Effects of Light Intensity, Oxygen Concentration, and Carbon Dioxide Concentration on Photosynthesis in Algae

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Abstract

The effects of various combinations of light intensity, oxygen concentration, and CO_2 concentration on photosynthesis and growth in several algal types were studied. The results suggest the following. (1) Different algae show different responses to high oxygen concentrations and high light intensities. (2) Inhibition of photosynthesis (CO_2 fixation and growth), if seen, increases with increasing oxygen concentration and with increasing light intensity (at light intensities greater than saturation). (3) The inhibition of net photosynthesis observed cannot be attributed to high light intensity alone. (4) The inhibition cannot be attributed to increased rates of excretion of organic materials under conditions of high oxygen concentration and high light intensity. (5) Increased concentrations of CO_2 can decrease the effect of high oxygen and light in some algae. (6) The decrease in net photosynthesis observed is probably the result of photorespiration. (7) The effect of light intensity, oxygen concentration, or CO_2 concentration on algal photosynthesis should not be studied without considering the effect of the other factors. Some implications of these results, as related to primary productivity measurements, are also discussed.

Zelitch [15] defines photorespiration as "the respiration (especially the CO_2 evolution) that differs biochemically from normal dark respiration and is specifically associated with substrates produced during photosynthesis." It has been the subject of several recent reviews [8, 10] and of a recent book by Zelitch [15]. Zelitch defines the Warburg effect as the increase in net photosynthesis when the oxygen content of the atmosphere is decreased from its normal of 21% to about 1-3%. He states that "the most likely explanation for this phenomenon is that there is an increase in the CO_2 evolution in the light resulting from photorespiration." Most workers in the field of photorespiration now believe the glycolate pathway to be the probable mechanism of photorespiration at least in higher plants. Essentially, this pathway involves the formation of glycolate from some intermediate in photosynthesis. The mechanisms in-

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volved in the metabolism of glycolate, once it is formed, are known in some detail. However, until recently, the evidence for the mechanism of glycolate formation was inconclusive. Andrews *et al.* [2] and Lorimer *et al.* [11] show that this photorespiratory substrate is produced by the action of an oxygenase on ribulose-1,5-diphosphate, producing phosphoglycolate and 3-phosphoglycerate. The fact that this oxygenase activity co-purified with ribulose diphosphate carboxylase activity, had an alkaline pH optimum, increased in activity with increased concentration of oxygen in the gas phase, and was specific for the substrate ribulose-1,5-diphosphate are strong indications of its role in photorespiration.

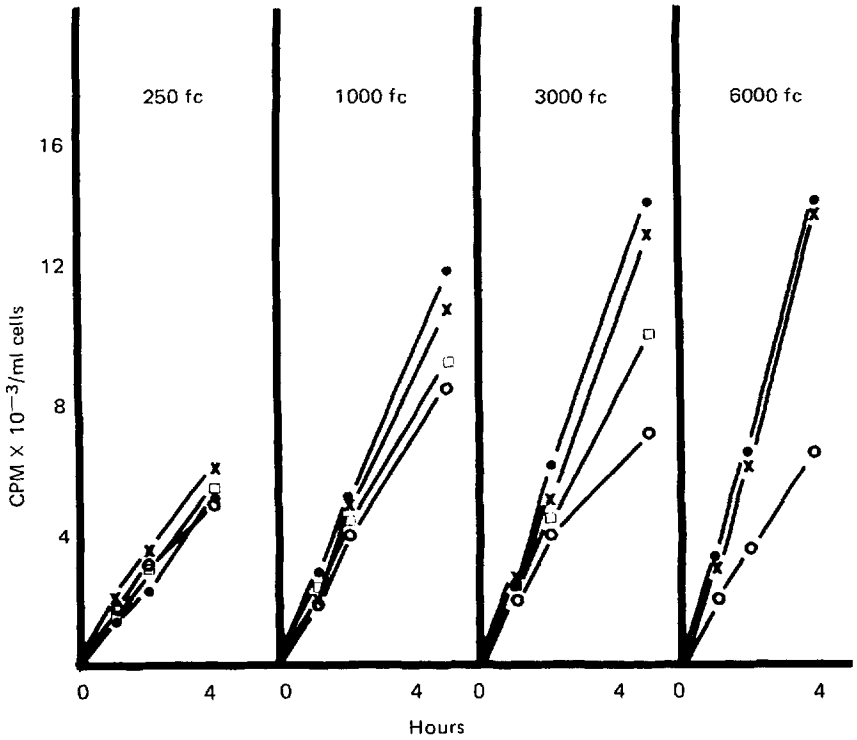

Fig. 1. CO_2 fixation by *A. nidulans* at 0.001% $NaHCO_3$ and various oxygen concentrations and light intensities. Cells were grown on a shaker at 25°C, 21% oxygen and 250 ft-c. Portions of the cell suspension were added to serum bottles which were then stoppered with serum stoppers. The suspensions were gassed for 10 min with the appropriate mixture of gases and returned to the shaker at the appropriate light intensity. NaH $^{14}CO_3$ "stock" was quickly added to provide a final concentration of approximately 0.001% $NaHCO_3$ and an activity of 0.42 μCi/ml. The temperature was maintained at approximately 25°-27°C throughout the experiment. •, gassed with nitrogen; X, gassed with CO_2 free air; □, gassed with 60% oxygen in nitrogen; ○, gassed with 100% oxygen.

The overall result of photorespiration is to cause the loss of previously fixed carbon with the concomitant uptake of 1 mole of oxygen per mole of CO_2 produced. This apparently wasteful process could have a significant effect on primary productivity and on the results obtained by conventional methods of measuring such productivity. Yet little work has been reported on these problems using algae or natural populations.

