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Photooxidative Death in Blue-Green Algae

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When incubated in the light under 100% oxygen, wild-type blue-green algae (*Anacystis nidulans*, *Synechococcus cedrorum*) die out rapidly at temperatures of 4 to 15 C, and at 35 C (or at 26 C in the case of *S. cedrorum*) in the absence of CO_2. Photosynthesis is impaired in these cells long before they die. Blocking of photosystem II at high temperatures in the presence of CO_2 sensitizes the algae to photooxidative death. Photooxidative death and bleaching of photosynthetic pigments are separable phenomena. Photooxidative conditions were demonstrated in Israeli fish ponds using *A. nidulans* as the test organism during dense summer blooms, when dissolved CO_2 is low, and in winter, when water temperatures generally drop below 15 C. This finding suggests that photooxidative death may be responsible for the sudden decomposition of blue-green blooms in summer, and may be a factor in the absence of blue-green blooms in winter.

The term "photooxidative death" refers to the lethal effect to cells exposed to light in the presence of oxygen and sensitized by internal or external dyes. Carotenoids were found to protect certain organisms from the photooxidative effects at physiological temperatures. Many organisms possessing carotenoids have been found to be sensitive to photooxidative reactions when deprived of this pigment (5-7, 11, 15, 17). Wild-type organisms, containing carotenoids, were sensitive to photooxidation when incubated at low temperatures (16, 17), indicating that photooxidation is prevented in these organisms by an enzymatic mechanism.

The frequent observations of sudden and spontaneous disintegration of the blue-green algal blooms, which appear in summer in Israeli fish ponds, raised the question of whether a photooxidative effect is involved in this phenomenon, since conditions of oxygen supersaturation and high light intensities prevail at that time (1). A characteristic feature preceding massive die-off of blue-green algal blooms is the low CO_2 content (as expressed in elevated *p*H values during the light period) of the highly alkaline waters. Therefore, the effect of CO_2 on die-off was investigated.

The investigations reported here show that several blue-green algae are susceptible to photooxidative effects when illuminated in an atmosphere of 100% oxygen in a CO_2-free medium. Conditions for photooxidation of blue-green algae were found in nature, and the possible ecological significance of this is discussed.

MATERIALS AND METHODS

Cultivation and enumeration of algae. Axenic *Anacystis nidulans* strain 6311 and *Synechococcus cedrorum* (from the collection of the Department of Bacteriology and Immunology, University of California at Berkeley) were used. Zehnder and Gorham medium no. 11 (25) with $NaNO_3$ concentration modified to 1.5 g/liter served as medium. Standard growth of algal cultures was under an atmosphere of air at 35 C in a New Brunswick Psychrotherm in 250-ml flasks containing 100 ml of medium. Illumination was provided by 40-w white fluorescent lamps giving an incident light intensity of 10^4 ergs per cm per sec at the flasks. Unless stated otherwise, all experiments were carried out with *A. nidulans* harvested at day 5 of growth (end of logarithmic phase; protein concentration, about 300 mg/liter; chlorophyll concentration, 3 mg/liter, and total carotenoids concentration, 1 mg/liter). Solid medium was prepared by mixing equal volumes of double-strength medium and 2% agar in distilled water, after they had been autoclaved separately. Viability counts were determined by conventional dilution and plating in soft agar technique. In certain cases, the most probable number of living cells was evaluated on the basis of growth in series of highly diluted suspension.

Photooxidation experiments. Photooxidation experiments were carried out in an illuminated reciprocal shaking bath (150 strokes/min). The light intensity at the flasks was 10^4 ergs per cm per sec (cool white fluorescent light). Cell suspensions were kept in complete medium or in Na_2CO_3-free medium under an atmosphere of 100% of O_2 (or N_2 in controls) in glass-stoppered 250-ml Erlenmeyer flasks. To remove CO_2, the flasks were equipped with a central well containing 0.5 ml of 50% KOH soaked on filter paper. Photooxidation experiments in

presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) were carried out on cells grown in an atmosphere of air + 5% CO_2.

Oxygen and pH measurements. Kinetic measurements of oxygen evolution and consumption by cells suspended in complete medium and CO_2-free medium were performed in a thermostatically controlled 20-ml Perspex cell, illuminated by a 500-w photoflood lamp (Tungsraphot-B, Tungsram, Germany). The light intensity was 1.5×10^5 ergs per cm per sec at the surface of the cell. The cell was equipped with a Clark-type oxygen electrode (YSI5331, Yellow Springs Instruments Co., Yellow Springs, Ohio) connected to Gilson oxygraph model KM (Gilson Medical Electronics, Middleton, Wisc.) and with a combined *p*H electrode (GK2320, Radiometer) connected to a Radiometer titrator (TTT2 + ABU 11, Radiometer, Copenhagen, Denmark).

