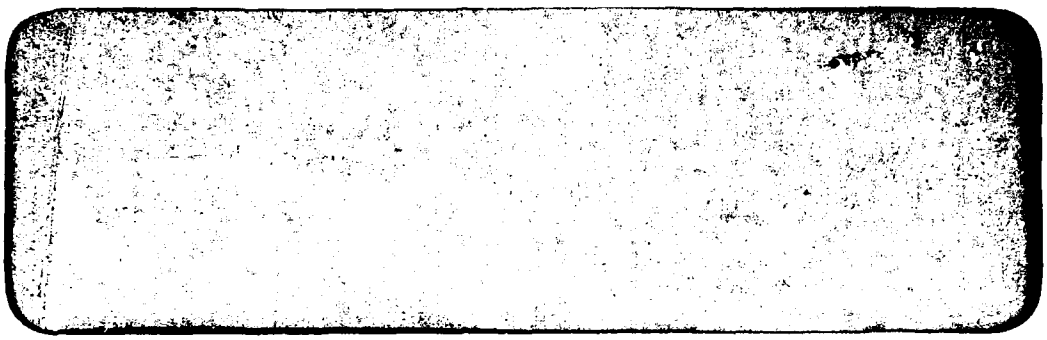

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Growth of *Chlorella sorokiniana* at Hyperbaric Oxygen Pressures

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The growth rate of *Chlorella sorokiniana* decreased in a linear fashion as the partial pressure of oxygen was increased from 711 to 1,478 mm of Hg. Under two atmospheres of oxygen pressure, growth ceased after 10 to 12 hr. This cessation of growth was not due to any permanent injury, as growth resumed when oxygen partial pressure was reduced to ambient levels. The inhibition occurred under both autotrophic and heterotrophic growth conditions and was not accompanied by an increase in cell size. The results indicated that the tolerance of *Chlorella* cells to elevated oxygen pressures was not an absolute immunity, and that inhibition of growth at very high oxygen pressures cannot be accounted for by an inhibition of photosynthesis alone.

The toxic effect of oxygen has been demonstrated in all forms of life, including unicellular microbes (4, 5, 12, 13). Although considerable literature and several theories exist concerning the phenomenon, relatively little is known about the mechanism of oxygen toxicity. Recently, we have been studying some physiological and biochemical aspects of an oxygen-tolerant strain (OTS) of the algal species *Chlorella sorokiniana* (Shihira and Krauss, 11). These studies demonstrated that OTS cells grew at an optimal rate of about 9 to 9.5 doublings per day when grown at a light intensity of about 1,500 ft-c in Knop's nutrient solution aerated with either air and 5%, CO_2 or 95%, O_2 and 5%, CO_2 (Wagner and Welch, *in preparation*). The experiments also suggested that partial pressures of oxygen ranging from 150 to 730 mm of Hg permitted optimal growth of OTS cells. To elucidate further the tolerance of *Chlorella* to elevated oxygen concentrations, it was desirable to determine the growth rates of OTS under varying partial pressures of oxygen.

MATERIALS AND METHODS

The growth experiments reported herein were performed in a model 614 hyperbaric chamber (The Bethlehem Corp., Bethlehem, Pa.) specially modified for growing algae. Temperature control was achieved by introducing a stainless steel heating coil into the chamber. The coil was passed through two openings in the chamber by means of pressure tight stainless steel fittings and connected to a circulating water bath. Chamber temperature was controlled and monitored by means of a model 73 resistance thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) wired in series with the water bath. To allow for independent aeration of the algal sample, two low-pressure hoses were attached from an oxygen regulator to the chamber. One of these was secured directly to the chamber and allowed for pressurization; the second gas hose was attached to a 0.63-cm length of stainless-steel tubing which passed into the hyperbaric chamber. Inside the chamber, this tubing was connected to the algal growth vessel by a piece of latex tubing. A controlled leak was used for accurate regulation of chamber pressure and to allow for gas flow through the algal culture. Pressure was monitored by means of a mercury U-tube manometer connected to the hyperbaric chamber.

