APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1983, p. 545–548
0099-2240/83/090545-04$02.00/0

Vol. 46, No. 3

Sensitivity of an Oligotrophic Lake Planktonic Bacterial Community to Oxygen Stress

ALFRED T. MIKELL, JR.,* BRUCE C. PARKER, AND GEORGE M. SIMMONS, JR.

Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received 13 May 1983/Accepted 22 June 1983

Dissolved oxygen at approximately four times normal saturation (42 mg liter^{-1}) inhibited the growth and metabolism of summer planktonic bacteria in the surface water of alpine oligotrophic Mountain Lake (Giles County, Va.). Data were derived from growth of CFU on membrane filters, D-[*U*-^{14}C]glucose incorporation into the extractable lipid of these CFU, and respiration and assimilation of D-[*U*-^{14}C]glucose by lake water samples. Statistically significant ($\alpha < 0.05$) differences were not detected in either CFU or ^{14}C incorporation in lipid when superoxide dismutase (30 U ml^{-1}) or catalase (130 U ml^{-1}) was added to the medium. Thus, exogenous oxygen by-products apparently are not responsible for the observed inhibition of growth and metabolism.

Oxygen, which is essential to life for all aerobic organisms, is also a very reactive molecule, often becoming toxic at concentrations exceeding atmospheric levels. The mechanisms for oxygen toxicity are not readily understood, but apparently they involve the reactivity of oxygen and its radical by-products at a variety of molecular sites within the cell (12). Most investigations of oxygen toxicity mechanisms concern organisms (anaerobes and microaerophiles) with high sensitivities to oxygen at or below the levels in normal atmospheres. In contrast, published studies involving community responses to naturally high dissolved oxygen (HDO) concentrations are lacking, apparently because of the rarity or ephemeral nature of such environments. For example, HDO concentrations occur ephemerally in cyanobacterial blooms with photosynthetic oxygen production, resulting in dissolved oxygen concentrations of 200 to 300% saturation (2). Prolonged existence of these HDO concentrations usually would induce photooxidative death, but they are normally brief. Among the relatively few known natural aquatic environments in which prolonged or permanent HDO conditions occur are certain oligotrophic, perennially ice-covered lakes in dry valleys of southern Victoria Land, Antarctica (9–11). To develop techniques for studying those Antarctic lakes and to obtain base-line data for comparison, we undertook this investigation of the planktonic microbial community in Mountain Lake, Giles County, Va. during the summer of 1982. Mountain Lake is a natural alpine oligotrophic lake with perennially oxygen-saturated surface waters, about which considerable information is known (1, 6, 8, 13).

MATERIALS AND METHODS

CFU were determined by filtering measured volumes of freshly collected lake water through 0.22-μm Millipore filters (47-mm diameter) at <26 cm of Hg (vacuum). Filters were placed onto Gelman absorbant pads saturated with medium. Medium consisted of autoclaved-sterilized Mountain Lake water with additions of 12.5 μCi of D-[*U*-^{14}C]glucose per liter (274 μCi μmol^{-1}), 0.02% Trypticase soy broth (BBL Microbiology Systems), and 0.002% yeast extract. These concentrations of nutrients gave the maximal CFU within 2 weeks of incubation. After preparation, one set of plates was incubated in a pressure cooker with oxygen added to give a final concentration of 42 mg liter^{-1} (11 lb/in^2) (HDO plates). A second set of plates was incubated in the dark at ambient atmospheric pressure with normal atmospheric dissolved oxygen (ADO plates). All plates were incubated at 12°C for 7 to 10 days.

After the CFU were counted, total lipids were extracted in scintillation vials by the method of McKinley et al. (5). The chloroform fraction was evaporated to dryness, Aquasol 2 was added, and the [^{14}C]lipid was counted by liquid scintillation.