Measurement of photosynthetic pigments. A 10-ml algal suspension in 0.1 M potassium phosphate buffer (*p*H 7.0) was disintegrated at 4 C using 0.1 to 0.11 mm ϕ glass beads in a Braun homogenizer (Model MSK, Braun Apparatbau Melsungen, Germany) at 4,000 cycles/min for 30 sec. The resulting extract was centrifuged at 12,000 $\times$ *g* for 10 min, and the *C*-phycocyanin in the supernatant was measured at 620 to 625 nm in a Perkin Elmer 137 UV spectrophotometer (Perkin-Elmer Corp. Norwalk, Conn.). Chlorophyll *a* and carotenoids were measured after acetone extraction as described by Parsons and Strickland (18).

RESULTS

Photooxidative death of A. nidulans. Photooxidative death of *A. nidulans* occurs when cells are incubated at 35 C under a 100% O_2 atmosphere in media devoid of CO_2, whereas cells remained viable when incubated at this temperature and oxygen concentration in a CO_2-containing medium (Fig. 1). At this temperature, the death rate becomes quite rapid after an initial lag period of 6 to 8 hr. On the other hand, photooxidative death at lower temperatures (4 C, 10 C, 15 C) occurs in media both containing and lacking CO_2 (Fig. 1; Table 1) with a considerably shortened lag period of 2 to 4 hr. *S. cedrorum* was also killed after 16 hr of incubation in photooxidative conditions (100% O_2 atmosphere in Na_2CO_3-free medium or in distilled water) at 26 C.

To determine whether oxygen partial pressure plays a role in susceptibility to photooxidative death, *A. nidulans* cells were incubated for up to 20 hr in CO_2-free medium under an atmosphere of air or under pure oxygen (Fig. 1). The algae remained viable in air, and were sensitive to photooxidation at 100% oxygen concentrations. This is in agreement with the findings of Franck and French (10), that photooxidative processes in hydrangea leaf tissue are fully expressed only at partial pressures of 60% O_2 saturation or more, as well as those of

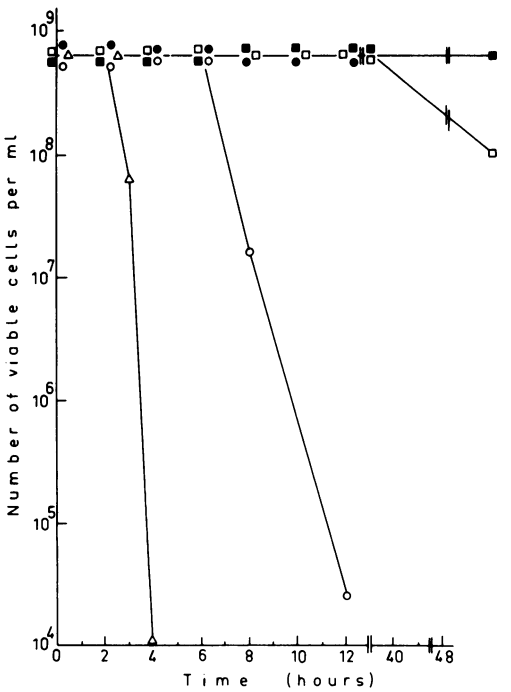

FIG. 1. *Viability of Anacystis nidulans after transfer from photosynthetic to photooxidative conditions. A. nidulans was grown in complete medium in air for 5 days under continuous illumination (10^4 ergs per cm² per sec), and then was exposed to the following environments:* (△), *complete medium and CO_2-free medium, 100% oxygen, light, 4 C;* (○), *CO_2-free medium, 100% oxygen, light, 35 C;* (●), *complete medium and CO_2-free medium, 100% oxygen, dark, 4 C and 35 C;* (●), *complete medium, 100% oxygen, light, 35 C;* (●), *CO_2-free medium, air or 100% N_2, light and dark, 4 C and 35 C;* (□) *and* (■), *A. nidulans grown in air enriched with 5% CO_2 and transferred to CO_2-free medium, 100% oxygen, 35 C in light* (□) *or dark* (■).

Stewart and Pearson (23), that growth of *Phormidium* and *Anabaena* is inhibited at this partial oxygen pressure. Because the *p*H of the CO_2-depleted medium rises to values of 10.5 and higher within 1 hr in light at 35 C, we studied the effect of *p*H on the system. Lethal photooxidation occurs as well at *p*H 7.0 (Fig. 2) and 9.2 (Table 1). Therefore, cells are sensitive to high oxygen partial pressure at lower *p*H values as long as CO_2 is absent.