Turner *et al.* [14] and Turner and Brittain [13] showed that in most of the 25 species of plants tested oxygen evolution was inhibited at increased levels

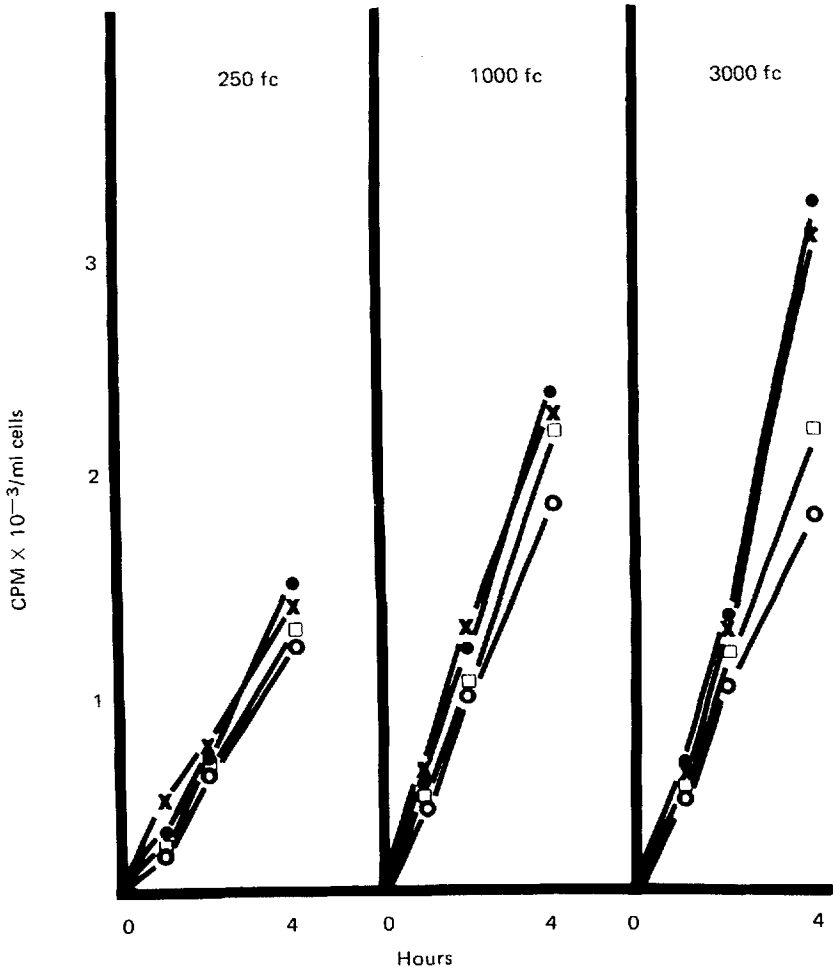

Fig. 2. CO_2 fixation by *A. nidulans* at 0.025% $NaHCO_3$ and various oxygen concentrations and light intensities. The data were obtained as described in Fig. 1, except that additional carrier $NaHCO_3$ was added to make the final concentration 0.025%. The activity was 0.42 μCi/ml. Figure legends are as in Fig. 1.

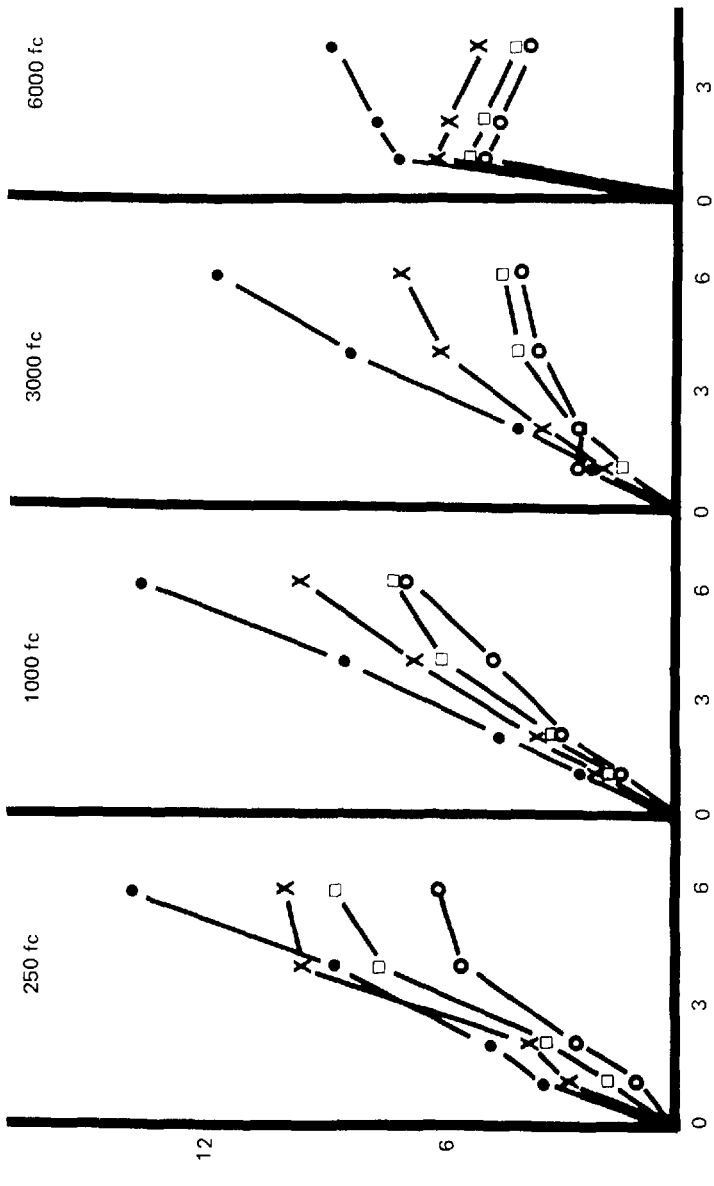

Fig. 3. CO_2 fixation by *P. tricornutum* at 0.001% $NaHCO_3$ and various oxygen concentrations and light intensities. The data was obtained as described in Fig. 1. The figure legend is as described in Fig. 1.

