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SOME FACTORS AFFECTING THE OXYGEN PRODUCTION BY ALGAE IN A SMALL CULTURE UNIT

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ABSTRACT

The performance of an algal culture unit having a 3/4-inch annular culture chamber has been evaluated in terms of its oxygen-producing capabilities. The oxygen production of cultures containing 0.5, 0.6, 0.7, and 1.0 percent of cells was determined at several light intensities. For a given light intensity, the rate of oxygen output was essentially the same for each cell density, indicating that all the cultures were in the linear phase of growth. The experimental results led to the conclusion that, for cultures in the linear phase of growth, the rate of oxygen evolution is a logarithmic function of light intensity.

At the highest light intensity used, 17,000 foot-candles, the rate of oxygen output was approximately 385 cc/hr/liter of suspension. Based on this figure, 65 liters of suspension and 53 kilowatts of power would be required to provide the oxygen needs of one man. However, at lower light intensities, power was utilized more efficiently for the production of oxygen. The optimum light intensity would depend, therefore, on whether space or power consumption was the more important consideration.

In these studies both urea and nitrate were used as nitrogen sources. Higher growth rates were obtained when urea was used, but more oxygen was produced by nitrate-grown cultures. In addition, the average photosynthetic quotient (O_2/CO_2) was significantly higher for nitrate cultures. These observations can be explained by assuming that the chlorophyll in the cells photochemically reduces the nitrate, releasing oxygen in the process (Hill reaction).

A preliminary study concerning the nutrient requirements of the cells has shown that the nitrogen source is the first culture nutrient to be depleted. This result was not unexpected considering the high requirement of nitrogen for the synthesis of cell material.

PROBLEM STATUS

This is an interim report; work is continuing on the problem.

AUTHORIZATION

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SOME FACTORS AFFECTING THE OXYGEN PRODUCTION BY ALGAE IN A SMALL CULTURE UNIT

INTRODUCTION

The development of a large scale algal system for the production of oxygen and the removal of carbon dioxide in nuclear submarines requires preliminary studies with laboratory pilot plants. Two such units of widely different designs have been built at this Laboratory and have been described in a previous report (1). As expected, the results obtained with these gas exchangers showed that under the conditions employed, the most important variable affecting the growth of the algae was the amount of light energy absorbed by the cell, and this was dependent to a large extent on the depth of suspension the light had to penetrate. The importance of the length of the light path was illustrated by a comparison of the oxygen productivities of the two units. In the first apparatus the suspension was contained in glass tubes about 1 centimeter in diameter, whereas the light in the second unit had to penetrate approximately 5 centimeters. The oxygen output of the first unit was about five times that of the second suggesting the oxygen production is inversely proportional to the thickness of the culture; this result is in line with the findings of Tamiya et al. (2). A third culture unit has been built based on the findings obtained with the previous ones. This unit is smaller and simpler in design than the first two, and is a better research tool for evaluating some of the factors associated with algal growth.

The primary purpose of this report was to evaluate this unit in terms of its oxygen-producing capability. The rates of oxygen evolution of cultures of different population densities have been determined at several light intensities. All the cultures were in the linear phase of growth where, under a given set of conditions, the increase in the mass of the cells with time is greatest, and the oxygen production, since it parallels growth, should also be at a maximum for the specified conditions.

Additional experiments have been conducted to show the influence of the type of nitrogen source in the nutrient medium on the oxygen yield. The oxidation level at which the algal cell finds the nitrogen atom is known to affect significantly both the growth rate and the gas exchanges occurring during growth. For example, higher growth rates occur with urea than with nitrate, whereas nitrate will raise the O_2/CO_2 ratio (photosynthetic quotient). It was of interest to determine which of these nitrogen sources gave the highest oxygen output in this unit. Ammonium salts can also be used as nitrogen sources by most algae, and are more easily assimilated than nitrate. However, an excess of these salts is toxic to some algae and may lower the pH of the medium enough to inhibit growth; consequently they were not considered in this study.

EXPERIMENTATION

Organism and Culture Medium

Chlorella pyrenoidosa, Sorokin strain 7-11-05, with an optimum temperature of 39° C was used in all these studies. Cell contentrations were estimated by measuring the turbidity in a Klett-Summerson photoelectric colorimeter and comparing the colorimeter reading with a standard curve of packed cell volume versus optical density. Except for

the work concerning the effect of nitrogen source, a modified form of Burk's medium* was used in which nitrate replaced urea so that an equivalent amount of nitrogen was present.