The kinetics of photooxidative death depends not only on experimental conditions, but also upon the preincubation history of the algae. For example, *A. nidulans* cells grown in air enriched with 5% CO_2 began to die only 48 hr after transfer to photooxidative conditions (light, $-CO_2$, + 100% O_2) although the *p*H rose to 11.0 within 6 hr after transfer.

TABLE 1. *Effect of light and O_2 on viability of A. nidulans at different temperatures*

Temperature (C)	Illumination	Viability of *A. nidulans* (%)[a]		
		Complete medium[b]	Distilled water	Sodium glycine (0.01 M) buffer (pH 9.6)
4	Dark	100	100	100
4	Light	0.1	1	1
10	Dark	100		
10	Light	0.1		
15	Dark	100		
15	Light	0.1		
35	Dark	100	100	100
35	Light	100	1	1

[a] Initial concentration, 10^2 cells/ml; incubation time, 12 hr under 100% oxygen.

[b] Final *p*H of the incubation suspensions at 35 C reached 10.5, whereas, at 4, 10, and 15 C, the *p*H remained 9.2.

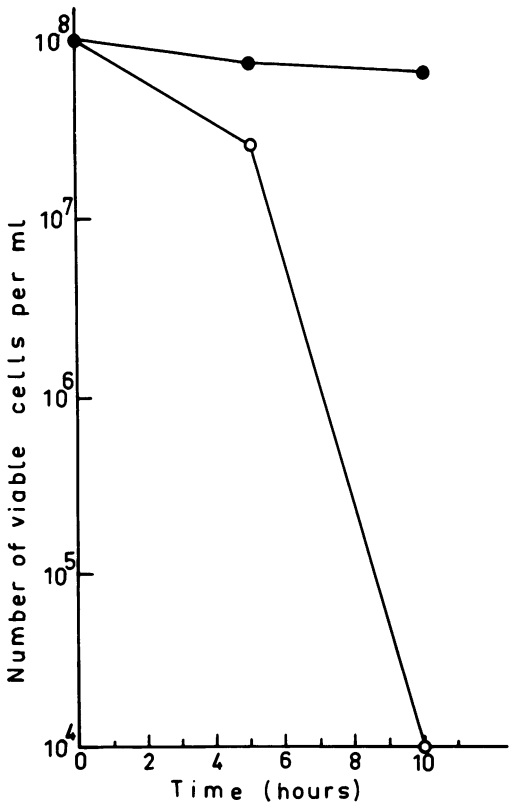

FIG. 2. *Photooxidative death at pH 7.0. A. nidulans cells were suspended in 0.05 M potassium phosphate buffer (pH 7.0) and incubated in the light at 35 C in an atmosphere of 99% O_2 + 1% CO_2 (●) or in 100% O_2 (○).*

Effect of DCMU on photooxidation. We found that 10^{-6} M DCMU, which inhibits oxygen evolution and CO_2 assimilation (3), inhibits within 10 sec both photoassimilation of CO_2 and oxygen evolution of *A. nidulans*, when incubated in complete medium under atmosphere of air. Therefore, DCMU was added to *A. nidulans* cells incubated under 100% oxygen in the light at 35 C (Table 2). The table shows that, in the presence of DCMU, the cells are sensitized to photooxidative death even in complete medium.

Effect of photooxidative conditions on photosynthetic oxygen evolution and dark respiration. When photosynthesizing *A. nidulans* cells are transferred to an atmosphere and medium devoid of CO_2, both the photosynthetic capacity and dark respiration are inhibited (Fig. 3) long before the onset of photooxidative death (cf. Fig. 1). Results identical to these were obtained when the cells were suspended in distilled water. Within 6 hr of incubation under photooxidative conditions, only part of the initial photosynthetic capacity (as measured by O_2 evolution) of these cells was restored when CO_2 (as sodium bicarbonate) was added to the medium. When the cells were illuminated under 100% N_2 in medium devoid of CO_2, the photosynthetic capacity of the algae remained stable at 20% of its initial rate (Fig. 4). Addition of the sodium bicarbonate had no effect on oxygen evolution by these cells, but restored the initial rate of oxygen evolution in cells incubated in distilled water instead of CO_2-free medium. The depressing effect on photosynthesis of incubation in CO_2-free medium under 100% N_2 is still not clear. In comparison, *A. nidulans* cells, which had been incubated in the dark under either 100% N_2 or O_2 and without CO_2, fully retained their photosynthetic capacities (Fig. 3, 4).

TABLE 2. *Effect of DCMU[a] on photooxidative death in Anacystis nidulans*

Illumination	DCMU (10^{-6} M)	Viability (%)[b]	
		Complete medium	Na_2CO_3-free medium
Light	+	1	0.01
	−	100	0.01
Dark	+	100	100
	−	100	100

[a] DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

[b] Initial cell concentration, 10^8 cells/ml; incubation time, 16 hr at 35 C, in 100% O_2 atmosphere.

