

added to experimental media. Double distilled water or pond water was used in preparing media for experiments. The natural algal flora of pond water was removed by filtration through a 20-cm column packed with glass wool. Glassware used in all experiments was washed in H_2SO_4-$Na_2CR_2O_7$ cleaning solution and rinsed in glass distilled water prior to use. Algae were grown in 25 ml of solution. Removal of CO_2 from media was accomplished by boiling for 15 min prior to transferring 25 ml aliquots to 125-ml erlenmeyer flasks. Evaporation losses were replaced with CO_2-free water. Limitation of the atmospheric CO_2 supply was achieved by sealing the flasks tightly with rubber stoppers (13). The only atmospheric CO_2 available to such cultures was that occurring above the medium (115 ml of air was present in the flasks). In order to maintain a surplus of atmospheric CO_2 in other flasks, openings were loosely covered with aluminum foil. Media were shallow (0.9 mm), and rapid equilibration with atmospheric CO_2 was possible.

Inoculation of experimental flasks with algae was accomplished by adding 0.5-ml aliquots from stock liquid cultures. Infection of the algae inoculum with bacteria was achieved by leaving it uncovered for 1 to 2 hr prior to use. Flasks were incubated at 26 C under 4300 lux illumination (16 hr light and 8 hr dark) on a mechanical shaker set at 60 oscillations/min. Flasks containing *Ankistrodesmus falcatus* (Corda) Ralfs were usually incubated 7 days, those containing *Chlamydomonas* sp. for 5 days, and flasks inoculated with other species were incubated for 4 days.

Cell density was ascertained by counting the number of individuals per milliliter (single cells or colonies) or measuring the length of filaments per milliliter in the case of *Anabaena flos-aquae* (Lyngbye) Breb. A counting cell (1 × 20 × 50 mm) and Whipple disk micrometer (1) were used to measure the length of *Anabaena flos-aquae* filaments per milliliter. *Staurastrum* sp. was enumerated in the counting cell. Ten 100 x microscope fields were evaluated for each sample when the counting cell was used. Other species were enumerated with a Spencer Bright-Line Hemacytometer (0.1 mm deep). Four 1 mm^2 grids were counted for each sample.

Experiment 1. This experiment was designed to determine the effect of sucrose on the growth rate of algae in CO_2 limited cultures. Four treatments (0 mg/L sucrose in stoppered flasks, 0 mg/L sucrose in foil covered flasks, 15 mg/L sucrose in stoppered flasks, and 15 mg/L sucrose in foil covered flasks) were prepared for species listed in Table 1. Five replications were used with each treatment for all species. The growth of *Scenedesmus dimorphus* Kutz, *Chlorella pyrenoidosa* Chick, and *Chlamydomonas* sp. was also determined in stoppered flasks (limited supply of atmospheric CO_2) at sucrose levels of 0, 10, 25, 50, 100, 150, 200, and 250 mg/L. Foil covered flasks containing no sucrose served as a control with abundant atmospheric CO_2. Three replications were used at each treatment level for all three species.

Experiment 2. In order to determine if decomposition of organic matter in pond water was a readily available source of CO_2, growth of six species of algae was determined in stoppered flasks containing media made with either distilled water or pond water. Five replications of both treatments were prepared for each species. Pond water was obtained from a pond which received heavy applications of fish feeds and presumably contained large concentrations of organic matter.

Experiment 3. Rates of production of CO_2 in various waters were determined by filling 300-ml BOD bottles with pond waters of known CO_2 concentration and determining the increase in CO_2 after 24 hr incubation in the dark at 26 C. Samples containing the native algal flora and samples which were filtered to remove all algae were used for each water source. Five replications were prepared for each sample. Ponds were all located on the Fisheries Research Unit, Auburn University.

A solution containing high levels of organic matter was prepared by adding 50 g of Auburn No. 3 fish feed (17) to a liter of water and allowing the feed to decay. Aliquots of the supernatant were added to a selected pond water at rates of 0 to 40 mg of organic matter per liter and the rates of CO_2 production determined. Three replications were used at each level of organic matter.

Concentrations of CO_2 were determined by titration with Na_2CO_3 (1). Organic matter concentrations were ascertained by H_2SO_4-$K_2Cr_2O_7$ digestion (1). Chlorophyll analyses were made according to Golterman and Clymo (8).