Total assimilated and respired carbon was determined by using lake water (50 ml) dispensed into 125-ml serum bottles stoppered with butyl rubber serum stoppers with inserted glass center wells and paper wicks (Bellco Glass, Inc.). A minimum of 10 bottles per treatment was used in each experiment. HDO bottles were then pressurized with oxygen (11 lb/in^2). Both HDO and ADO bottles were agitated at 12°C for 2 h, which had been predetermined to be an adequate time to achieve equilibrium of the dissolved oxygen. Controls were fixed with glutaraldehyde (2% final concentration, vol/vol). All HDO and ADO bottles received 20 μCi of D-[*U*-^{14}C]glucose per liter (274 μCi μmol^{-1}). The bottles were incubated for 4 to 6 h at 12°C. After incubation, unfixed ADO and HDO treatments were fixed with glutaraldehyde. Phenethyl-

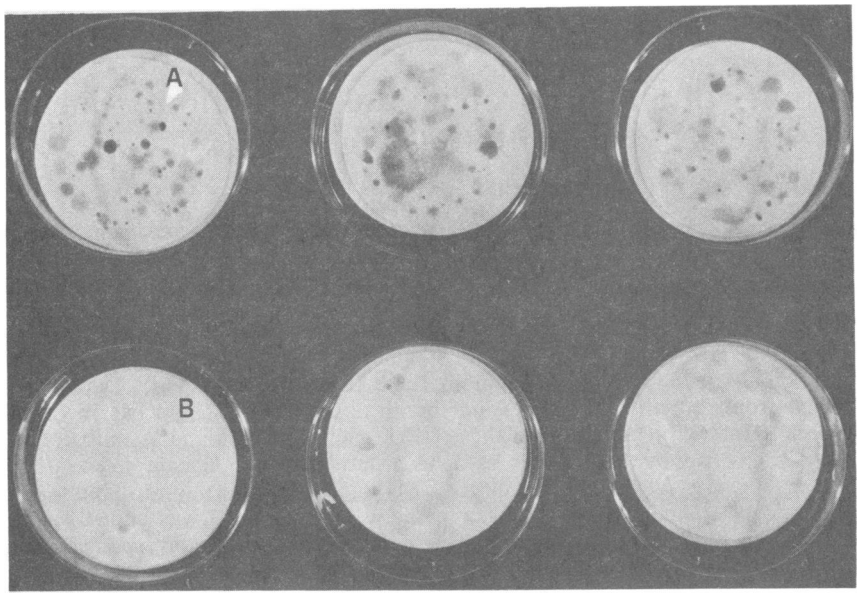

FIG. 1. Photograph of representative membrane filters after development of CFU with ADO (A) or HDO (B).

amine (150 μl) was added to the paper wick in each center well, after which all bottles were acidified with 200 μl of 2 N H_2SO_4. The bottles were slowly agitated for 2 h to enhance both release of CO_2 from the acidified water and trapping by the phenethylamine. Water from each bottle was filtered through 0.2-μm Nuclepore filters and then rinsed with an equal volume of autoclaved lake water. Aquasol 2 was added to the filters for liquid scintillation counting. Center wells and paper wicks were placed into Aquasol 2, and the total assimilated and respirated carbon was counted by liquid scintillation. Fixed control values were subtracted, and all counts were corrected to disintegrations per minute by the channels ratio method.

The total lipid fraction of the ^{14}C assimilated by lake water was determined by filtering (0.2-μm Nuclepore filter, <26 cm of Hg [vacuum]) one-half the volume of each bottle and rinsing with an equal volume of autoclaved lake water. Total lipid was extracted from filters by the previously described technique (5).

RESULTS AND DISCUSSION

CFU were significantly ($\alpha < 0.05$) reduced with HDO as compared with ADO (Fig. 1 and Table 1). CFU differences do not necessarily indicate relative differences in biomass, because an equal number of small and large colonies on HDO and ADO plates may occur regardless of differences in colony size. Relative differences in biomass and metabolic activity can be determined by extracting the total lipid incorporated in cells on the same filters after counting CFU. Table 1 presents a comparison of CFU development and total lipid incorporation.