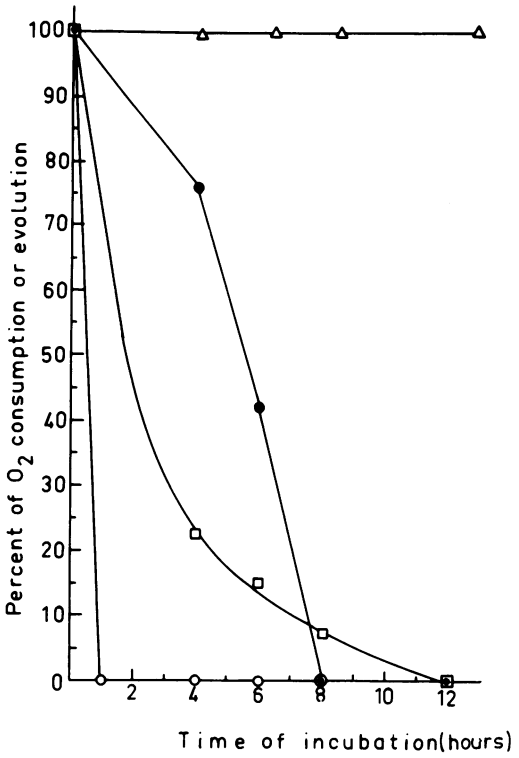

FIG. 3. *Rate of O_2 evolution and dark respiration of A. nidulans (10^8 cells/ml) incubated in both the light and dark under an O_2 atmosphere in Na_2CO_3-free medium. Initial O_2 evolution and dark respiration rates were 90 nmoles of O_2 per min per ml and 40 nmoles of O_2 per min per ml, respectively. Oxygen evolution of cells incubated in photooxidative conditions (○), and after addition of $NaHCO_3$ (up to 0.05 M) (●); rate of dark respiration (□) and rate of oxygen evolution of control cells incubated in the dark and transferred to light (△).*

Light-dependent O_2 consumption. When transferred to CO_2-free medium under an atmosphere of 100% oxygen at 35 C, *A. nidulans* cells developed a light-dependent oxygen consumption (Fig. 5) after 4 hr, a time when all the cells are still viable and capable of recovering most of their initial rate of photosynthetic oxygen evolution (cf. Fig. 3). The light-dependent oxygen consumption proceeds at an increasing rate as the cells die off (6 to 12 hr, cf. Fig. 1). A typical tracing of one such experiment is shown in Fig. 6. As seen in Fig. 5, light-dependent oxygen consumption does not occur after the cells were incubated under a N_2 atmosphere in the CO_2-free medium or in distilled water, when the cells were incubated under 100% oxygen in distilled water, or when incubated in CO_2-free medium under an atmosphere of air. This light-dependent oxygen consumption could be imitated by raising the *p*H of the medium, commencing within 5 min (Fig. 7) after the *p*H of the cells in standard growth medium was raised to 12.0. The light-induced oxygen consumption ceased after the *p*H of the medium was reduced to 9.5 (by bubbling CO_2 through the medium), and the original oxygen evolution rate was once again obtained. The photosynthetic capacity of the cells kept at *p*H 12 remained unimpaired for at least 25 min. The light-dependent oxygen consumption was found not to be affected by KCN (10^{-2} M), dinitrophenol (4×10^{-6} M) or DCMU (5×10^{-6} M) under these conditions.