Experiment 4. The influence of alkalinity on growth of *Scenedesmus dimorphus* in stoppered flasks was determined at additions of 5, 10, 20, 30, 40, and 50 mg/L of a 1 to 1 mixture of $NaHCO_3$-Na_2CO_3 to the basic medium. The basic medium was boiled for 15 min and cooled before aliquots of the $NaHCO_3$-Na_2CO_3 solution were added. A second series was prepared to include the same $NaHCO_3$-Na_2CO_3 range, but with the addition of 15 mg of sucrose per liter to the basic medium. Controls consisted of flasks containing 0 or 15 mg of sucrose per liter of basic medium (without $NaHCO_3$-Na_2CO_3) in stoppered flasks and open flasks containing the basic medium. Three replications were used for all treatments.

RESULTS

Experiment 1. The effect of 15 mg of sucrose per liter on growth of algae in stoppered and open flasks is presented in Figures 1 and 2. The length of filaments of *Anabaena flos-aquae* (a blue-green algae) was increased in both open and closed flasks by addition of sucrose; however, growth was greater in open flasks. There was signifi-

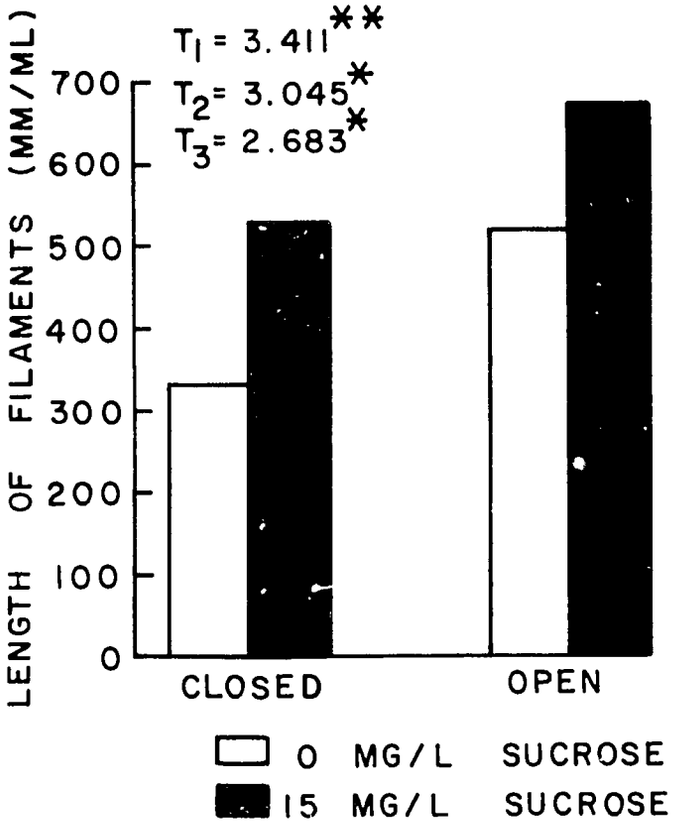

Figure 1. Growth of *Anabaena flos-aquae* in stoppered flasks (closed) and flasks loosely covered with aluminum foil (open) which received either 0 or 15 mg/L sucrose. Designation of t-tests are: T_1 – closed flasks, 0 vs 15 mg/L sucrose; T_2 – open flasks, 0 vs 15 mg/L sucrose; T_3 – open vs closed flasks, 15 mg/L sucrose. Significance at the 5% and 1% levels of probability is indicated by one and two asterisks, respectively.

cantly greater growth of all green algae except *Scenedesmus quadricauda* (Turp.) Breb. when 15 mg/L of sucrose were added to stoppered flasks. Sucrose additions did not increase growth of green algae in open flasks. The amount of growth obtained in open flasks with 0 or 15 mg/L of sucrose was always much greater than that obtained in stoppered flasks.

Sucrose additions up to 25 mg/L to stoppered flasks increased growth of *Scenedesmus dimorphus* and *Chlorella pyrenoidosa,* while *Chlamydomonas* sp. grew best at 50 mg/L sucrose (Figure 3). Increases in sucrose concentrations above these levels increased bacterial activity to the extent that algal growth decreased. Sucrose treatments above 100 mg/L developed heavy white turbidities from bacterial growth. Maximum growth in cultures which were dependent upon bacterial respiration for CO_2 was considerably less than growth of the same species when atmospheric CO_2 was abundant.

