ABSTRACT

The CO₂ exchange of several species of fresh water and marine algae was measured in the laboratory to determine whether photorespiration occurs in these organisms. The algae were positioned as thin layers on filter paper and the CO₂ exchange determined in an open gas exchange system. In either 21 or 1% O₂ there was little difference between ¹⁴CO₂ and ¹³CO₂ uptake. Apparent photosynthesis was the same in 2, 21, or 50% O₂. The compensation points of all algae were less than 10 μl 1⁻¹. CO₂ or ¹⁴CO₂ evolution into CO₂-free air in the light was always less than the corresponding evolution in darkness. These observations are inconsistent with the proposal that photorespiration exists in these algae.

Algae are C₃ plants but there is still controversy regarding the existence or nature of photorespiration (7, 8, 33). Many algae have been shown to produce glycolate (28, 33) and to possess the enzymes of the glycolate pathway (28, 33). The inhibition of photosynthesis by O₂ or the Warburg effect was first shown in algae, and has since been extensively studied (2, 34). Some workers, however, have found no effect of O₂ on CO₂ fixation in algae (1, 16, 18) and many algae show low compensation points (4, 25, 35) and release less CO₂ in the light compared to the dark (7, 17).

In higher plants, CO₂ evolution and CO₂ exchange are the most extensively studied aspects of photorespiration (36). In algae, although CO₂ evolution during photosynthesis has been implied (33), few studies have been performed on CO₂ exchange because of the difficulties imposed by the aquatic medium. In view of the paucity of information and the diversity of observations and opinions on CO₂ exchange in algae, we decided to investigate the CO₂ exchange of several species of algae using an open gas analysis system such as that used for measurement of CO₂ exchange in higher plants (15, 26).

MATERIALS AND METHODS

The following algae were obtained from the Indiana University Culture Collection, and were grown on Gorham's culture medium No. 11 (21): Chlorella pyrenoidosa Chick (strain 252), Scenedesmus quadricauda (Turp.) Breb. (strain 76), Chlamydomonas reinhardtii (-) (strain 90), and Anabaena flos-aquae (Lyngbya) Breb. (strain 1444). Navicula pelliculosa (Breb.) Hilse was supplied by T. Murphy at Canada Centre for Inland Waters, Burlington, Ontario, and was grown on Chlo No. 10 medium (9). All of the above species were grown with continuous aeration and light. Chlorella and Anabaena were also grown in cultures bubbled with 0.5 and 5% CO₂ in air. Mougeotia sp. was grown as a unialgal culture and supplied by B. Colman, Department of Biology, York University, Downsview, Ontario. Anacystis nidulans (Richt. Drouet was grown on Gorham's medium (21) bubbled with 1.5% CO₂ and supplied by K. Budd, Queen's University. Dunaliella tertiolecta, Thalassiosira fluvialis, and Porphyridium sp. (Levin strain) were supplied by J. Craigie at the Atlantic Regional Laboratory, Halifax, Nova Scotia. These marine species were grown on a shaker without aeration with a 16-hr photoperiod.

All species were grown at 25 C and a quantum flux density of 80 to 100 μeinstein m⁻² sec⁻¹. All cultures were axenic with the exception of Mougeotia.

Cells were harvested during linear growth phase, and concentrated, where necessary, by settling and decanting the medium. Algae were suspended as an "artificial leaf" (12) to facilitate gas exchange and rapid changes in gas composition. The cells were filtered on Whatman No. 3 filter paper, producing an even double layer of cells (4 x 4 cm). This square was cut out and enclosed in the leaf chamber for the gas analysis measurements. The total volume of medium on the filter paper was approximately 0.3 ml. Similar results were obtained when the algae were suspended on 10-μm Nitex nylon mesh (B and S. H. Thompson & Co., Montreal, Quebec).