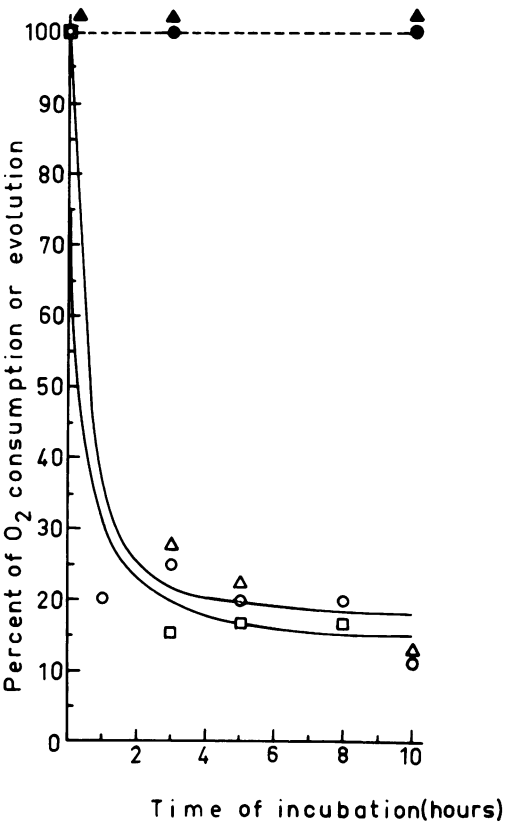

FIG. 4. *Rate of O_2 evolution and dark respiration of A. nidulans under N_2 atmosphere in Na_2CO_3-free medium or distilled water. Initial O_2 evolution and dark respiration rates were 115 nmoles O_2 per min per ml and 40 nmoles per min per ml, respectively. Oxygen evolution of cells in medium and distilled water (○); oxygen evolution after addition of $NaHCO_3$ to cells incubated in Na_2CO_3-free medium (△), or in distilled water (▲); rate of dark respiration (□); rate of O_2 evolution of cells incubated in Na_2CO_3-free medium in the dark (●) upon illumination.*

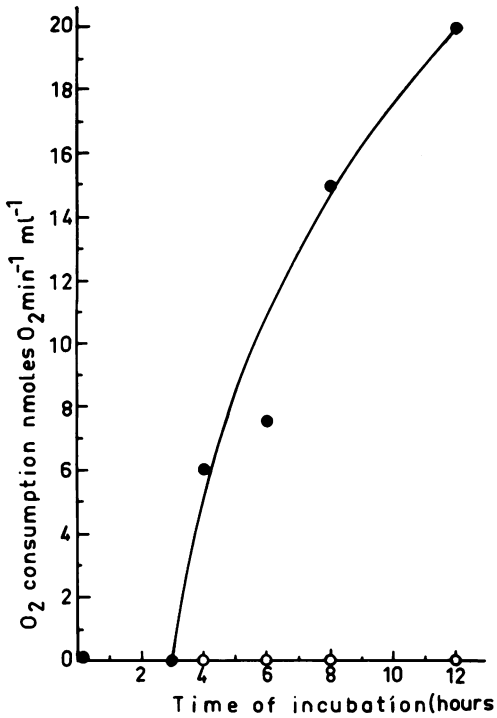

FIG. 5. *Rate of light dependent oxygen consumption in A. nidulans. Oxygen consumption in cells incubated in 100% oxygen in Na_2CO_3-free medium in the light (●); oxygen consumption of cells incubated in Na_2CO_3-free medium in N_2 or air, in complete medium under O_2 or N_2, or in distilled water under N_2 or O_2 (○) in light or dark.*

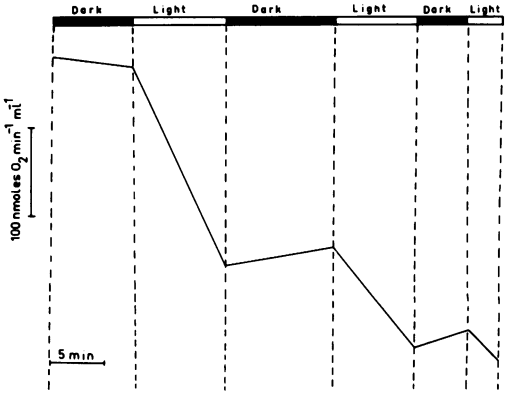

FIG. 6. *Oxygraph recording of light-dependent oxygen consumption in A. nidulans. Cells (10^9) were incubated for 12 hr in Na_2CO_3-free medium at pH 10.5 in oxygen.*

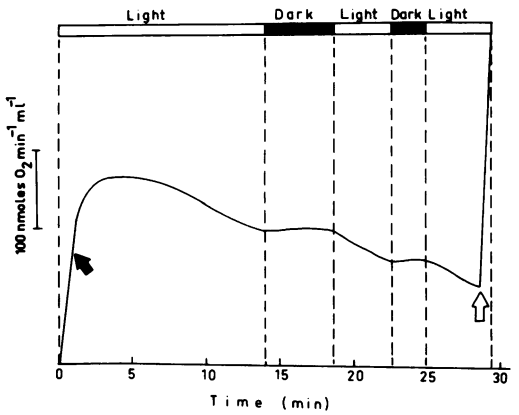

FIG. 7. *Oxygraph recording of the light-dependent oxygen consumption at elevated pH in A. nidulans. A. nidulans grown in standing conditions and the pH of the medium shifted to 12 (➡) and to 9.5 (⇨) by the addition of 0.01 N NaOH and by bubbling with CO_2, respectively.*

Effect of pH and photooxidative death on the pigmentation of the cells. Only those *A. nidulans* cells kept in the light under a pure O_2 atmosphere devoid of CO_2 at high *p*H values (10 to 11) and at 35 C bleach rapidly and almost totally (Table 3).