of oxygen. The inhibition was rapidly produced and rapidly reversed. The effect was absent or small between 0 and 20% oxygen, and large and variable between 20 and 100% oxygen. Hoch *et al.* [9] using mass spectrometry demonstrated that in *Anacystis nidulans* there are two mechanisms for the uptake of oxygen. One of these (probably the same as dark respiration) is inhibited by relatively low light intensities. Superimposed on the inhibited dark respiration is a second uptake mechanism for oxygen. The rate at which oxygen uptake proceeds by this second mechanism is dependent on light intensity and according to the authors "without the release of CO_2." This failure to "release CO_2" seems in conflict with the results of others [6]. These authors postulated that some of the reduced pyridine nucleotide is (a) either reoxidized by oxygen with concomitant phosphorylation or (b) it is reoxidized by oxygen without phosphorylation and the resulting oxidized pyridine nucleotide is reduced by the chloroplast with its accompanying production of ATP. Bjorkman and his colleagues [5] failed to show inhibition of photosynthesis by oxygen in the algae *Chlorella* or *Ulva*. Brown and Tregunna [6], using an infrared CO_2 analyzer, measured CO_2 compensation points (i.e., that CO_2 concentration at which no net CO_2 is evolved or fixed) at various concentrations of oxygen in several algae. They observed that dark respiration was inhibited in the light, but was replaced by another CO_2 producing mechanism. They also noted that the CO_2 compensa-

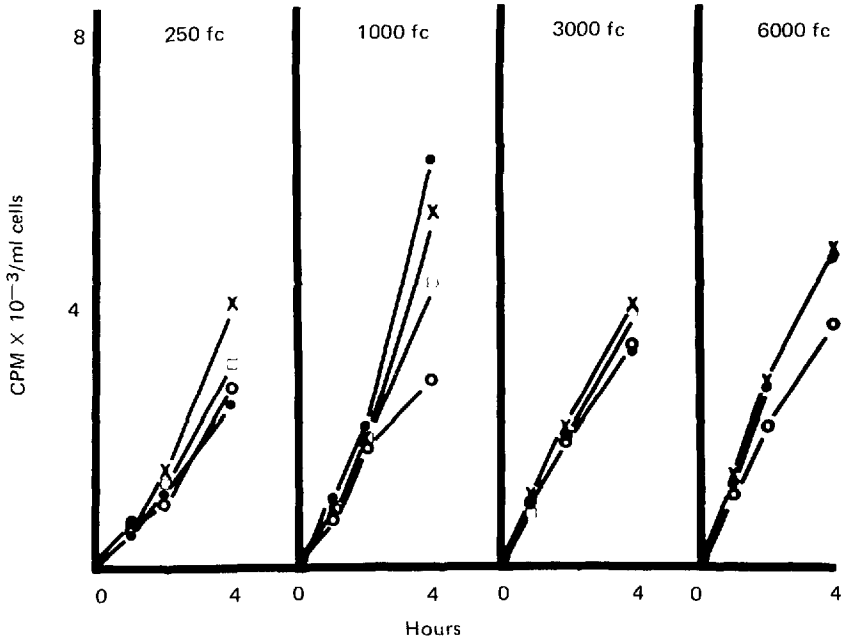

Fig. 4. CO_2 fixation by *Cricosphaera sp.* at 0.001% $NaHCO_3$ and various oxygen concentrations and light intensities. The data was obtained as described in Fig. 1. The figure legend is as described in Fig. 1.

tion point decreased with decreasing oxygen concentration. However, some of the algae such as *Chlorella* had CO_2 compensation points so low as to be unmeasurable with any accuracy using their system.

The rate of photorespiration typically increases with increasing oxygen concentration, increasing light intensity, and decreasing CO_2 concentration. For this reason, the conclusions drawn from some of the more recent studies on the effects of oxygen or light intensity in algae are questionable. Bunt [7] measured the rates of CO_2 fixation by several species of marine microalgae as a function of oxygen and carbon dioxide concentrations. He tested the organisms at 0, 21, and 100% oxygen. The concentrations of CO_2 used were those obtained by adding 7.6 or 103 mg/liter of $NaHCO_3$. He reported that some of the species were inhibited at increased oxygen concentrations, while others showed no inhibition, and still others showed other patterns. Unfortunately, the effect of light intensity was not considered in this study. Only one light intensity was used for each of the organisms studied, i.e., for the Antarctic species he used 107 foot-candles (ft-c) and for the other organisms only 860 ft-c. These are both relatively low light intensities compared to those prevailing at the surface of a body of water during most of the daylight hours.