It is generally recognized that pressure effects begin to be of physiological significance at 100 atm (1,500 lb/in^2) (4). The pressure applied

TABLE 1. Comparison of ADO and HDO conditions on development of CFU and D-[U-^{14}C]glucose incorporation into lipid

Date	Treatment	CFU liter^{-1}	% Reduction[a]	dpm	% Reduction[a]
6/28/82	ADO	4.9×10^4		10,393	
6/28/82	HDO	2.2×10^4	54	1,191	88
8/10/82	ADO	1.4×10^5		8,206	
8/10/82	HDO	2.5×10^4	82	2,120	74
9/22/82	ADO	4.2×10^5		8,873	
9/22/82	HDO	2.1×10^5	50	1,302	85

[a] Percent reduction = (1 − (HDO/ADO)] × 100.

TABLE 2. Comparison of the effects of ADO and HDO on total carbon (T) and total lipid (L) assimilated

Date	Treatment	Total carbon assimilated (dpm)	% Reduction[a]	Total lipid fraction (dpm)	% Reduction[a]	L/T (%)
7/6/82[b]	ADO	35,350		6,102		17
7/6/82	HDO	7,709	78	1,109	82	14
7/12/82[c]	ADO	3,437		735		21
7/12/82	HDO	1,041	70	232	68	22
7/22/82[c]	ADO	3,884		774		20
7/22/82	HDO	1,532	61	345	55	23

[a] See Table 1, footnote *a*, for definition.
[b] 6-h incubation.
[c] 4-h incubation.

(HDO conditions) did not exceed 12 lb/in^2 (≅83 kPa), which is less than that encountered in 28 feet (8.53 m) of fresh water. Nevertheless, 50 lb/in^2 of helium was applied and compared with ADO in one experiment. No significant difference ($\alpha > 0.05$) between these treatments existed as measured by CFU development and total lipid incorporated into CFU.

The influence of HDO on lipid incorporation need not necessarily reflect an influence on the total carbon assimilated. Whereas total carbon assimilation cannot be measured accurately with CFU owing to high levels of abiotic absorption, the total carbon assimilated by lake water can be measured by standard procedures which also allow a portion to be fractionated for the determination of total lipid incorporation. The results of three such experiments (Table 2) indicated that 42 mg of dissolved oxygen per liter was inhibitory to both carbon and lipid assimilation ($\alpha < 0.05$). However, the percent lipid to total carbon assimilated was not significantly different ($\alpha > 0.05$) within each treatment (HDO versus ADO).

Respiration of D-[*U*-^{14}C]glucose was also inhibited in two experiments (Table 3). It cannot be determined whether respiration was more or less inhibited than carbon assimilation on the basis of these experiments.

Superoxide dismutase and catalase were added to the standard medium in one experiment to determine whether superoxide or hydrogen peroxide was being generated exogenously to cells in inhibitory concentrations. Whereas positive evidence for such exogenous generation exists for the microaerophiles *Spirillum volutans* and *Campylobacter fetus* (3, 7), the same enzyme concentrations had no effect ($\alpha > 0.05$) on the Mountain Lake community treated with or without the enzymes, as measured by CFU development and ^{14}C incorporation into total lipid (Table 4). Endogenous generation and activity of superoxide and hydrogen peroxide remains a possibility.

On the basis of several criteria (CFU development, total lipid incorporated into CFU, and total carbon assimilated and respired), 42 mg of dissolved oxygen per liter (HDO) appeared to inhibit the growth and metabolism of heterotrophic microorganisms in this alpine oligotrophic lake. This constitutes the first investigation of the effects of HDO on natural heterotrophic plankton communities and has laid the basis for comparative studies with the naturally perennial HDO concentrations in lakes of the dry valleys of Antarctica.

TABLE 3. Comparison of the effects of ADO and HDO on total carbon assimilated and respired

Date	Treatment	Total carbon assimilated (dpm)	% Reduction[a]	Total carbon respired (dpm)	% Reduction[a]
9/3/82[b]	ADO	10,724		5,145	
9/3/82	HDO	6,652	38	2,612	49
9/21/82[c]	ADO	7,228		7,884	
9/21/82	HDO	3,277	55	4,480	43

[a] See Table 1, footnote *a*, for definition.
[b] 6-h incubation.
[c] 10-h incubation.