Photooxidative death of A. nidulans in fish ponds. We tried to determine whether photooxidative death is related to the sudden disintegration and decay of waterblooms of blue-green algae observed in natural water bodies and, in particular, in Israeli fishponds. Three neighboring fishponds having different diurnal *p*H curves were chosen to test this possibility. In September 1971, the water temperature in the profile examined ranged from 28 to 32 C. *A. nidulans* cells were suspended in dialysis tubing at different depths. Algae incubated in pond 1 showed marked mortality in conditions on high illumination. Photooxidative death was also observed in pond 2, whereas no killing occurred in pond 3 (see Table 4). A comparison of the *p*H fluctuations in each pond to the respective mortalities indicated that viability depends on the presence of available CO_2, since *p*H fluctuations are primarily due to changes in CO_2 concentration.

In the light of the experimental finding that at low temperature photooxidative death occurs even in the presence of CO_2, field observations were made in winter during the month of January in these ponds, at low temperatures (11 to 14 C), and at low *p*H values (8.1 to 8.4). When suspensions of *A. nidulans* were kept for 8 hr in dialyzing tubing in the sunlight, submerged at a depth of 5 cm, more than 99% of the algae died. Suspensions kept in the same conditions, but shaded from the light, remained fully viable.

TABLE 3. *Effect of photooxidative conditions on bleaching of photosynthetic pigments in Anacystis nidulans in the absence of CO_2*[a]

Photosynthetic pigment	Pigment concentration (%)				
	Complete medium-CO_2 at 35 C[b]		Medium at 4 C[c]	Potassium phosphate (0.05 M, pH 7.0), at 35 C[d]	
	O_2	N_2	O_2	O_2	N_2
C-phycocyanin	5	50	100	75	100
Chlorophyll *a*	50	50	100	75	100

[a] 8×10^8 cells/ml were incubated in the light for 12 hr before chlorophyll *a* and *C*-phycocyanin were extracted and measured.
[b] Final *p*H, 10.5.
[c] Final *p*H, 9.2.
[d] Final *p*H, 7.0.

DISCUSSION

Photooxidative damage has been observed in many photosynthetic organisms including bacteria, eukaryotic algae, and higher plants, after interference or suppression of carotenoid biosynthesis (22), thus establishing the protective function of carotenoid pigments in photosynthetic organisms kept under air. (However, it has been recently shown that *Micrococcus roseus* and *Acholeplasma laidlawii* B are subject to photooxidative killing although they are carotenoid-containing organisms [Schwartzel and Cooney, Cooney and Krinsky, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 291–292, 1972].) The photooxidative death under atmosphere of pure oxygen in blue-green algae which is described in this paper can proceed in carotenoid-containing organisms (12) at physiological temperatures only in the absence of CO_2. It is possible that under these conditions carotenoids do not afford sufficient protection against photooxidation. Thus, the role of CO_2 in preventing photooxidative death in *A. nidulans* and *S. cedrorum* indicates that the protective mechanism(s) is connected with the photosynthetic activity of the cell. Indeed, addition of DCMU, which blocks photosystem II, leads to photooxidative death even in complete (CO_2-containing) medium. It may be that the photooxidation at physiological temperatures in the absence of CO_2 involves a peroxide or a superoxide radical produced by direct reduction of oxygen by some reduced electron carrier that accumulates when photosynthesis is inhibited by absence of CO_2.

This hypothesis, however, fails to explain the lethal photooxidation at low temperatures, which probably proceeds by direct photosensitization of the cellular photosynthetic pigments. The death at low temperatures has been observed (5, 6, 16) in many organisms studied, including nonphotosynthetic microorganisms (such as *Sarcina lutea*, *Mycobacterium marinum*, *Halobacterium salinarium*), as well as in several photosynthetic organisms (17). In the case of carotenoid-containing *A. nidulans* and *S. cedrorum*, photooxidative death occurred at a much more rapid rate at the lower temperature. The considerable lag (ca. 8 hr) before the onset of mortality at 35 C and the even longer lag (48 hr) when cells were preincubated in an atmosphere of 5% CO_2 indicate that the protective mechanism(s) may be sustained by metabolic reserves possibly supplying endogenous CO_2 by respiration, even after photoassimilation of exogenous CO_2 ceases. Stewart and Pearson (23) have found that nitrogen fixation and photosynthetic carbon fixation are inhibited by high O_2 levels. We found that, under photooxidative conditions, *A. nidulans* cells show progressive damage to their photosynthetic capacities during the period preceding onset of death. Yet another indication of early photooxidative damage to *A. nidulans* cells is the induction of light-dependent oxygen consumption.