Experiment 2. Growth of six species of algae was much greater in media prepared from pond water than in media made from distilled water (Table 3). The pond water contained 29.9 mg/L of organic matter and had a CO_2 production rate of 3.53 mg/L per 24 hr (Table 4). This water

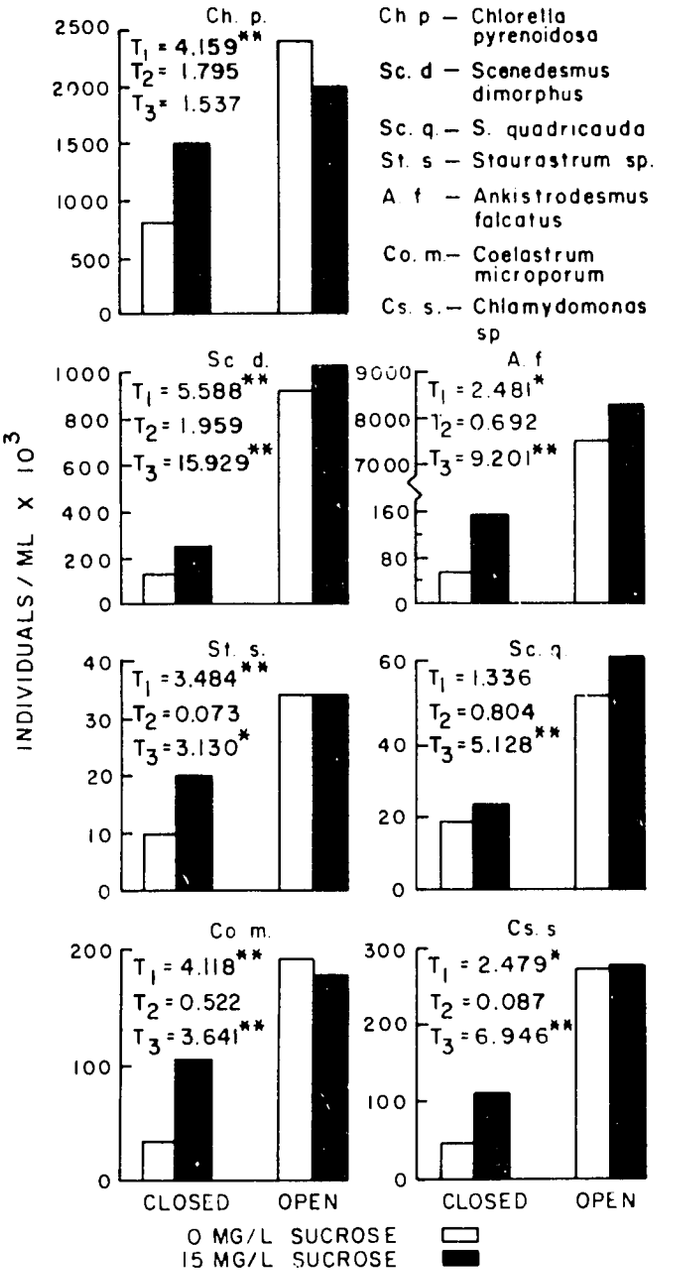

Figure 2. Growth of seven species of green algae in stoppered flasks (closed) and flasks loosely covered with aluminum foil (open) which received either 0 or 15 mg/L sucrose. Designations of t-tests are: T_1 – closed flasks, 0 vs 15 mg/L sucrose; T_2 – open flasks, 0 vs 15 mg/L sucrose; T_3 open vs closed flasks, 15 mg/L sucrose. Significance at the 5% and 1% levels of probability is indicated by one and two asterisks, respectively.

had a total alkalinity of only 17 mg/L (as $CaCO_3$) which was all in the bicarbonate form. Boiling of media converted bicarbonate to carbonate and essentially destroyed the carbonate-bicarbonate buffer system. Therefore, increased growth in media prepared from pond water was assumed to be the result of increased CO_2 from bacterial activity.

