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## THE EFFECTS OF LIGHT INTENSITY ON THE GROWTH RATES OF GREEN ALGAE<sup>1,2,3</sup>

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Among the environmental factors affecting the growth rates of unicellular algae, light is frequently at an improper level. In many laboratory cultures used for physiological research the light intensity is too low to permit logarithmic growth. In nature the intensity is well above saturation and may be high enough to inhibit growth during much of the day. The intensities for saturation and inhibition depend on the suitability of other factors of the environment, e.g., temperature, CO<sub>2</sub> level, and nutrient supply. In attempting to predict the performance of an alga under a given set of conditions it is necessary to know its potential under optimum conditions. Such information is basic to the evaluation of physiological studies and to the design and operation of culture apparatus. This paper describes the growth responses of five green algae to different light intensities under comparable environmental conditions.

### MATERIAL AND METHODS

The algae for these studies included: *Chlorella pyrenoidosa* from the collection of Dr. Van Niel, Code No. Z.2.2.1. BAD 12/11; *Chlorella vulgaris* from Dr. Pringsheim's collection, No. 211/8b, listed as the one isolated by R. Emerson and used in his work on iron deficiency and photosynthesis; *Scenedesmus obliquus*, No. WH-50, isolated from fresh waters in Woods Hole by R. W. Krauss in 1950; *Chlamydomonas reinhardtii*, plus strain, obtained from Dr. G. M. Smith through Dr. Harold C. Bold; and *Chlorella pyrenoidosa*, high-temperature strain 7-11-05, isolated in Texas in 1951 (10).

The cultures were grown in a medium of the following composition (in grams per liter): KNO<sub>3</sub>, 1.25; KH<sub>2</sub>PO<sub>4</sub>, 1.25; MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 1.00; CaCl<sub>2</sub>, 0.0835; H<sub>3</sub>BO<sub>3</sub>, 0.1142; FeSO<sub>4</sub> · 7 H<sub>2</sub>O, 0.0498; ZnSO<sub>4</sub> · 7 H<sub>2</sub>O, 0.0882; MnCl<sub>2</sub> · 4 H<sub>2</sub>O, 0.0144; MoO<sub>3</sub>, 0.0071; CuSO<sub>4</sub> · 5 H<sub>2</sub>O, 0.0157; Co(NO<sub>3</sub>)<sub>2</sub> · 6 H<sub>2</sub>O, 0.0049; ethylene-

diaminetetraacetic acid (as a chelating agent), 0.5. The pH of the medium was 6.8. Test tubes with inner dimensions of 16 × 150 mm were used as growth vessels and the 10 ml of culture medium which they contained was inoculated from stock cultures maintained on agar slants. The test tubes were fitted with cotton plugs, through which cotton-plugged bubbling tubes were passed to supply the cultures with a 4% CO<sub>2</sub>-in-air mixture. After inoculation the cultures were placed in a constant temperature water bath made of lucite.