The open gas analysis system was as previously described (15, 26). Measurements were started about 30 min after the algae were placed in the leaf chamber because the rate of apparent photosynthesis increased about 30% during that time but thereafter remained constant. All measurements, with the exception of studies on the effect of temperature, were done at 25 C and light saturation (see Fig. 1). The relative humidity of the air entering the leaf chamber was maintained above 90% to avoid desiccation of the cells. Quantum flux densities were measured with a Lambda quantum sensor.

Rates of true photosynthesis, apparent photosynthesis, and photorespiration were calculated as previously described (26) except that it was necessary to do a control measurement with filter paper and medium alone, and to subtract the rate of ¹⁴CO₂ uptake obtained from that obtained with the algae included. This correction was only necessary during the first min of ¹⁴CO₂ supply and eliminated any error caused by uptake of ¹⁴CO₂ by the medium. Rates are the means of three or four determinations.

Compensation points were obtained by measuring CO₂ exchange at CO₂ concentrations both above and below the compensation point and interpolating to the point where there was zero net photosynthesis.

Chlorophyll content was determined after extraction with 80% acetone by the method of Bruinsma (5) or by extraction with acetone, methanol, H₂O (80:15:5, v/v/v) with sonication according to the method of Daley et al. (14).

RESULTS

Light response curves of photosynthesis for Anabaena, Navicula, and Chlorella are shown in Figure 1. Apparent photosyn-

1 Supported in part by the National Research Council of Canada and Environment Canada, Fisheries and Marine Service.
2 Present address: Department of Biology, Carleton University, Ottawa, Ontario Canada.
3 Present address: Department of Zoology, Ohio State University, Columbus, Ohio.
thesis of all species of algae was saturated at about 200 μeinstein m⁻² sec⁻¹ and no inhibition was observed at high light intensities. Light compensation points were about 20 μeinstein m⁻² sec⁻¹ with both Anabaena and Navicula and the O₂ concentration had no effect on photosynthesis of Anabaena or Navicula at any quantum flux density.

The response of apparent photosynthesis to CO₂ concentration for Navicula, Anacystis, and Chlorella is shown in Figure 2. Although the photosynthesis of Navicula was not close to saturation until 1,000 μl 1⁻¹ CO₂, Anacystis and Chlorella appeared to be close to saturation at 350 μl 1⁻¹. The latter concentration was used for the ¹⁴CO₂ uptake measurements and there could then be some variation in the degree of CO₂ saturation for the various algae. This should not have much effect on the attempts to measure photorespiration, however, as photorespiration has been shown to be independent of CO₂ concentration in that range (27).

When a minimum estimate of true photosynthesis was determined with ¹⁴CO₂ and apparent photosynthesis with ¹²CO₂, most of the algae had rates of true photosynthesis approximately equal to apparent photosynthesis and therefore no, or very little photorespiration in either 1% or 21% O₂ (Table I). These measurements were obtained at 42 sec after the introduction of the ¹⁴CO₂ but similar results were obtained 3 to 4 min after exposure to ¹⁴CO₂. In those cases where rates of true photosynthesis were larger than apparent photosynthesis, i.e. where there appeared to be photorespiration, there was no effect of O₂ on apparent photosynthesis; rather the effect was due to changes in true photosynthesis. If the algal photosynthesis responded similarly to O₂ as higher plants, then both true and apparent photosynthesis should be higher in 1% O₂ (27). As the ¹⁴CO₂ fluxes were very small (about 5% of the rates determined with sunflowers) they were subject to considerable uncertainty and this may be the cause of the few differences noted.

In the above experiments, there was little effect of O₂ on the rates of apparent photosynthesis (Table I), but since two preparations of algae were often involved some variation could be expected. To reduce this variation, it was desirable to study the effect of O₂ on apparent photosynthesis of the same preparation and to vary the O₂ concentration rapidly over a wider range. Measurements at each O₂ concentration were continued for 10 to 15 min before switching to another concentration. The order of O₂ concentration exposure had no effect and rates for each O₂ concentration were constant throughout the measurement period. The results of this systematic study are presented as rates of photosynthesis relative to the rate that occurred in 21% O₂ (Table II). In all cases, there was little or no difference between the rates of photosynthesis in 2, 21, or 50% O₂ (Table II). Also, there was no interaction between the CO₂ concentration and the O₂ concentration (Table II).