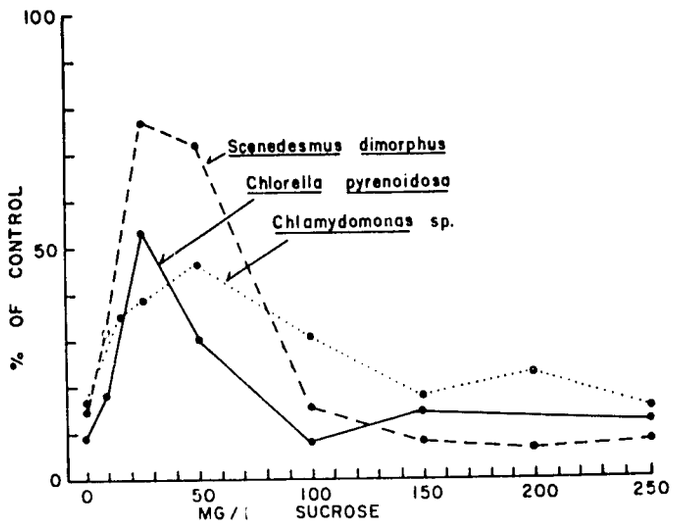

Figure 3. Effect of sucrose on growth of three species of green algae in stoppered flasks. Growth of each species in open cultures which received plentiful CO_2 was used as the control.

Table 3. Growth of algae in media prepared from glass distilled water (control) and pond water. Algae were cultured in stoppered flasks with a limited supply of CO_2 from inorganic sources.

Species	Control	Pond water	t-value[a]
	(Individuals/ml × 10^3)		
Scenedesmus dimorphus	156	289	8.365**
Chlorella pyrenoidosa	114	257	5.842**
Coelastrum microporum	48	83	4.648**
Chlamydomonas sp.	59	99	7.463**
Ankistrodesmus falcatus	97	144	6.657**
Staurastrum sp.	10	20	3.792**

[a]**Significant at the 0.01 level of probability.

Table 4. Production of CO_2 in raw pond water and in pond water that was filtered to remove algal cells. Samples were incubated in the dark at 26 C. Chlorophyll and organic matter concentrations are also given.

Pond[a]	Chlorophyll content of raw water	Organic matter content of filtered water	CO_2 produced[b]	
			Raw water	Filtered water
	(ug/L)	(mg/L)	(mg/L/24 hr.)	(mg/L/24 hr.)
I-11	2.7	9.0	0.43 ± 0.08	0.32 ± 0.18
S-10	12.6	18.6	2.23 ± 0.02	2.13 ± 0.07
S-1	24.3	18.8	3.63 ± 0.18	2.55 ± 0.07
S-7	37.8	13.8	2.30 ± 0.20	0.57 ± 0.02
M-3	102.4	29.9	5.73 ± 0.23	3.53 ± 0.12
S-3	175.0	16.8	8.78 ± 0.23	2.70 ± 0.07

[a]Designations refer to ponds on the Fishery Research Unit Auburn University.
[b]± 1 Standard error.

Experiment 3. Amounts of CO_2 produced by filtered pond waters varied from 0.32 to 3.53 mg/L per 24 hr (Table 4). Rates generally increased with increasing concentrations of organic matter. Differences between CO_2 production in filtered and raw water were related to algal respiration in raw water. With chlorophyll values as indices of algal density, respiration increased with algal density. Large amounts of CO_2 were produced by algal respiration in waters with high chlorophyll levels.

When decomposing fish feed was added to water, a marked increase in CO_2 production resulted (Table 5).

Table 5. Effect of added organic matter (decomposing fish feed) on CO_2 production in a pond water. Samples were incubated in the dark at 26 C.

Added organic matter	CO_2 produced[b]
(mg/L)	(mg/L/24 hr.)
0.0[a]	0.43 ± 0.008
5.4	2.57 ± 0.02
13.4	5.87 ± 0.06
26.8	6.17 ± 0.02
40.2	5.56 ± 0.04

[a]Water used in experiment contained 12.8 mg/L organic matter.
[b]± 1 Standard error.

Maximum production of CO_2 occurred when 26.8 mg/L of organic matter (as decomposing fish feed) was added. Excessive levels of organic matter resulted in oxygen depletion, and respiratory by-products other than CO_2 were produced.

