Effects of Hypobaric and Hyperbaric Helium Atmospheres on the Growth of Chlorella sorokiniana

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Growth of Chlorella sorokiniana is unaffected by the choice of atmospheric diluent gas. Reduced total pressure has no inhibitory effect and may be slightly stimulatory.

Helium has been proposed as a constituent of spacecraft cabin atmospheres and as a diluent in undersea operations (8). Since the unicellular algae are considered for use in bioregenerative life support in such closed systems (2), it is essential that the effect of helium atmospheres and variations in total pressure on the growth of such organisms is elucidated.

Literature concerning the biological effects of inert gases is devoted principally to animal experiments and suggests minor abnormalities induced by the physical nature of the gas (9). Substitution of helium or argon for the nitrogen in air at one atmosphere apparently has no effect on the growth and gas exchange of Chlorella pyrenoidosa (1). Experiments with Neuspora crassa grown in atmospheres of helium, neon, nitrogen, argon, krypton, and xenon showed an inverse relationship between growth rate of the organism and the square root of the molecular weight of the gas (6). Latterell (3) reported a significant decrease in germination when rye seeds were exposed to a helium atmosphere at 760 mm of Hg. Mansell et al. (4) found an increase in per cent dry weight when turnip plants were exposed to 375 mm of Hg pressure. This difference was attributed to an increased transpiration rate at the lower pressure. Apparently the only studies concerning the effect of pressure on algae are those of Hannan (2) and Richardson et al. (5), who found that increases in pressure above ambient resulted in slight but progressive decreases in growth rates. No work on the effects of reduced pressure on algae was reported.

In this study, C. sorokiniana was cultured in a system identical to that described by Richardson et al. (5), with the exception that hypobaric conditions were achieved by connecting a small vacuum pump in series with a house vacuum jet to the culture chamber. Cultures were started from log-phase inocula and maintained at 38 C in a modified Knops medium with nitrate as the nitrogen source, as prepared by Vela and Guerra (7).

Total pressure was maintained by pressurization or evacuation of the chamber, and the desired partial pressures of the various gases were achieved by the use of prepared gas mixtures, constantly bubbled through the algal suspension. The mixtures were prepared in such a way that carbon dioxide was 23 to 38 mm of Hg under all conditions. Culture populations were determined by hemocytometer cell counts, and growth rates were calculated as doublings per day.

When compared with nitrogen as a diluent gas at one atmosphere total pressure, helium had no effect on growth rate (Table 1). As reported previously (5), nonoxygenated cultures showed a slight increase in growth rate over those in which oxygen was approximately equivalent to ambient.

To determine the effects of total pressure on growth rate, mixtures of He and CO2 were prepared which would permit variations in He par-
**TABLE 3. Growth of Chlorella sorokiniana in argon, helium, and neon at 2250 mm of Hg**

| Gas mixture (mm of Hg) | Growth rate (doublings per day) |
|------------------------|--------------------------------|
| He | Ar | Ne | N₂ | CO₂ | Run 1 | Run 2 | Run 3 | Mean |
| 2,198 | 2,205 | 52 | 8.5 | 9.8 | 11.4 | 9.9 |
| 2,205 | 2,207 | 45 | 10.1 | 9.0 | 10.0 | 9.7 |
| 2,205 | 2,207 | 45 | 10.9 | 9.5 | 10.6 |
| 2,205 | 2,207 | 43 | 9.0 | 9.6 | 9.8 | 9.8 |

medium (total pressure) and total pressure while maintaining CO₂ partial pressure at 37 to 38 mm of Hg. As shown in Fig. 1, cultures at 188 and 375 mm of Hg total pressure exhibited mean growth rates of 13.4 and 10.8 doublings per day, respectively, compared with growth rates of 9.3 to 9.9 for cultures grown at 750 to 2250 mm of Hg. Evapo-

**Fig. 1. Effects of pressure on the growth rate of C. sorokiniana in He-CO₂ atmospheres with a constant CO₂ partial pressure of 37 to 38 mm of Hg.**

Finally, algal cultures subjected to atmospheres of helium, argon, neon, and nitrogen at 2250 mm of Hg exhibited no appreciable differences in growth rate (Table 3).

From these data, it seems reasonable to conclude that the choice of inert diluent gas has little bearing on the utility of algae in closed systems. Reduced pressure has no inhibitory effect and may, in fact, be beneficial to the growth of *Chlorella*.

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