Culture Apparatus

A schematic diagram of the unit used in these studies is shown in Fig. 1. It may be divided into three main sections:

1. A cylindrical chamber containing the culture and light source, together with a centrifugal pump† to circulate the algal suspension.

2. A system for adding fresh nutrient medium to the culture at a constant rate.

3. A system for analyzing the inlet and vent gases for concentration of oxygen and carbon dioxide (not shown in Fig. 1).

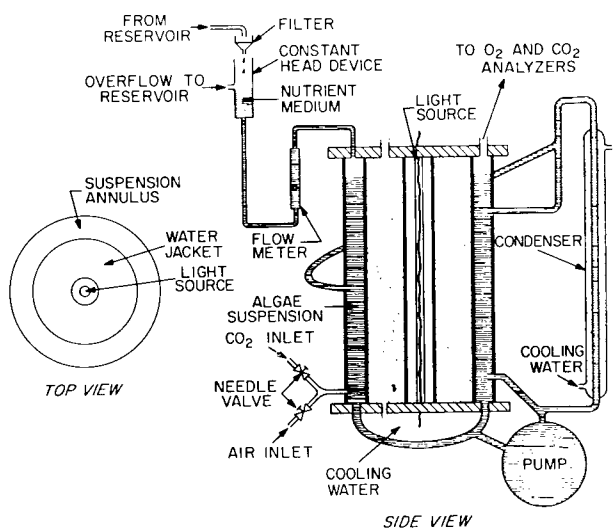

Fig. 1 - Schematic diagram of culture unit

Culture Chamber - The culture chamber with an outside diameter of 6 inches consists of three concentric Lucite tubes. The suspension is illuminated by a high intensity incandescent lamp‡ suspended in the center of the innermost tube. Heat generated by the lamp is largely dissipated by passing water through the 1.5-inch inner annulus surrounding the light source. Prior to the assembly of the whole unit, light intensities were measured at the outside of this water jacket with the use of a General Electric Exposure Meter. The outer annulus contains approximately 1600 milliliters of suspension and is 3/4 inch thick. An additional 175 milliliters of suspension is required to fill the pump and connecting tubing to give the unit a capacity of about 1775 milliliters.

* The composition of this medium may be found in Ref. 1.
† Eastern Industries, Pump Model D-11.
‡ General Electric Co., Model 1500 T3Q/CL.

A heat exchanger in the form of a condenser placed outside the culture chamber functions as a thermostat for the suspension. When the temperature reaches 38.5 °C, a thermoregulator opens a solenoid valve, allowing cooling water to flow through the condenser. This external cooling system lowers the oxygen output since approximately ten per cent of the cells are in comparative darkness at any given time.

Dilution System - In the study of the influence of light intensity on the rate of oxygen evolution (Fig. 2 only), the concentration of cells was kept constant by continuously diluting the culture with nutrient medium as fast as it grew. The medium was added at a constant rate, and this brought to light special problems. The medium has a tendency to form a fine precipitate upon standing, and, although this precipitate probably is not dense enough to cause a deficiency of any nutrient in the medium, the particles formed not only will contribute to the optical density to give erroneous concentration readings but will impede the flow of the medium through small constrictions such as a needle valve, resulting in varying flow rates. To eliminate this problem, a dilution system (shown in Fig. 1) was devised so that the medium could be filtered prior to entering the culture chamber and the flow could be manually controlled by adjusting the height of a constant-head reservoir. In this system the medium need not flow through a small orifice and any sediment formed will not interfere with the flow rate. In the continuous dilution experiments (Fig. 2) the flow rate of culture medium necessary to maintain a certain cell concentration was determined experimentally.