Experiment 4. Growth of *Scenedesmus dimorphus* in stoppered flasks increased in response to bicarbonate-carbonate alkalinity over the range of 5 to 30 mg/L of $NaHCO_3$-Na_2CO_3 (Figure 4). Additions of 15 mg/L of

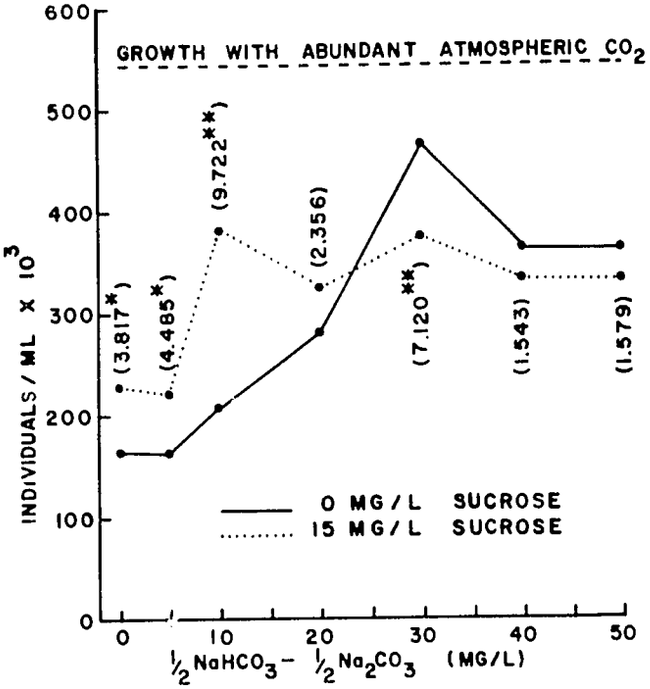

Figure 4. Effect of increased $NaHCO_3$-Na_2CO_3 alkalinity on growth of *Scenedesmus dimorphus* in stoppered flasks. Media contained 0 or 15 mg/L sucrose. Values in parentheses are t-tests comparisons of 0 vs 15 mg/L sucrose at different alkalinity levels. Significance at the 5% and 1% levels of probability is indicated by one and two asterisks, respectively.

sucrose increased algal growth at levels of 0 to 10 mg/L of $NaHCO_3$-Na_2CO_3. At higher levels of alkalinity, sucrose was not effective in increasing growth. Growth was even decreased by additions of sucrose at 30 mg/L of $NaHCO_3$-Na_2CO_3. Growth at additions of 30 mg/L of $NaHCO_3$-Na_2CO_3 buffer in stoppered flasks without sucrose was 85.7% as great as growth in flasks receiving abundant atmospheric CO_2. However, this difference was statistically significant (t= 4.761**).

Discussion

Lange (13) found that seven species of blue-green algae grew at considerably greater rates when 10 mg/L of sucrose were added to inorganic media in stoppered flasks. Results for *Anabaena flos-aquae* (Figure 1) corroborate his findings, and data in Figure 2 indicate that CO_2 from bacterial metabolism of sucrose also increases growth of green algae in CO_2-starved cultures. Kuentzel (12) emphasized that bacteria were always present in sheath material of blue-green algae and suggested that sheath-dwelling bacteria were important in supplying CO_2 to blue-green algae. However, in a stoppered, CO_2-limited culture, it appears obvious that CO_2 produced by free-living bacteria would be beneficial to algal growth. This was true in the present study since only one green alga *(Chlamydomonas* sp.) was surrounded by sheath material.

Sucrose concentrations of 10 mg/L were reported to produce as much growth of blue-green algae as was obtained with abundant atmospheric CO_2 (13). Flasks reported to receive abundant CO_2 were loosely covered with aluminum foil but not agitated (13). Atmospheric CO_2 supplied by agitating loosely covered flasks produced considerably more growth than was obtained by adding 15 mg/L sucrose to stoppered flasks (Figures 1 and 2). Initial experiments also indicated a greater growth rate in agitated than in stationary open flasks. Therefore, it is assumed that cultures reported by Lange (13) to receive abundant CO_2 were actually CO_2 limited.