Carbon dioxide compensation points were determined for the algae by interpolation to the point of zero net photosynthesis (Fig. 3). In all cases, the compensation points were below 10 μl 1⁻¹ CO₂ (Table III) and were not changed at O₂ concentrations of 2 or 50% nor by temperature changes between 15 and 35 C.

Rates of release of CO₂ into CO₂-free air in light and darkness are shown in Table III. In all cases CO₂ evolution in the light was less than in the dark with the light/dark ratios ranging from 0.04 to 0.44. ¹⁴CO₂ release after 3 min in the light is also less than that in darkness with light/dark ratios ranging from 0.20 to 0.74 (Table IV). As the relative specific radioactivity of the ¹⁴CO₂ released in the light and darkness is about the same, the ratio of CO₂ released at 3 min of flushing would be similar to the ratios of ¹⁴CO₂ release. Thus, at no time during a CO₂-free flush does CO₂ or ¹⁴CO₂ release in the light exceed that released in darkness.

Chlorella and Anabaena cultures were also grown on 0.5 and 5% CO₂ to determine if the CO₂ concentration during growth affected the CO₂ gas exchange characteristics of the algae. Photosynthesis of these cultures was measured in air. The rate of photosynthesis of the 0.5 and 5% CO₂-grown Chlorella was 51% and 0%, respectively, of the rate of photosynthesis of air-grown cells. The 5% CO₂-grown cells were not dead, though, as over a period of 3 hr, the photosynthetic rate increased to that observed in the air-grown cells. In 0.5% CO₂-grown Anabaena, the rate of photosynthesis was 158% of the rate of air-grown cells but an inhibition to 48% of the rate of photosynthesis of air-grown cells was observed with the 5% CO₂-grown cultures. In all cultures where measurements could be performed, no change was found in the compensation points. The low rates of photosynthesis and the adaptation in photosynthetic rate that was observed in the algae on changing from a high to low CO₂ concentration are well known in green (3) and blue-green (24) algae.
Table I. True photosynthesis, apparent photosynthesis and photorespiration in several species of algae.
All determinations were made 42 sec after the introduction of 14CO2 at 25°C, 350 μl l-1 CO2 270 μeinstein m-2 sec-1 quantum flux at either 21 or 1% O2.

<table>
<thead>
<tr>
<th>Algae</th>
<th>21% O2</th>
<th>1% O2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPS1</td>
<td>APS1</td>
</tr>
<tr>
<td></td>
<td>μmol mg-1 chl hr-1</td>
<td></td>
</tr>
<tr>
<td>Chlorella</td>
<td>61 ± 102</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>104 ± 22</td>
<td>115 ± 5</td>
</tr>
<tr>
<td>Chlamydomonas</td>
<td>73 ± 5</td>
<td>55 ± 0.8</td>
</tr>
<tr>
<td>Mougeotia</td>
<td>29 ± 3</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>Anabaena</td>
<td>312 ± 16</td>
<td>264 ± 4</td>
</tr>
<tr>
<td>Anacystis</td>
<td>185 ± 41</td>
<td>196 ± 7</td>
</tr>
<tr>
<td>Navicula</td>
<td>237 ± 17</td>
<td>236 ± 4</td>
</tr>
<tr>
<td>Dunaliella</td>
<td>11.4 ± 0.6</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>Porphyridium</td>
<td>27 ± 3</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Thalassiosira</td>
<td>14.5 ± 2.3</td>
<td>17.3 ± 1.1</td>
</tr>
</tbody>
</table>

1. True photosynthesis, apparent photosynthesis and photorespiration.
2. Mean ± standard deviation, n = 3 or 4.

Table II. Relative rates of apparent photosynthesis at 3 oxygen concentrations in several species of algae.
Temperature was 25°C and quantum flux 270 μeinstein m-2 sec-1.