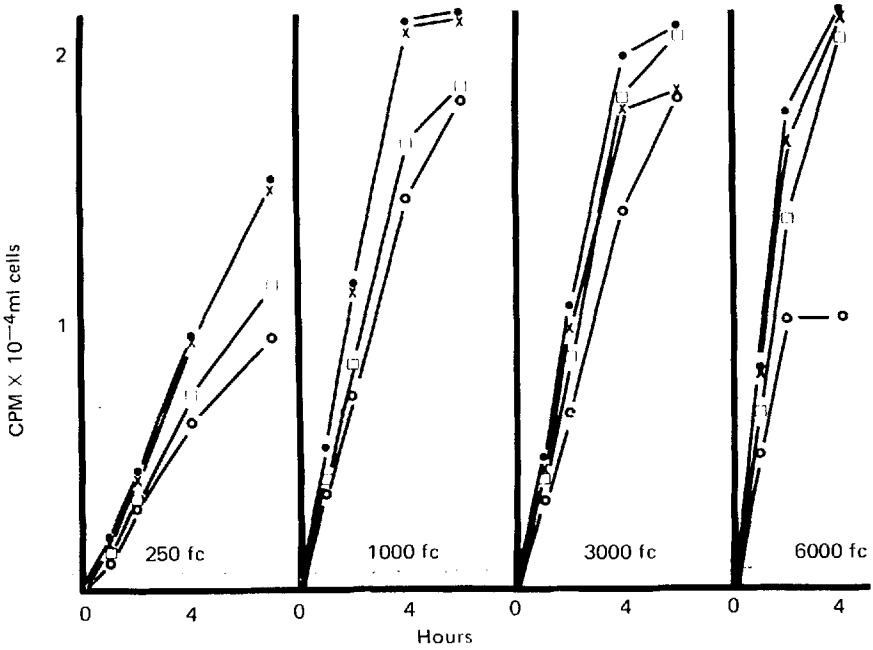

Fig. 5. CO_2 fixation by *D. tertiolecta* at 0.001% $NaHCO_3$ and various oxygen concentrations and light intensities. The data was obtained as described for Fig. 1. The figure legend is as described for Fig. 1.

Takahashi *et al.* [12] attempted to determine whether the effect of high light intensities on algal photosynthesis could be attributed to an increase in the amounts of organic materials being excreted, or to a direct effect on photosynthesis. They used the organism *Phaeodactylum tricornutum* for laboratory studies and also studied natural populations of algae. They found little increase in the amount of extracellular organic material excreted by the algae under conditions of high light intensity, i.e., up to 7000 ft-c. However, a severe inhibition of photosynthesis occurred after about 1 hr of incubation at high light intensities. These authors also showed inhibition of photosynthesis (CO_2 fixation) when concentrated samples of natural populations of phytoplankton were used, but

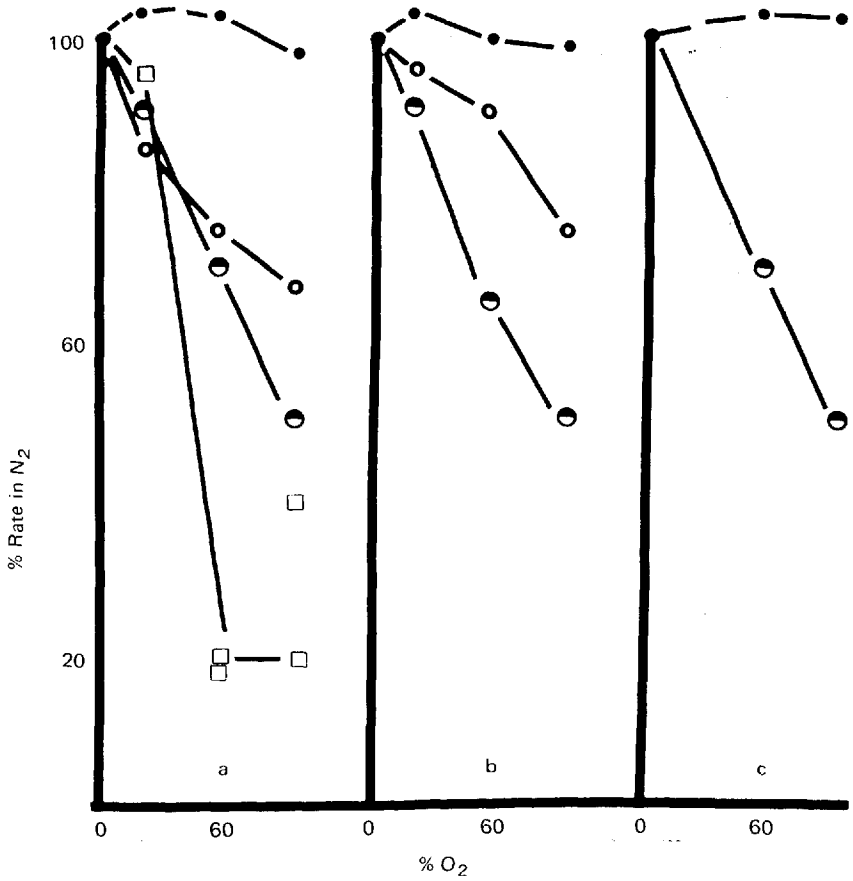

Fig. 6. Summary of the effects of light intensity, oxygen concentration, and CO_2 concentration on CO_2 fixation by *A. nidulans*. Data are summarized from the experiments described in Figs. 1 and 2. The data for 0.1% $NaHCO_3$ were obtained as described in Fig. 1, except that additional carrier $NaHCO_3$ was added to provide a final concentration of 0.1%. ●, 250 ft-c; ○, 1000 ft-c; ◒, 3000 ft-c; □, 6000 ft-c.