The algal growth vessel, a "lollipop" about 0.5 cm thick and 8 cm in diameter, was placed inside the chamber directly in front of a glass window. The vessel was illuminated from outside the hyperbaric chamber by two fluorescent lamps (high output, cool white). Light intensity at the surface of the growth vessel was about 1,500 ft-c. For dark studies, the chamber window was covered with a double thickness of aluminum foil. The lollipop had one port at the top to allow excess gas to escape from the vessel and two ports at the bottom, one for aeration and one for sampling. The sample outlet of the growth vessel was connected to a port on the wall of the hyperbaric chamber, allowing for sampling without disruption of the atmosphere inside the chamber.

Stock cultures of OTS cells were maintained at 38 C in illuminated culture tubes aerated with 95%, O_2 5%, CO_2. Cells of the wild type or oxygen sensitive strain (OSS), were aerated with 95%, air 5%, CO_2. To determine the growth rate of cells at any particular atmospheric condition, a sterile lollipop was inoculated with about 25 ml of a dilute, axenic, log phase culture

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about 10 hr before the growth measurements were to begin. During this time, the sample was equilibrated to the temperature of the chamber (38 C) and aerated with 3% CO_2-20.9% O_2-76.1% N_2 at ambient pressure (748 ± 4 mm of Hg). The gas regulator was then transferred to a cylinder containing the gas mixture desired for study. For the hyperbaric experiments, chamber pressure was adjusted in the range of 770 to 1,540 mm of Hg above ambient to provide a total pressure of 2 to 3 atm. The first growth measurements were taken not less than 1 hr after the change in gas or total pressure, thus allowing sufficient time for the dissolved gases to come into equilibrium with the new gas phase. Although the chamber pressure varied somewhat and required periodic adjustment, it was easily maintained within 5 mm of Hg of the desired level. CO_2 concentrations of gas mixtures used at ambient pressure were approximately 5%. Those used at 2 atm were 2.5 to 3% and those at three atmospheres were 1.5 to 2% CO_2; thus, the pCO_2 was essentially the same in all cases.

Culture populations were determined by counting cells with a hemocytometer. Growth rates were calculated in terms of doublings per day.

RESULTS

The influence of oxygen tension on growth rate during the first 24 hr of exposure to the various gas mixtures studied is illustrated by Fig. 1. The growth rate of OTS cells was unaffected by variations in oxygen pressure in the range of 131 to 711 mm of Hg. At zero-oxygen tension, the growth rate was accelerated about 12%, from 8.5 to 9.2 doublings/day to 10.0 doublings/day. As pO_2 was increased from 711 to 1,478 mm of Hg, growth rate decreased in a linear fashion. Tests with the OSS indicated that after an initial adaptation period of 4 to 8 hr, its response to oxygen is similar to that of the OTS, although growth rate reduction occurs at somewhat lower oxygen pressures.

When pO_2 was maintained in a range of 156 to 711 mm of Hg, nitrogen partial pressures of as much as 3 atm resulted in a relatively slight reduction growth. The slope of the least-squares line is significantly different from zero, but it represents only about a 10% decrease in growth rate at 2,055 mm of Hg (Fig. 2). Thus, the inhibitory effects of high-oxygen partial pressures on the growth of OTS cells appear to be due at least primarily to oxygen per se, rather than to a pressure or inert gas effect.

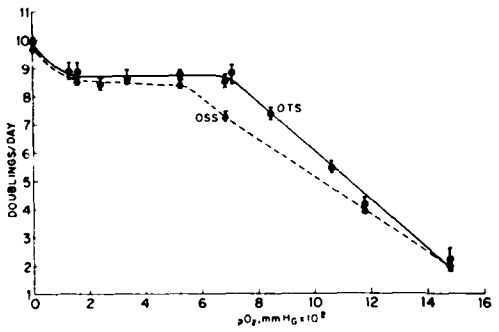

FIG. 1. *Effects of oxygen partial pressure on the growth rates of oxygen-tolerant (●) and oxygen sensitive (▲) strains of C. sorokiniana. Each point represents the mean of three determinations.*

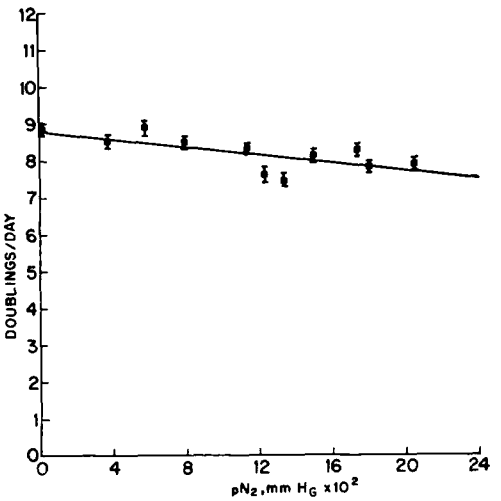

FIG. 2. *Effect of nitrogen partial pressure on the growth rate of C. sorokiniana OTS.*