<table>
<thead>
<tr>
<th>Algae</th>
<th>Apparent Photosynthesis</th>
<th>350 μl l-1 CO2</th>
<th>150 μl l-1 CO2</th>
<th>1000 μl l-1 CO2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative Rate</td>
<td>21% O2</td>
<td>9% O2</td>
<td>50% O2</td>
</tr>
<tr>
<td>Chlorella</td>
<td>102</td>
<td>100</td>
<td>100</td>
<td>96</td>
</tr>
<tr>
<td>Scenedesmus</td>
<td>106</td>
<td>100</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Chlamydomonas</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mougeotia</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anabaena</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Anacystis</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Navicula</td>
<td>97</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Dunaliella</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Thalassiosira</td>
<td>104</td>
<td>100</td>
<td>100</td>
<td>108</td>
</tr>
</tbody>
</table>

1. Apparent photosynthetic rate shown in Table I.

DISCUSSION

Higher plants, which fix carbon via the C3 pathway, exhibit the process of photorespiration (8, 36). In terms of CO2 exchange, which is the best studied aspect, the presence of photorespiration is shown by a higher rate of 14CO2 uptake than 13CO2 uptake when 14CO2 is initially supplied (27), a marked effect of O2 on photosynthesis (1, 16, 17, 27), a CO2 compensation point exceeding 40 μl l-1 (8, 36), and CO2 or 14CO2 evolution into CO2-free air in the light at a rate that exceeds the rate of evolution of these molecules in the dark (15, 27).

These characteristics of photorespiration have not been systematically investigated in algae, primarily because of the difficulties of CO2 exchange between the gas phase and the aqueous medium in which the algae are suspended. Over short periods of time, if physical support and adequate moisture are provided, most of the aqueous medium could be removed without harmful effects. Diffusion of CO2 to the cells would be facilitated and CO2 exchange could be investigated. That photosynthesis of the algae was not adversely affected by removal of the medium was shown by typical responses to light (Fig. 1) and CO2 (Fig. 2) (30, 35, 36).

Using the 14CO2/13CO2 technique (26), which is presently the only technique for detecting photorespiration during photosynthesis, we could find no consistent evidence for the presence of CO2 evolution in the light by the algae (Table I). In addition, when the O2 concentration was decreased to 1%, there was little effect on the relationship between the measurements (Table I), a finding in sharp contrast to that observed with higher plant leaves where both true and apparent photosynthesis is stimulated and photorespiration is eliminated (27).

The apparent scarcity of CO2 evolution in the light was confirmed by rates of CO2 or 14CO2 evolution into CO2-free air (Tables III and IV) in the light which were lower, in all cases, than CO2 or 14CO2 evolution in darkness. These observations are consistent with those of several other workers (7, 17) but inconsistent with those of Zelitch (36). The lower CO2 or 14CO2 evolution in the light could be ascribed to refixation but the insensitivity of this CO2 or 14CO2 evolution in the light to changes in O2 concentration (17) indicates that the process producing the CO2 is quite different from that in higher plants.

Fig. 3. Rate of net photosynthesis of Anabaena and Chlorella at low concentrations of CO2. Compensation points were determined from such plots. Quantum flux 270 μeinstein m-2 sec-1 and O2 concentration 21%.
The characteristics of the CO₂ evolved indicate that it is more likely due to residual dark respiration, which, while apparently suppressed (23, 32), may continue in the light.

The low compensation points (Table III) are not typical of C₃ plants that show substantial CO₂ evolution in the light (36). Further, they are not sensitive to O₂ concentration or temperature such as those of C₃ plants (8, 36). These characteristics of the compensation point are similar to those observed for C₄ plants (36) and it has been suggested that blue-green algae may fix CO₂ by the C₄ pathway (10) but other work shows that this is not so (22).