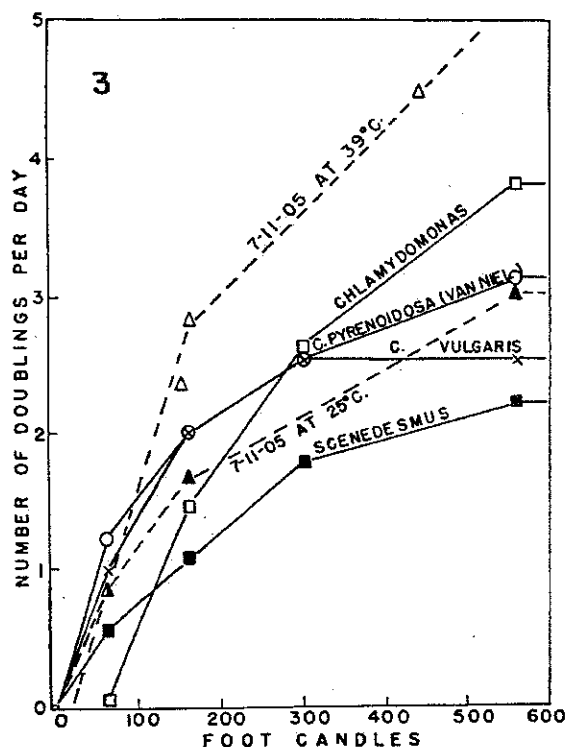
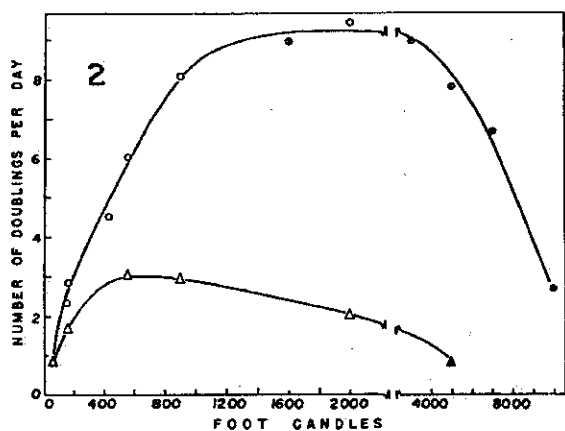
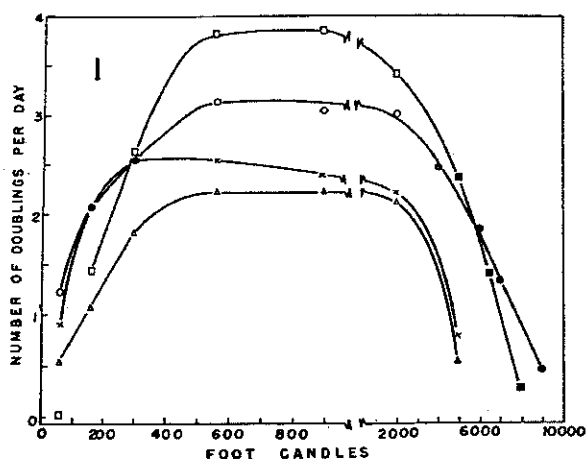
Illumination was provided by two batteries of lamps placed on opposite sides of the bath. Light intensities up to 2,000 ft-c were obtained with two batteries of four, white, 40-watt Champion, fluorescent lamps in each. Intensities above 2,000 ft-c were obtained with General Electric, 300-watt, medium-flood lamps. Reduced intensities were achieved by placing wire screens between the light source and the water bath. Light intensities were measured by a recently calibrated Weston illumination meter with the photocell inside the bath at the level of the culture tubes. It is recognized that the actual light intensity received by any cell in the algal suspension may differ from that measured by the photocell. There are two reasons for this discrepancy. First, cultures receive light from both sides of the bath as well as from reflection and scattering of light from other points around the culture tube. This situation is constant during each of subsequent experiments as long as the technique remains unchanged. Nevertheless, data obtained in different laboratories with different sources, different geometry of illumination, and different culture vessels may not be directly comparable. Second, as the culture grows, mutual shading of the cells reduces the actual amount of light received by a particular cell. It has been assumed that this factor does not influence growth as long as measurements indicate that the culture is in the logarithmic phase. Frequent transfers to fresh medium are required to prevent a reduction in growth rate. Deviations of curves from the exponential may also be due to transition phenomena taking place after transfer of cells from agar slants to liquid media. Therefore more than one transfer to fresh liquid media and a

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losses by respiration are just compensated by formative gains. For *Chlamydomonas* this compensation point for growth is above 60 ft-c; for other strains it lies at lower light intensities.

It can be concluded from table I that considered in terms of growth the maximum efficiency of utilization of incident energy, observed at the lowest light intensity, differed widely with various strains. It was more than twice as high in *C. pyrenoidosa* (van Niel) than in *Scenedesmus*. In *C. pyrenoidosa*, strain 7-11-05, it was higher at 39° than at 25° C.

A relatively high efficiency of utilization of incident energy at low light intensities is of great ecological importance. Because of this, an alga may be able to survive better in shaded or deep water. Efficiency must also be considered in predicting yields of mass cultures, since the density of algal populations often limits the light intensity received by the algal cells. It is obvious that any discussion on the utilization of light energy may be of little value unless the dependence of the "efficiency" of the species or strain on the environmental factors employed is clearly recognized.