Gas Analysis - A mixture of approximately 2 percent carbon dioxide in air is admitted at the bottom of the culture annulus through a fritted glass filter to facilitate dispersion of the gas. After passing through the suspension, a portion of the gas is withdrawn by an aquarium pump, and sent through several drying columns into a paramagnetic oxygen analyzer (Beckman Model F-3) and an infrared carbon dioxide analyzer

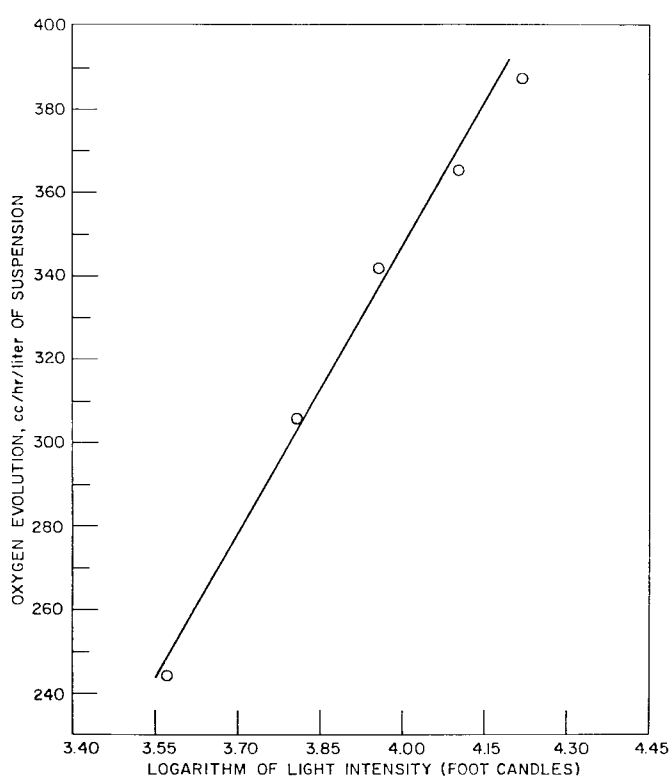

Fig. 2 - Variation of oxygen production with logarithm of light intensity

(Mine Safety Appliances Model 300). These analyzers are connected to a multipoint Brown recorder so that the concentrations of these gases are plotted continuously. The volume of gas passing through the suspension is measured on the outflow periodically with the use of a wet test meter. No correction is made for the water vapor in the gas. In this way the oxygen output, the carbon dioxide uptake, and the gas exchange ratio are easily determined. In this report the oxygen output of either a liter of suspension or a cubic centimeter of cells is given, depending on the purpose of the experiment. In all cases the gas volumes are reported for room temperature (22°C) and pressure (approximately 1 atmosphere).

Operation - In the operation of the culture unit, the suspension is withdrawn from the bottom of the algal chamber by a centrifugal pump and returned to the chamber through three tubes positioned at different heights in the outer wall. These inlet tubes are bent at right angles inside the annulus so that a rapid swirling motion is imparted to the suspension.

RESULTS AND DISCUSSION

Oxygen Production as a Function of Light Intensity

The oxygen yields of four cultures containing 0.5, 0.6, 0.7, and 1.0 percent of cells at five light intensities provided the data for Fig. 2. It is seen that a fairly straight line results when the rate of oxygen evolution is plotted against the logarithm of the light intensity. Within experimental error, the oxygen output at each light intensity was the same for each cell density, and the values shown represent the average value of the four densities. The oxygen production would be expected to be the same for each cell concentration if the cultures were in the linear phase of growth, i.e., when mutual shading by the cells is significant. In this case, the growth rate obeys zero-order kinetics, that is,

$$\frac{dN}{dt} = k_1 \tag{1}$$

where N is the number of cells, t is time, and k_1 is a constant. Since it is assumed that the rate of oxygen output is proportional to the growth rate,

$$\frac{dO_2}{dt} = k_2\frac{dN}{dt} = k_3 . \tag{2}$$

The rate of oxygen production should therefore be constant and independent of cell concentration for a given light intensity, as long as the culture remains in the linear phase of growth.

The deviation of the observed values from the line at the higher light intensities may be due to the solarization* of some of the cells at these intensities. Sorokin and Krauss (3) have estimated that the saturation intensity for this strain of algae is 1400 foot-candles, with the growth rate decreasing at light intensities above 3000 foot-candles.