TABLE 4. Comparison of the effects of ADO and HDO with or without enzymes on CFU development and D-[U-^{14}C]glucose incorporation into total lipid

Treatment[a]	CFU liter^{-1}	% Reduction[b]	dpm	% Reduction[b]
ADO (N)	1.4×10^5		8,206	
HDO (N)	2.5×10^4	82	2,120	74
ADO (C)	1.3×10^5		7,256	
HDO (C)	3.0×10^4	77	2,456	66
ADO (S)	1.5×10^5		8,041	
HDO (S)	2.6×10^4	83	3,062	62
ADO (CS)	1.3×10^5		7,986	
HDO (CS)	2.3×10^4	82	1,940	76

[a] N, No enzyme added; C, plus 130 U of catalase per ml; S, plus 30 U of superoxide dismutase per ml; CS, plus 60 U of catalase and 15 U of superoxide dismutase per ml.

[b] See Table 1, footnote *a*, for definition.

ACKNOWLEDGMENT

We thank the Division of Polar Programs, National Science Foundation, for support of this research (grant DPP-80-12988).

LITERATURE CITED

1. **Dubay, C. I., and G. M. Simmons, Jr.** 1979. The contribution of macrophytes to the metalimnetic oxygen budget of a montane, oligotrophic lake. Am. Midl. Natl. **101**:108–117.
2. **Eloff, J. N., Y. Steinitz, and M. Shilo.** 1976. Photooxidation of cyanobacteria in natural conditions. Appl. Environ. Microbiol. **31**:119–126.
3. **Hoffman, P. S., H. A. George, N. R. Krieg, and R. M. Smibert.** 1979. Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. II. Role of exogenous superoxide anions and hydrogen peroxide. Can. J. Microbiol. **25**:8–16.
4. **Marquis, R. E.** 1976. High-pressure microbial physiology. Adv. Microb. Physiol. **14**:159–241.
5. **McKinley, V. L., T. W. Federle, and J. R. Vestal.** 1982. Effects of petroleum hydrocarbons on plant litter microbiota in an arctic lake. Appl. Environ. Microbiol. **43**:129–135.
6. **Obeng-Asamoa, E. K., and B. C. Parker.** 1972. Seasonal changes in plankton and water chemistry of Mountain Lake, Virginia. Trans. Am. Microsc. Soc. **91**:363–380.
7. **Padgett, P. J., W. H. Cover, and N. R. Krieg.** 1982. The microaerophile *Spirillum volutans*: cultivation on complex liquid and solid media. Appl. Environ. Microbiol. **43**:469–477.
8. **Parker, B. C.** 1976. Assessment of the trophic state of Mountain Lake, Giles County, Virginia, p. 102–109. *In* B. Parker and M. Roane (ed.), Algae and fungi of the southern Appalachians. Systematics and ecology. Fourth symposium on biota of the southern Appalachians. The University Press of Virginia, Charlottesville.
9. **Parker, B. C., G. M. Simmons, Jr., G. Love, K. G. Seaburg, and R. A. Wharton, Jr.** 1981. Modern stromatolites in Antarctic dry valley lakes. BioScience **31**:656–661.
10. **Parker, B. C., G. M. Simmons, Jr., K. G. Seaburg, D. D. Cathey, and F. C. T. Allnutt.** 1982. Comparative ecology of plankton communities in seven Antarctic oasis lakes. J. Plankton Res. **4**:271–286.
11. **Parker, B. C., G. M. Simmons, Jr., R. A. Wharton, Jr., K. G. Seaburg, and F. G. Love.** 1982. Removal of organic and inorganic matter from Antarctic lakes by aerial escape of bluegreen algal mats. J. Phycol. **18**:72–78.
12. **Shilo, M. (ed.).** 1979. Strategies of microbial life in extreme environments, p. 137–241. Dahlem Konferenzen, Berlin.
13. **Simmons, G. M.** 1973. Observations on limnetic carbon assimilation rates in Mountain Lake, Virginia during its thermal stratification period. Va. J. Sci. **24**:206–211.