The conclusion that oxygen uptake in the light in photooxidatively damaged cells may be a nonphysiological reaction is also sup-

TABLE 4. *Photooxidative death of A. nidulans in fishponds*[a]

Depth of immersion (cm)	Percent of viable cells[b]		
	Pond 1[c]	Pond 2[d]	Pond 3[e]
5	0.001	10	100
15	10		
25	10		
40		10	100
Dark control (5 cm)	100	100	100

[a] The experiments were carried out in three adjacent fishponds in Beit Shan valley in September 1971 when water temperature ranged from 28 to 32 C. *A. nidulans* were suspended in closed, clear dialysis tubing (1-inch diameter) at different depths in the ponds. Each bag initially contained 50 ml of suspension of the algae (10^7 cells/ml) in logarithmic growth phase, washed in distilled water. Samples were kept in the ponds from sunrise to sunset (0500–1800 hr).
[b] Initial concentration, 10^7 cells/ml.
[c] *p*H shift (0500–1400 hr), 9.4 → 9.8.
[d] *p*H shift (0500–1400 hr), 8.0 → 9.1.
[e] *p*H shift (0500–1400 hr), 8.0 → 8.6.

ported by observations of such oxygen uptake in boiled leaf tissues of hydrangea (11) and *A. cylindrica* heterocysts (Bradley and Carr, Proc. Soc. Gen. Microbiol., p. XII, 1971), and on transfer of the *A. nidulans* cells to very high *p*H.

The destructive nature of photooxidative conditions is further emphasized by the bleaching of photosynthetic pigments observed in *A. nidulans*. However, this bleaching bears no direct relation to the photooxidative death of the algae, since unbleached cells die at 4 C and at 35 C at low *p*H values, and *A. nidulans* has been observed to bleach in the absence of nitrogen source (nitrogen chlorosis) while remaining viable (2). Bleaching and photooxidative death are thus clearly separable phenomena.

The dependence of blue-green algae on CO_2 for protection against photooxidative effects at physiological temperature could partially explain why planktonic blooms of blue-green algae in eutrophic waters are frequently denser and longer-lasting than in oligotrophic waters (8), although the algae are obligate photoautotrophs. The preference of blue-green algae for polluted waters could well be related to the high evolution of CO_2 by bacteria in such conditions (13). This assumption is further strengthened by the finding of Lange (14) that carbohydrates enhance growth of blue-green algae when associated with bacteria. He found that both carbohydrates and bacteria could be replaced by CO_2 added to the algal cultures. M. E. Meffert (Abstr. 13th Limnological Congress, Leningrad, p. 72, 1971) found that *Oscillatoria redekei* lysed in axenic cultures when CO_2 was removed, but not in bacterized cultures.

The requirement for CO_2 as protection against photooxidative death is indicated by the finding that *Anabaena variabilis* and *A. nidulans* die even in the presence of acetate when deprived of CO_2 (19).

The broad sensitivity of blue-green algae to photooxidation may also explain why this group thrives in environments of reduced oxygen supply (23), and why certain blue-green algae, such as *Gleocapsa*, are killed at high light intensities and can grow only in dim light (22). Furthermore, the low light penetration and reducing conditions may be factors for enrichment of blue-green algae in eutrophic waters, as stated by Bozniak and Kennedy (4). Massive water blooms of blue-green algae tend to disintegrate spontaneously and suddenly without preliminary visible symptom. The conditions proven experimentally to be required for photooxidative death (presence of light and oxygen, lack of CO_2) are not uncommon in the upper layers of dense algal water blooms in ponds and lakes. Oxygen concentrations of 300 to 400% saturation and high *p*H values (*p*H 10) following CO_2 depletion frequently occur in freshwater ponds during the summer (1). A possible mechanism for avoiding photooxidative death of blue-green algae which contain gas vacuoles in oxygen-rich layers could be the change in buoyancy of the algae due to changes in gas volume, described for *Aphanizomenon* (20) and *Anabaena flos aqua* (8, 9, 24), causing migration from such layers.

Since light intensity is high the year-round in Israel, photooxidative conditions prevail in winter when water temperatures generally remain below 15 C, and in summer when heavy algal blooms cause CO_2 depletion.

Therefore, photooxidative conditions in nature might be tested in terms of sensitivity of *A. nidulans*. A test system using this organism suspended in dialysis tubing, as described in the experimental part, which allows for rapid equilibration with external conditions, can serve for this purpose.

The sudden and sporadic die-off of summer blooms of blue-green algae, often with catastrophic consequences to fish populations, is most probably due to photooxidation which occurs when CO_2 deficiency, caused by algae photosynthesis, becomes critical.

In addition, the high sensitivity of blue-green algae, such as *A. nidulans*, to photooxidation at the low temperatures at which protective or repair mechanisms are nonfunctional is most probably a factor in the absence of blue-green algal blooms observed in winter in Israeli fish ponds.