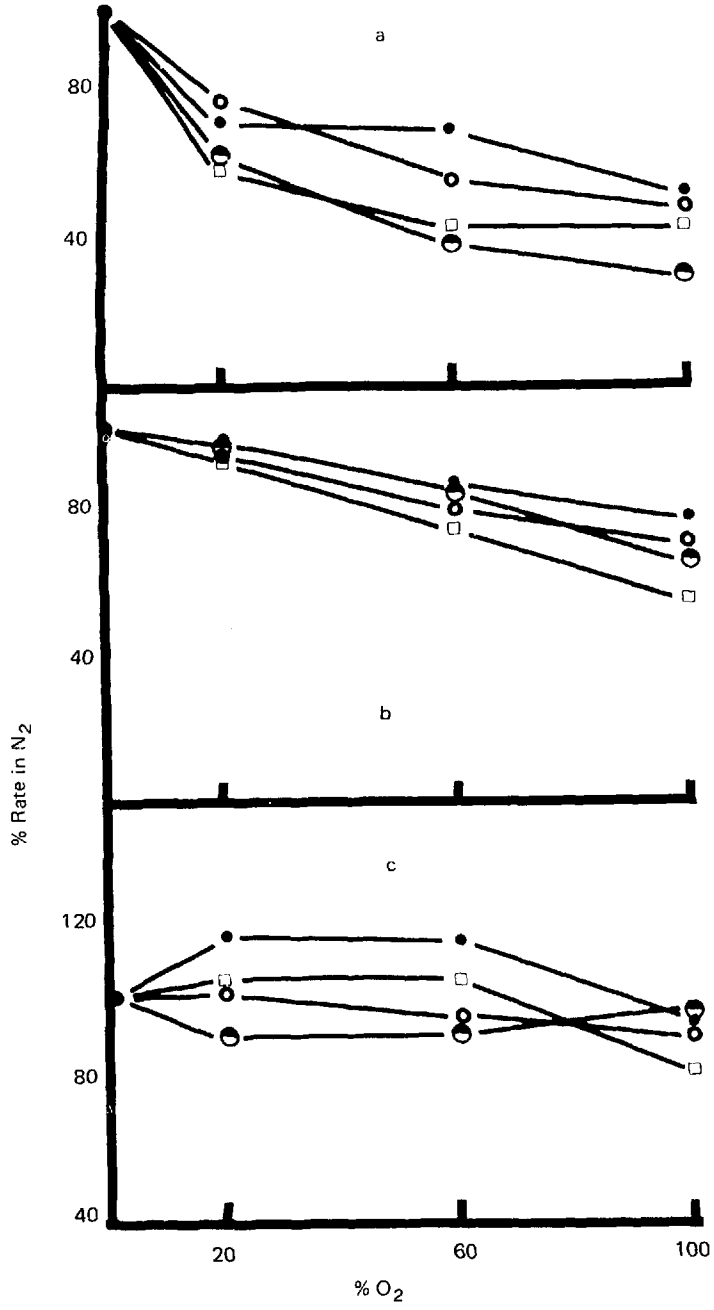

Fig. 7. Summary of the effects of light intensity and oxygen concentration at 0.001% $NaHCO_3$ on CO_2 fixation by (a) *P. tricornutum*, (b) *D. tertiolecta*, and (c) *Cricosphaera sp.* The data are summarized from the experiments described in Figs. 3, 4, and 5. Figure legends are given in Fig. 6.

not when unconcentrated samples were employed. Unfortunately, it is difficult to determine from their methodology what concentrations of oxygen in the sample containers were used throughout the experiments.

The purpose of our studies was to test the effects of various combinations of light intensity, oxygen concentration, and carbon dioxide concentration on the rates of photosynthesis by a variety of freshwater and marine algae, and to show that all these factors must be considered and controlled when attempting to describe the effects of any one of the factors on photosynthesis.

Materials and Methods

The organisms used in these studies were *Anacystis nidulans* strain 625, from the culture collection, Indiana University, Bloomington Indiana and *Cricosphaera sp., Dunaliella tertiolecta*, and *Phaeodactylum tricornutum*, from the Hawaii Institute of Marine Biology, Oahu, Hawaii. *Anacystis nidulans* was grown in modified Detmer M medium [4], and the other organisms in AM medium [3]. Unless otherwise specified, the organisms were grown axenically in 200 ml volumes of the growth medium contained in 1 liter cotton-stoppered flasks. These were placed on a rotary shaker at 100 rpm, 25°C, and 250 ft-c.

In preparation for testing, the organisms were diluted to the desired density with fresh growth medium. The density of organisms was kept low to prevent significant changes (greater than 1%) in the oxygen content of the medium during the experiment. Twenty milliliter portions of this suspension were then dispensed into 150 ml serum bottles and sealed with serum stoppers. This provided approximately 1 part of cell suspension to 10 parts of gas phase, thus helping to prevent the oxygen concentration in the culture from changing significantly during the experiment. Calculations based on the rates of oxygen production, cell concentration, and sample and container sizes showed that the oxygen concentration would not increase at a rate greater than 0.5% per hour. These suspensions were then bubbled with filtered nitrogen, CO_2-free air, 60% oxygen in nitrogen, or 100% oxygen to provide final "concentrations" of 0, 21, 60, and 100% oxygen, respectively. This procedure also removed most of the CO_2 from the bottles.

The stock solution of NaH $^{14}CO_3$ was prepared by adding 2 ml (5 μCi) of carrier-free $NaHCO_3$ to 10 ml of 0.025% $NaHCO_3$. To begin the experiment 1 ml of the stock suspension was added to each of the test bottles containing the algae. This provided a final concentration of approximately 0.001% $NaHCO_3$. For concentrations greater than 0.001%, additional unlabeled $NaHCO_3$ was added. The bottles were immediately placed on the shaker at 100 rpm.

Light at the required intensity was provided by banks of 150 W flood lights, connected to a variable transformer to control the intensity. To maintain "white light" at low intensities the distance from the lamps to the bottles was increased. An aquarium filled with water was placed between the lights and the samples to act as a heat trap. To check the temperatures in the bottles during incubation, a control bottle containing the same proportions of water and gas phase was sealed with a stopper through which a thermometer was passed. This was placed with the other bottles. Several fans were employed to help maintain the desired temperature when 3000 and 6000 ft-c intensities were used. Light intensities were measured using a Luna-pro (P. Grossen & Co. Erlangen, Germany) incident light meter.