Typical growth curves for cultures in 1 atm of oxygen, 2 atm of oxygen, and 2 atm of nitrogen are represented in Fig. 3. Under 2 atm of oxygen, growth, in terms of cell division, ceases after about 10 to 12 hr. Resumption of growth never occurred in cultures maintained up to 120 hr. This was also the case with OSS cells. If oxygen pressure was reduced to 156 mm of Hg, growth was resumed and proceeded at a normal rate (Fig. 4). Cell size distribution, determined by means of a Coulter counter, was found to be the same before and after 8 hr of exposure to 1,478 mm of O_2, and microscopic examination revealed no formation of giant cells or other gross abnormalities in the oxygen-treated cells.

Because of the well-documented inhibition of photosynthesis by oxygen (14), it appeared advisable to determine whether the oxygen-induced inhibition of growth would also occur under heterotrophic conditions. Typical growth curves of OTS grown on 1% (w/v) glucose under 711 and 1,478 mm of pO_2 are shown in Fig. 5. The effects of the higher oxygen level in either light or darkness were essentially identical to those obtained with autotrophic conditions.

Since succinate and lactate have been reported

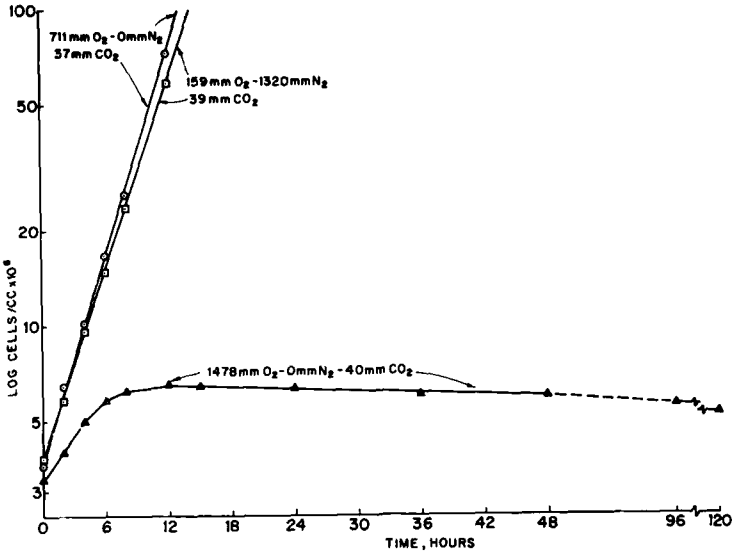

FIG. 3. *Typical growth curves of OTS cells when pO_2 is 1 atm or less (○), pN_2 is 2 atm (□), and pO_2 is 2 atm (△).*

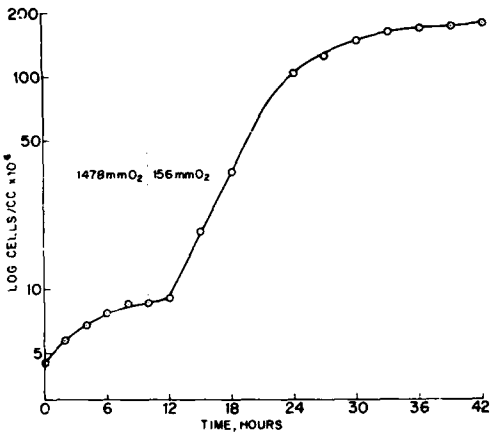

FIG. 4. *Growth pattern of OTS cells when oxygen partial pressure is reduced from 2 to less than 1 atm.*