The percentage of cells exposed to a light intensity of 3000 foot-candles or greater in this culture unit may be estimated by assuming that light intensity decreases with suspension depth according to Beer's law of light absorption:

$$I = I_0 e^{-\epsilon C \ell}$$

where I_0 is the incident light intensity, I is the light intensity at distance ℓ, C is the cell concentration, and ϵ is the extinction coefficient of the algal suspension. For a culture containing 1 percent of cells (the densest culture used in this study) and illuminated by 17,000 foot candles, the light intensity equals 3000 foot-candles at a suspension depth of

*Solarization occurs when excessive light intensity damages the cells, inhibiting the growth rate.

about 2.4 mm, if Beer's law is obeyed.* Approximately 9 percent of the total cells are found in this 2.4-mm annulus at any given time, so it is possible that a significant number of cells are receiving too much light, inhibiting their growth and lowering the overall growth rate of the culture. The solarization effect could theoretically be reduced by using denser cultures since according to Beer's law the distance that light of a given intensity I will penetrate is inversely proportional to the cell concentration C. Thus, in a dense culture, relatively fewer cells would be exposed to growth-inhibiting light than in more dilute suspensions. However, the culture should not be so dense that linear growth is impossible, for it is only in the linear phase that the oxygen yield is maximum for a given light intensity. The use of dense cultures should not raise the nutrient or carbon dioxide requirements, since the growth rate is independent of cell concentration for a culture in the linear phase.

Tamiya et al. (2) have predicted a logarithmic relation between growth and light intensity on theoretical grounds assuming that Beer's law is applicable. If a logarithmic relationship exists between oxygen production and light intensity, there is no saturation intensity for cultures in the linear phase of growth. Nevertheless, at very high light intensities, the growth rate would be expected to decrease due to solarization of some of the cells, and the optimum light intensity would have to be determined experimentally. The fact that there is no theoretical saturation point in intensity for cultures in the linear phase can be easily deduced from the rate expression describing growth of cultures in this stage of growth. The rate of oxygen evolution by a culture in the linear phase has already been given as

$$\frac{dO_2}{dt} = k_3 \,. \tag{2}$$

To obtain the rate of oxygen evolved by each of the N number of cells, both sides of the equation are divided by N,

$$\frac{1}{N}\frac{dO_2}{dt} = \frac{k_3}{N} \tag{3}$$

or,

$$N \times \frac{1}{N}\frac{dO_2}{dt} = k_3 \,. \tag{4}$$

These equations show that the oxygen production rate of each cell is a function of the number of cells and that the rate of oxygen evolved by the culture is the product of the number of cells and the oxygen output of each cell. If the culture were light-saturated, the oxygen output of each cell should be constant and independent of cell concentration, since the cells are presumably growing without limitation by light or nutrients. Equations (3) and (4) show that this constancy of the rate of oxygen output per cell is not possible for cultures in the linear phase of growth.

Although a culture in the linear phase of growth can, by definition, never be saturated with light, light saturation is required for exponential growth. In this case, first-order kinetics are obeyed, so that

$$\frac{dN}{dt} = k_4 N \,. \tag{5}$$

Since it is assumed that the rate of oxygen evolution is proportional to the growth rate,

$$\frac{dO_2}{dt} = k_5 \frac{dN}{dt} \,. \tag{6}$$

*For this calculation the extinction coefficient was taken as 0.41 liter/ml-cm, the average value estimated for Chlorella ellipsoidea by Tamiya et al. (2) in the wavelength range 434-729 millimicrons.

Substituting Eq. (5),

$$\frac{dO_2}{dt} = k_4 k_5 N = k_6 N \ . \tag{7}$$

Dividing by N,

$$\frac{1}{N}\frac{dO_2}{dt} = k_6 \ . \tag{8}$$

In the exponential phase of growth, the oxygen rate of each cell is seen to be constant and independent of the number of cells present. This condition can only exist if the culture is light-saturated so that mutual shading by the cells is negligible. Even though a cell evolves more oxygen during exponential growth than during linear growth, the oxygen output of a suspension in the linear phase is greater at a given light intensity. This is because at constant light intensity exponential growth necessarily precedes linear growth. At low cell concentrations, the culture is light-saturated permitting exponential growth at which stage the rate of oxygen output of each cell is at a maximum. As growth proceeds, a cell density will be reached when the cells begin to shade each other, and the culture will then enter the linear phase of growth. Under these conditions the rate of oxygen output per cell is less than maximum but, since a large number of cells are evolving oxygen, the overall rate of oxygen production is higher than that obtainable during exponential growth. In both phases, the total rate of oxygen production is determined by the product of the number of cells and the rate of oxygen output per cell; this product is always higher when the culture is in the linear phase since there are a greater number of cells present in this phase of growth as opposed to the number of cells that can exist in the exponential phase of growth. This statement implies from Eqs. (4) and (6) that

$$k_3 \text{ (Overall rate of oxygen production for linear phase)} > k_6 N \text{ (Overall rate of oxygen production for exponential phase)}$$

because of an upper limit imposed upon N when the culture is in the exponential phase of growth.