Samples of 0.5 or 1.0 ml were removed through the serum stoppers using 1 ml tuberculin syringes with number 21 needles. These samples were immediately placed in the dark until filtered. It took about 5 min to collect samples from all 12 bottles. These samples were then rapidly filtered through 13 mm 0.22 μm Millipore membrane filters, washed once with 2 ml of 0.001 *N* HCl and twice with growth medium. The filters were dried, added to 10 ml of Spectafluor scintillation fluid (Amersham-Searle), and counted using a Packard Tri-carb scintillation counter at 90% efficiency. In all these studies background was always less than 20 cpm and was not subtracted from the experimental counts.

Since the primary aim of these studies was to compare the effects of various combinations of light intensity and oxygen and carbon dioxide concentrations, all determina-

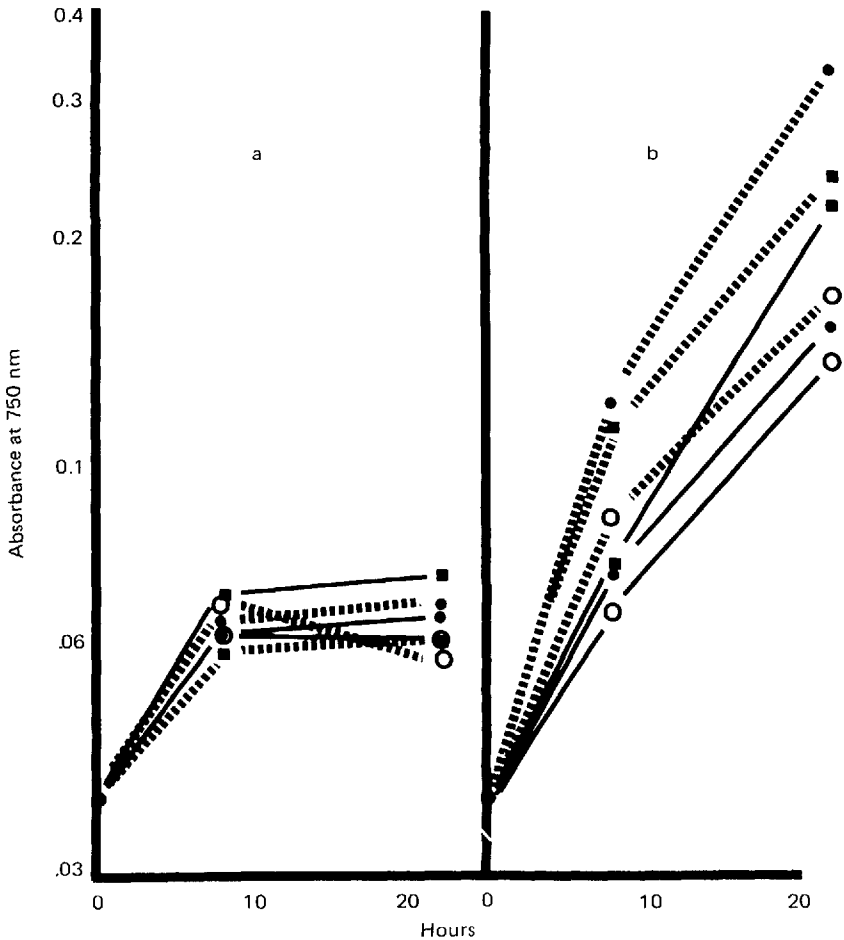

Fig. 8. Growth of *A. nidulans* at various light intensities, oxygen concentrations, and CO_2 concentrations. Experimental procedures were as described in Fig. 1. (a) 0.001% $NaHCO_3$ and (b) 0.1% $NaHCO_3$. ●, gassed with nitrogen; ■, gassed with CO_2-free air; ○, gassed with 100% oxygen. Solid lines at 250 ft-c, broken lines at 3000 ft-c.

tions were made at the same time using portions of a single cell suspension. This eliminated those problems which are inherent in the use of different batches of cells on different days.

The amount of soluble organic material being produced by the organism under these conditions was determined by collecting 1 ml of the filtrate (obtained by filtering 1 ml of the cell suspension through a 0.22 μm Millipore membrane filter contained in a Swinney adapter) directly in a scintillation vial containing 10 ml of Bray's scintillation fluid. This was followed by a rinse consisting of 1 ml of the growth medium. One-half milliliter of 0.001 *N* HCl was added to the vial, and the solution was bubbled with filtered air for 5 min. The activity remaining after this procedure was considered the result of soluble organic materials. Standards consisting of [^{14}C]-glucose and NaH $^{14}CO_3$ treated in this manner showed that none of the organic materials was lost while the inorganic ^{14}C-labeled material was completely removed. These procedures were also used to construct a quenching curve for the AM and DM media. Using the sampling method described above, the DM and Am media caused a 49-54% and 60-64% quenching, respectively. To obtain background counts, blanks containing scintillation fluid were included in all runs. Background was without exception 20 cpm or less.

Growth was determined by measuring the absorbancy of the cell suspension at 750 nm, using a Beckman model DU spectrophotometer.