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LITERATURE CITED

1. Abeliovich, A. 1969. Water blooms of blue-green algae and oxygen regime in fish ponds. Verh. Int. Verein. Limnol. **17**:594–601.
2. Allen, M. M., and A. J. Smith. 1969. Nitrogen clorosis in blue-green algae. Arch. Mikrobiol. **69**:114–120.
3. Bishop, M. I. 1958. The influence of the herbicide, DCMU, on the oxygen evolving system of photosynthesis. Biochim. Biophys. Acta **27**:205–206.
4. Bozniak, E. G., and L. L. Kennedy. 1968. Periodicity and ecology of the phytoplankton in an oligotrophic and autrophic lake. Can. J. Bot. **46**:1259–1271.
5. Burchard, R. P., and M. Dworkin. 1966. Light-induced lysis and carotenogenesis in *Myxococcus xanthus*. J. Bacteriol. **91**:535–545.

6. Dundas, I. D., and H. Larsen. 1963. A study on the killing by light of photosensitized cells of Halobacterium salinarium. Arch. Mikrobiol. **46**:19–28.
7. Dworkin, M. 1959. Function of carotenoids in photosynthetic bacteria. Nature (London) **184**:1891–1892.
8. Fogg, G. E. 1969. The physiology of an algal nuisance. Proc. Roy. Soc. Ser. B. Biol. Sci. **173**:175–189.
9. Fogg, G. E., and A. E. Walsby. 1971. Buoyancy regulation and the growth of planktonic blue-green algae. Mitt. Int. Verein. Limnol. **19**:182–188.
10. Franck, J., and C. S. French. 1941. Photooxidation processes in plants. J. Gen. Physiol. **25**:309–324.
11. Griffiths, M., W. R. Sistrom, G. Cohen-Bazire, and R. Y. Stanier. 1955. Function of carotenoides in photosynthesis. Nature (London) **176**:1211–1214.
12. Halfen, L. N., and G. W. Francis. 1972. The influence of culture temperature on the carotenoid composition of the blue-green alga, *Anacystis nidulans*. Arch. Mikrobiol. **81**:25–35.
13. Kuentzel, L. E. 1969. Bacteria, carbon dioxide and algal blooms. J. Water Pollut. Contr. Fed. **41**:1737–1747.
14. Lange, W. 1967. Effect of carbohydrates on the symbiotic growth of planktonic blue-green algae with bacteria. Nature (London) **215**:1277–1278.
15. Mathews, M. M. 1963. Studies on the localization, function, and formation of the carotenoid pigments of a strain of *Micobacterium marinum*. Photochem. Photobiol. **2**:1–8.
16. Mathews, M. M. 1964. The effect of low temperature on the protection by carotenoids against photosensitization in *Sarcina lutea*. Photochem. Photobiol. **3**:75–77.
17. Mathews, M. M., and W. R. Sistrom. 1960. The function of the carotenoid pigments of *Sarcina lutea*. Arch. Mikrobiol. **35**:139–146.
18. Parsons, T. R., and J. D. H. Strickland. 1963. Discussion of spectrophotometric determination of marine plant pigments, with revised equations for ascertaining chlorophylls and carotenoids. J. Mar. Res. **21**:155–163.
19. Pearce, J., and N. G. Carr. 1967. The metabolism of acetate by the blue-green algae, *Anabaena variabilis* and *Anacystis nidulans*. J. Gen. Microbiol. **49**:301–313.
20. Sirenko, L. A., N. M. Stetsenko, V. V. Arendarchuk, and M. I. Kuzmenko. 1968. Role of oxygen conditions in the vital activity of certain blue-green algae. Microbiology **37**:199–202.
21. Spikes, J. D. 1968. Photodynamic action, p. 33–64. *In* A. C. Giese (ed.), Photophysiology, vol. 3, Academic Press Inc., New York.
22. Stanier, R. Y., R. Kunisawa, M. Mandel, and G. Cohen-Bazire. 1971. Purification and properties of unicellular blue-green algae (order *Chroococcales*). Bacteriol. Rev. **35**:171–205.
23. Stewart, W. D. P., and H. W. Pearson. 1970. Effects of aerobic and anaerobic conditions on growth and metabolism of blue-green algae. Proc. Roy. Soc. Lond. Ser. B. **175**:293–311.
24. Walsby, A. E. 1969. The permeability of gas vacuole membranes to gas. Proc. Roy. Soc. Lond. Ser. B. **173**: 235–255.
25. Zehnder, A., and P. R. Gorham. 1960. Factors influencing the growth of *Microcystis aeruginosa* Kutz. Can. J. Microbiol. **6**:645–660.