Because of the gradual transition from the light-dependent to light-independent portion of an intensity curve, the lowest intensity which gives light saturation can only be approximated. For *Chlorella vulgaris* the data obtained in these experiments (table II) indicate that an increase in light intensity above 250 to 300 ft-c did not result in any increase in growth rate. For other strains at 25° C, the value was about 500 ft-c. This value is close to that given by Myers (6) and by Phillips and Myers (9) for the light-saturating intensity for the Emerson strain of *Chlorella pyrenoidosa*. Myers (6) estimated it to be in the neighborhood of 400 ft-c and Phillips and Myers (9), using different technique, found it at approximately 600 ft-c. It should be recognized, of course, that such determinations depend on the light source and the geometry of illumination. A characteristic feature of *Chlorella pyrenoidosa*, strain 7-11-05, is its higher requirement for light saturation at 39° C than at 25° C. Figure 2 shows that higher temperature will be of ad-

FIG. 1. The growth rates of four species of algae at 25° C measured at limiting, saturating, and inhibiting light intensities. The symbols are as follows: *Chlorella pyrenoidosa* (van Niel), circles; *Chlorella vulgaris*, crosses; *Scenedesmus obliquus*, triangles; and *Chlamydomonas reinhardtii*, squares. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light.

FIG. 2. The growth rates of *Chlorella pyrenoidosa*, strain 7-11-05, measured at limiting, saturating, and inhibiting light intensities. Circles plot rates at 39° C; triangles plot rates at 25° C. Open symbols show growth under fluorescent light; closed symbols show growth under incandescent light.

FIG. 3. The growth rates of five strains of green algae measured at limiting light intensities. Data are those from figures 1 and 2 replotted on a larger scale.

species. It appears that a decrease in the slope of the light intensity curve is a characteristic common to these algae and others that have been studied previously.

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## THE OXYGEN AFFINITY OF A FLAVIN OXIDASE INVOLVED IN THE RESPIRATION OF *STREPTOCOCCUS FAECALIS*<sup>1,2</sup>

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The characterization of the oxidases involved in cell respiration has been based largely on experiments with enzyme inhibitors. In addition, it is possible to characterize an oxidase by its affinity for oxygen, which may be determined by measuring the oxygen consumption as a function of oxygen concentration. This information is especially valuable in those cases where the tissue respiration is insensitive to such classical inhibitors as cyanide and carbon monoxide (see 1, 4, 6). Although it has often been assumed that this "insensitive" respiration is mediated by oxidases which are flavoproteins, recent studies (15, 17) have shown that these same tissues may exhibit a high oxygen affinity, much greater than that exhibited by most isolated flavin oxidases (12). This poses the following question: are there some other flavin oxidases with a relatively high oxygen affinity which could account for this type of respiration? To answer this, a study was made with *Streptococcus faecalis* (B33A) whose respiration is known to be mediated by a flavoprotein oxidase (2, 3). The great advantage of this particular organism is that it does not contain any cytochromes (13), which might complicate the interpretation of the results.

The culture methods employed were those described previously by Seeley and Vandemark (11). The cells were harvested in a centrifuge, resuspended in distilled water, and their respiratory rates deter-

mined, in the presence of glucose, by standard manometric techniques at 37.5° C (16). To test the effect of cyanide on respiration Robbie's Ca(CN)<sub>2</sub>—Ca(OH)<sub>2</sub> mixtures were added to the center well (10). Cell-free extracts were obtained by grinding freshly harvested cells with alumina (A-301, Alcoa) in phosphate buffer (0.05 M, pH 7). The slurry was centrifuged at 2,000×G for 30 minutes in the cold and the supernatant fraction, referred to as the enzyme solution, was used in the spectrophotometric assays. The oxidation of reduced diphosphopyridine nucleotide (DPNH) was followed at 340 m $\mu$  in a Beckmann DU spectrophotometer at room temperature, using cuvettes with a 1 cm light path. The effects of oxygen concentration were determined in vaccine-stoppered cuvettes which had been flushed 10 minutes with the appropriate gas mixtures. To obtain complete anaerobiosis, the special cell of Lazarow and Cooperstein was employed (8). Optical density units were converted to DPNH concentration by assuming a molecular extinction coefficient of 6.22×10<sup>6</sup> sq cm/mole (5). Protein was determined by the method of Stadtman et al (14).

In preliminary experiments, the well known fact that the respiration of *S. faecalis* is not inhibited by cyanide (9, 11) was confirmed using 0.001 M HCN. Following this, the effect of oxygen concentration on the oxygen consumption of whole cells was determined manometrically. The respiratory rates in vessels gassed with 5, 20 and 100% O<sub>2</sub> showed only slight differences, whether the vessels were gassed initially or after a period in air. The rate in 5% O<sub>2</sub> was not

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