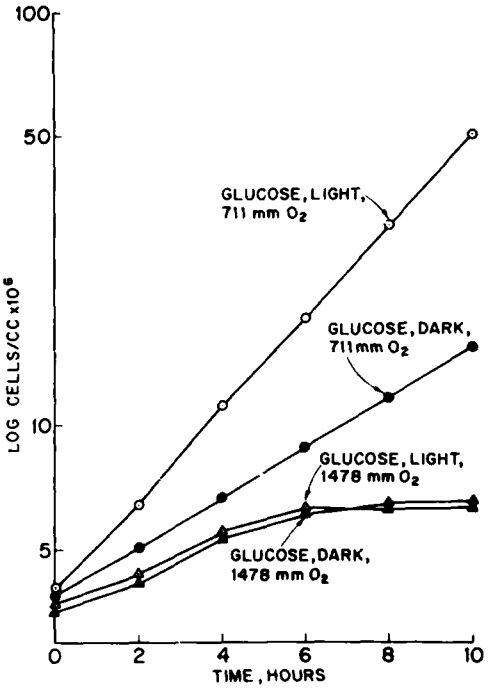

FIG. 5. *Typical growth curves of OTS cells grown heterotrophically in the light at 1 atm of oxygen (○), in the dark at 1 atm of oxygen (●), in the light at 2 atm of oxygen (△), and in the dark at 2 atm of oxygen (▲).*

to protect against oxygen toxicity in animals (3, 10), an attempt was made to determine if these materials would protect OTS cells against high oxygen pressures. At concentrations of 0.01 and 0.1 M, these materials had no effect on growth at 1,478 mm of pO_2 in either light or darkness. However, neither succinate nor lactate would support growth in darkness at ambient oxygen pressures, suggesting that they are not taken up by *C. sorokiniana* and, therefore, cannot serve as either carbon sources or protectants. Samejima

and Myers (9) reported that these materials will not support the growth of other *Chlorella* species.

DISCUSSION

The maximal oxygen tension under which the OTS is capable of sustained growth lies between 1 and 2 atm. *Chlorella*, therefore, appears to be considerably more resistant to the effects of hyperbaric oxygen than are mammalian systems and, in this respect, resembles the aerobic bacteria. Ollsdart (8) reported that the growth rate of *Escherichia coli* declines as oxygen pressure is raised above 1 atm, and Bornside (1) found the growth rate of *Staphylococcus aureus* to be reduced 60% in 3 atm of oxygen. Kaye (7) reported diverse bacterial responses, with several species unaffected by oxygen at 3 atm.

The only previous work with pressure effects on *Chlorella* appears to be that of Hannan (6), who reported that when carbon dioxide is not limiting, pressures of more than 10 psi above ambient progressively inhibit oxygen production. Since oxygen production and cell growth are concomitant phenomena, the effects of pressure, depicted in Fig. 2, can be said to substantiate Hannan's findings. This effect of pressure may be of some importance in oceanographic studies of phytoplankton.

The ability of the cells to resume growth when oxygen tension was lowered from 2 to 1 atm seems indicative that cessation of growth was not due to any permanent injury. This, too, is paralleled by bacterial studies. Caldwell (2) reported that growth of *Bacillus subtilis* is inhibited at 11 atm of O_2 but resumes when pressure is released.

That elimination of oxygen from the gas phase results in an accelerated growth rate was first noted by C. H. Ward *(unpublished data)* during the initial isolation of the oxygen-tolerant strain. This phenomenon may be due to the inhibitory effects of even moderate partial pressures of oxygen.

Since cessation of growth in 2 atm of oxygen occurs under both heterotrophic and autotrophic conditions, it does not seem that the inhibition of photosynthesis is sufficient to account for this effect. Therefore, the possibility of a common mechanism of oxygen toxicity in algal and mammalian systems remains open, and algae may be useful in elucidating the problem of oxygen toxicity in man.

ACKNOWLEDGMENTS

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The growth rate of the oxygen tolerant strain (OTS) of Chlorella sorokiniana decreases in a linear fashion as the partial pressure of oxygen is increased from 711 to 1478 mm Hg. Under two atmospheres of oxygen pressure growth ceases after 10 to 12 hours. This cessation of growth is not due to any permanent injury, as growth is resumed when oxygen partial pressure is reduced to ambient levels. The inhibition occurs under both autotrophic and heterotrophic growth conditions and is not accompanied by an increase in cell size. The results indicate that the tolerance of OTS cells to elevated oxygen pressures is not an absolute immunity, and that inhibition of growth at very high oxygen pressures cannot be accounted for by an inhibition of photosynthesis alone.

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