Results and Discussion

The objective of these studies was to determine the effects of light intensity, oxygen concentration, and CO_2 concentration in various combinations on the rate of photosynthesis of various algae. As described above, a single batch culture was used to test CO_2 fixation under all the various conditions, at one time. The results are shown in Figs. 1 through 5. Summaries of these data as a function of oxygen concentration are given in Figs. 6 and 7. From these results several conclusions can be made. (1) Photosynthesis (as measured by CO_2 fixation) is light saturated, at 0.001% $NaHCO_3$, by approximately 1000, 250, 1000, and 1000 ft-c in *A. nidulans, P. tricornutum, Cricosphaera sp.*, and *D. tertiolecta,* respectively. (2) The rates of CO_2 uptake in nitrogen are approximately linear over the test period for all light intensities and organisms, except for *P. tricornutum* at 6000 ft-c. Therefore, it is possible to conclude that high light intensity alone does not result in lowered rates of net CO_2 fixation, except perhaps in the case of *P. tricornutum* at 6000 ft-c. (3) At light intensities above saturation, for all organisms tested except *Cricosphaera sp.*, there is a decreased rate of CO_2 fixation with increased oxygen concentration. This is not true for *Cricosphaera,* however, since this organism is little affected by increased oxygen at any light intensity except at 6000 ft-c in 100% oxygen. (4) The effect of increased concentrations of oxygen on the rate of CO_2 fixation by *A. nidulans* and *P. tricornutum* increases with increasing light intensity above light saturation. This is not true for *Cricosphaera* which shows no effect except at 6000 ft-c in 100% oxygen, or for *D. tertiolecta* which shows approximately the same degree of inhibition by increased concentrations of oxygen at all light intensities tested. (5) In the case *A. nidulans* (the most thoroughly studied of the organisms) there

is little difference in the degree of inhibition by oxygen at high light intensity when the $NaHCO_3$ concentration is raised to 0.025 or 0.10%. (6) Since the levels of organic matter in the test suspensions were in all cases low, i.e., of the order of 3-5% of the total activity in the cells, and remained constant throughout the experiments (these results are not shown in the figures), the inhibition of net CO_2 fixation cannot be attributed to increased excretion of soluble organic matter under these conditions of high light and high oxygen.

Experiments similar to the above mentioned were done with *A. nidulans* and *P. tricornutum* grown at 250 ft-c and 1000 ft-c (data not shown). There was essentially no difference between the response of the low-light-grown cells and the high-light-grown cells to increased concentrations of oxygen at any light intensity.

Experiments were done to determine whether the inhibition of photosynthesis observed at increased oxygen concentrations was paralleled by the growth of the organisms. Other experiments were done to determine whether increased concentrations of CO_2 would have any effect on the rates of growth or of CO_2 fixation under these conditions. CO_2 fixation and growth were determined concurrently in several cultures. *Anacystis nidulans* was tested under

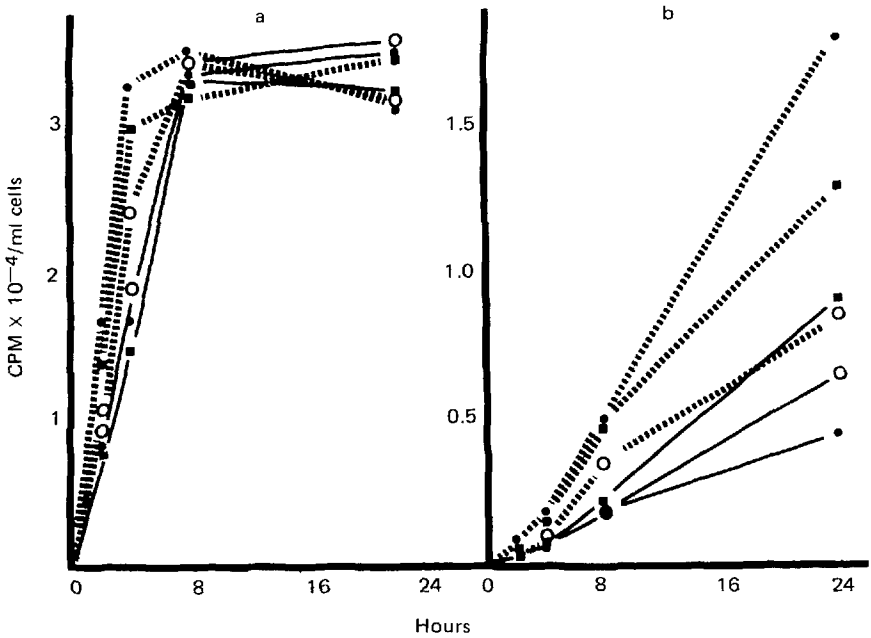

Fig. 9. CO_2 fixation by *A. nidulans* at various light intensities, oxygen concentrations, and CO_2 concentrations. The data are obtained concurrently with the growth data presented in Fig. 8. Experimental procedures and figure legends are as described in Fig. 8.

0, 60, and 100% oxygen, all at 250 and 3000 ft-c. Duplicate sets were run at the same oxygen and light conditions, 0.001% and 0.1% $NaHCO_3$ were used. Experiments with *P. tricornutum* were done as above except that the oxygen concentrations were 0, 21, and 100%. The growth data for *A. nidulans* is shown in Fig. 8. The CO_2 uptake data is shown in Fig. 9. Comparison of these data show that CO_2 fixation and growth paralleled each other under all conditions tested except at 3000 ft-c and 0.001% $NaHCO_3$. Lack of correlation in this case probably resulted from the exhaustion of the CO_2 from the medium in about 4 hr. This also explains the fact that the sample under 100% oxygen and 3000 ft-c grew for 8 hr whereas those at 0 and 60% stopped after approximately 4 hr. That is, since the sample saturated with 100% oxygen is fixing CO_2 at a slower rate, it does not use up all the CO_2 until approximately the eighth hour. However, as expected, the rates of growth and CO_2 fixation for this organism are greater at 3000 ft-c than at 250 ft-c (250 ft-c is about 1/2 light saturation). There was little effect of increased oxygen on either process at 250 ft-c, but a large decrease in the rate of both processes at 3000 ft-c under high oxygen tensions. Also, it is apparent that increased levels of CO_2 do not change the level of inhibition caused by high oxygen concentrations. The fact that the absorbancy of the culture at 3000 ft-c under 100% oxygen drops after the CO_2 is exhausted is probably the result in part of the phenomenon of photooxidative death [1]. They incubated *A. nidulans* in the light under 100% oxygen and found that the cells died rapidly at 4, 15 and 35°C in the absence of CO_2. They reported that