Sucrose is a highly available carbon substrate for bacteria. Organic matter present in natural waters will likely vary in chemical composition and in suitability as a substrate. However, organic matter in water from a fish pond was utilized by bacteria at a rate sufficient to produce fairly dense algal growth in stoppered flasks (Table 3). Rates of CO_2 evolution were fairly large in waters with 18.6 to 29.9 mg/L of dissolved organic matter (Table 4). When a readily available substrate (decomposing fish feed) was added to a water of low organic matter content, maximum CO_2 production occurred after addition of only 26.8 mg/L of organic matter. Limitation of CO_2 production at higher levels of organic matter resulted from oxygen depletion in darkened BOD bottles. In a natural system, O_2 from photosynthesis would normally prevent O_2 depletion at such low concentrations of organic matter. These findings substantiate the claim by Kuentzel (12) that large amounts of CO_2 are produced by bacterial activity. The CO_2 from algal respiration can also be reused in photosynthesis. Respiration of other organisms also releases considerable CO_2. For example, CO_2 evolution by white catfish *(Ictalurus catus* L.) at 28 C is 1.8 mg/kg per min[1]. White catfish populations often attain densities of 2,000 kg/ha in production ponds. Such populations would produce 5.18 kg CO_2/ha per day.

Data in Figure 4 reveal the importance of the carbonate-bicarbonate buffer system in supplying CO_2 for photosynthesis of *Scenedesmus dimorphus* in stoppered flasks. At low concentrations of this buffer system, additional CO_2 from bacterial activity was of no benefit. The decrease in growth at high alkalinities and the inhibition of growth by sucrose at 30 mg/L $NaHCO_3$-Na_2CO_3 is puzzling. The pH varied only slightly (7.2 at 0 mg/L to 7.8 at 50 mg/L of $NaHCO_3$-Na_2CO_3), so abundant bicarbonate ion was present to supply CO_2 at high treatment levels. Increased $NaHCO_3$-Na_2CO_3 concentrations also greatly enhanced the growth of a submersed vascular plant *(Najas* sp.) in greenhouse cultures (16). Increased growth of *Scenedesmus* in cultures which received abundant atmospheric CO_2 supports the idea that CO_2 from the buffer system cannot produce maximal growth (12). Abundant atmospheric CO_2 also supported heavier growth than was achieved with sucrose additions (Figure 3). The equilibrium concentration of atmospheric CO_2 in natural water is about 0.7 mg/L, and diffusion of the gas through the water column is slow (10). Except during periods of turbulence due to wave action, atmospheric CO_2 is probably a minimal source of CO_2. Blue-green algae often occur at the surface of lakes and ponds as a scum and probably benefit more from atmospheric CO_2 than do algae which occur at greater depths.

The present study indicates that CO_2 from bacterial activity can stimulate algal growth in waters of low alkalinity where CO_2 from inorganic sources is in short supply. The rate of production of CO_2 by bacteria in natural waters containing excessive organic matter is likely sufficient to support massive algal blooms. Likewise, the carbonate-bicarbonate buffer system can support dense growth of algae in flasks and presumably in nature. Many waters of medium to high alkalinity contain enough available carbon to support nuisance blooms without addition of organic matter. Most of the CO_2 in waters of high alkalinity and pH is bound in carbonate form, and little CO_2 is supplied by the buffer system. Photosynthesis in such waters would likely respond to additions of organic substrate and ensuing bacterial activity. Respiratory CO_2 is important in replenishing CO_2 in the carbonate-bicarbonate equilibrium system in all waters (10).

Findings of the present study neither support nor discredit the hypothesis that eutrophication with associated algal blooms is a carbon related process. Development of massive algal blooms require plentiful supplies of all resources necessary for algal growth. Production in any plant community is limited by some genetic or environmental factor(s). Alleviation of this limitation allows increased growth until another resource is exhausted. It is unreasonable to assume that the same factor limits growth in all aquatic systems. However, wastewater effluents responsible for eutrophication usually contain high levels of nitrogen, phosphorus, and carbon (15). One or more of these nutrients are likely responsible for most cases of accelerated eutrophication effected by human influence.

Proponents of the CO_2 hypothesis contend that lakes presently contain adequate phosphorus to support nuisance blooms (10, 12). Phosphorus is assumed to be recycled rapidly, and continued additions of this nutrient are not necessary to sustain high levels of primary production. This assumption appears incorrect since phosphorus is rapidly absorbed by bottom muds (4, 5, 9). Even in highly fertile fish ponds, high levels of photosynthesis depend upon periodic additions of phosphorus (9). Production in fertilized ponds decreases drastically within 3 years if applications of inorganic fertilizers cease (20). Nevertheless, CO_2 relationships must be given critical consideration in case studies of artificial eutrophication.

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