Changes in O₂ concentration had only a slight effect on the rate of apparent photosynthesis (Tables I and II) and this was entirely consistent with a lack of CO₂ evolution. This result was surprising in view of previous work (34) and in view of the fact that the experimental system should allow ready access of the new O₂ concentration to the cells.

The lack of O₂ effect on apparent CO₂ fixation of algae has been noted before (1, 16, 18) but these observations are greatly overbalanced by a vast literature showing an inhibition of photosynthesis (2, 34) by increased concentrations of O₂. In most of the earlier studies, the inhibition of photosynthesis by O₂ has been determined at O₂ concentrations between 21 and 100% and by measuring photosynthesis as O₂ evolution (34). An inhibition of O₂ evolution at increased O₂ concentrations with little effect on CO₂ fixation has been noted by Fock et al. (16, 18). While this would, in part, reconcile our observations with earlier results, the position is weakened by the fact that within our present understanding of photosynthesis, an adequate explanation cannot be formulated for net CO₂ fixation to exceed net O₂ evolution (16, 18) for a substantial period of time. In addition, at least some of the earlier studies (2, 34) have shown inhibition of CO₂ fixation by increased O₂ concentrations. While the significance of it is not yet clear, one should also point out that the O₂ inhibition of photosynthesis observed in algae is not affected by temperature (34) whereas there is a clear effect of temperature on the O₂ inhibition of photosynthesis in higher plant leaves (8). It is perhaps no coincidence that all of the studies (Table I and II; 1, 16, 18) in which no effect of O₂ on photosynthesis could be detected used algae suspended as thin layers on a solid support but more work will be necessary before the contradictory observations can be reconciled.

We have concentrated our efforts on trying to determine CO₂ evolution in the light in algae because we have the equipment and have used it for extensive measurements on higher plant leaves, and because we believe that the CO₂ exchange is the best characterized aspect of photorespiratory gas exchange. Other workers (6, 25) have studied photorespiration in algae by determining the O₂ consumption that occurs in the light. This O₂ uptake can be quite substantial (6, 25) but it would appear to be due to the oxidation of some component in the photosynthetic electron transport chain (6, 19, 25, 31) rather than to having anything to do with either glycolate formation, its subsequent oxidation, or CO₂ evolution. While we do not deny that this O₂-consuming process has equal claim to the term “photorespiration,” it is misleading to assume that CO₂ photorespiration through the glycolate pathway accompanies it. Perhaps, it is time that our terminology in this area should be refined.

Strong cases have been made (28, 33) for the presence in algae of photorespiration, which, except for a few modifications, is similar to that in higher plants. In part, this position is based on observations on glycolate excretion by algae. However, it seems that significant glycolate excretion only occurs upon transfer of the algae from a high CO₂ concentration to a low CO₂ concentration (2, 11, 13, 20, 24, 29), a condition in which photosynthesis is greatly suppressed (3, 24). Even then glycolate excretion cannot account for the observed inhibition of photosynthesis by O₂ (2) and either a direct inhibition is involved (2, 8) or a large portion of the glycolate is metabolized. Air-grown algae do not excrete glycolate (11, 13, 24, 29) but since glycolate excretion can be forced using inhibitors (13, 29, 30), it is implied that in air, the glycolate is metabolized via the glycolate pathway (29, 33). Most of our algae were grown in air and we could still not find any indication of CO₂ evolution due to metabolism of glycolate in the glycolate pathway. Inasmuch as glycolate is not excreted under these conditions and because we find no evidence for its metabolism, it may not be produced. We are aware that such a suggestion raises questions concerning the proposed origin of glycolate via the ribulose-1,5-diphosphate oxygenase (8) in higher plant leaves as some mechanism would now be necessary to protect the enzyme from O₂ in the algae. Nevertheless, our results, including the lack of effect of O₂ on apparent photosynthesis, are consistent with the view that under normal conditions of growth, glycolate formation and photorespiration are absent in algae.

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