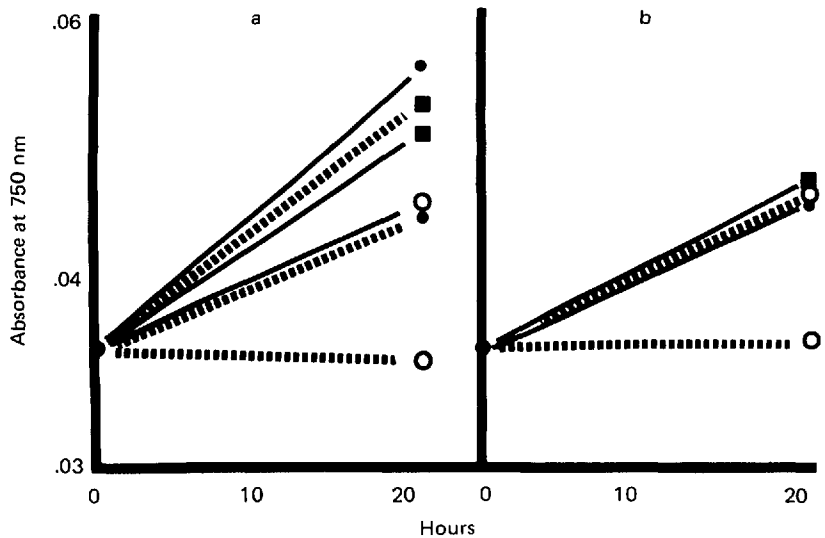

Fig. 10. Growth of *P. tricornutum* at various light intensities, oxygen concentrations, and CO_2 concentrations. Experimental conditions and procedures are as given in Fig. 1. (a) 0.001% $NaHCO_3$ and (b) 0.1% $NaHCO_3$. Solid lines at 250 ft-c, broken lines at 3000 ft-c. ●, gassed with nitrogen; ■, gassed with 60% oxygen in nitrogen; ○, gassed with 100% oxygen.

photosynthesis is impaired long before death occurs. They were also able to show this phenomenon, using *A. nidulans* as a test organism in an Israeli fish pond.

The results obtained for the growth of *P. tricornutum* under the conditions described above are shown in Fig. 10. The results for CO_2 uptake are omitted here since they paralleled the growth curves more closely than those with *A. nidulans*. This is because even at low (0.001% $NaHCO_3$) the cultures did not exhaust all the CO_2 from the medium during the experiment. From the growth data it is apparent that the response of this organism is quite different from that of *A. nidulans*. The yield of cells after 20 hr decreased with increased oxygen concentration at both 250 and 3000 ft-c. The inhibition was much more severe when the cells were incubated at 3000 ft-c and when the level of CO_2 was low. However, when the level of CO_2 was increased by 100-fold (CO_2 was still limiting), there was little difference in the final yields obtained at any of the conditions tested, except under 100% oxygen at 3000 ft-c where no growth occurred. Therefore, this organism shows the ability to overcome, to some degree, the effects of high oxygen and high light intensity if the level of CO_2 is increased sufficiently. With *P. tricornutum* significant inhibition of net CO_2 fixation is evident even at 250 ft-c and 21% oxygen. Since in open waters light intensities of the magnitude used in these studies are common and oxygen concentrations of 21% or slightly greater are normal, growth and activity of this organism might be expected to be inhibited by these factors in the natural environment.

The results reported, here, have demonstrated that the effect of light intensity, oxygen concentration, and carbon dioxide concentration on photosynthetic or photorespiratory processes in algae can best be studied when one controls, or at least measures throughout the experiment, all these parameters. Some of the previously published data are therefore questionable since some of the parameters were either uncontrolled or unmeasured. These results also raise some questions concerning primary productivity measurements. (1) One of the traditional methods for this determination makes use of closed light and dark bottles in which the concentrations of oxygen are measured after a period of incubation. Alternatively, the rates of $^{14}CO_2$ fixation are determined in both bottles. It seems apparent that if samples from highly productive waters are used, the concentration of oxygen may increase and that of CO_2 may decrease during the incubation period. Under these conditions productivity measurements may be in serious error. (2) The use of light cabinets which provide only one light intensity for mixed algal samples from different areas and depths may also lead to errors in productivity estimates. (3) Although these traditional methods give estimates of the net productivity of an area, it has not been possible to correlate the rates obtained in the different samples with light intensity, oxygen concentration, or CO_2 concentration to which the sample is exposed since accurate measurements of these parameters are not usually made with each of the pro-

ductivity measurements. The potential importance of photorespiration to the various algal types and therefore to the environment as a whole (in terms of net productivity, selection for species less affected by photorespiration, etc.) is apparent from these studies, but needs to be measured using natural populations.

The results presented here show that the effect of increased oxygen, especially at high light intensity, is not the result of increased excretion of soluble organic materials by the algae studied. The results also suggest that net CO_2 fixation (apparent photosynthesis) is affected and not gross (real) photosynthesis. Support for this conclusion comes from the results obtained by other workers as reviewed in the introduction. However, the direct demonstration that CO_2 production in the light is responsible for the lowered rates of net CO_2 fixation has not been possible using the techniques employed in these studies.

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