Estimate of Suspension Volume and Power Requirements for a One-Man Unit

In the design of a large scale algal system, the power requirements may have to be considered as well as oxygen productivity, and the brightest usable light probably would not be the most practicable. This is illustrated by the results shown in Table 1. The calculations were based on the foregoing experiments, and since the cultures were in the linear phase of growth, cell concentration would not be a factor in determining oxygen productivity.

Table 1
Oxygen Output and Power Requirements at Several Light Intensities

Light Intensity (foot-candles)	Oxygen Output (cc/hr)	Power Input (kilowatts)	Oxygen Output per kw (cc/hr)	Estimate of Requirements for One-Man Unit*	
				Suspension Volume (liters)	Power (kw)
3,700	426	0.58	734	103	34
6,400	535	0.75	714	82	35
9,000	598	0.88	679	73	37
12,500	641	1.20	534	69	47
17,000	672	1.42	473	65	53

*Based on a consumption of 25 liters of oxygen per hour.

It may be seen that the oxygen production per kilowatt decreases sharply with light intensity above 9000 foot-candles. For the hypothetical one-man unit, there is a relatively small increase in the power requirement up to 9000 foot-candles, whereas the suspension volume requirement decreases considerably. Above 9000 foot-candles, the large increase in power is not compensated by a corresponding decrease in the suspension volume requirement. The choice of the best light intensity would depend, therefore, on whether economy of space or power was the more important consideration.

Effect of Nitrogen Source on Oxygen Production

In this study a comparison was made of the oxygen output and growth rates of urea- and nitrate-grown cells. A control test was also made in which the medium contained no added nitrogen. The results are shown in Fig. 3.

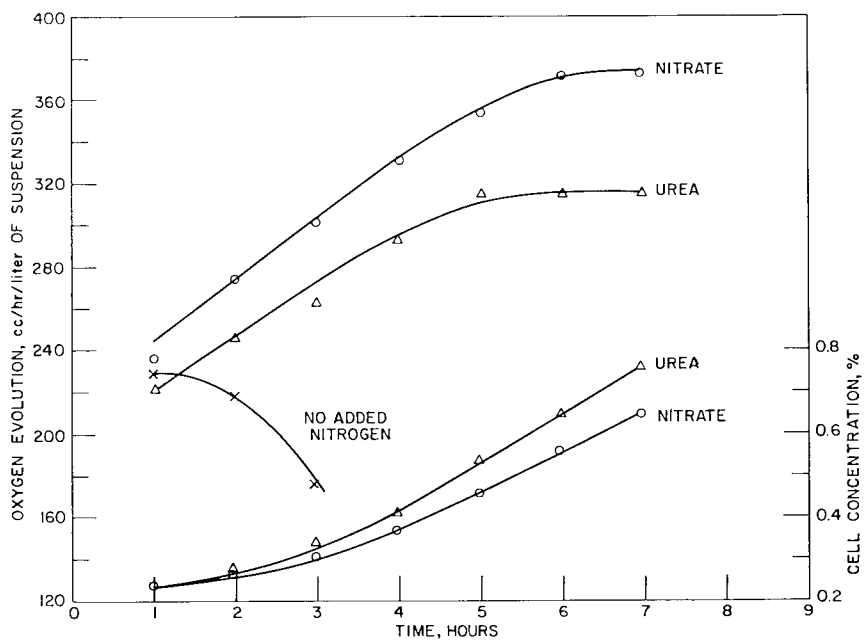

Fig. 3 - Effect of nitrogen source on oxygen production and growth rate at a light intensity of 12,500 foot-candles. The top three lines represent oxygen output (left ordinate); the lower two lines represent growth (right ordinate).

Although cells grown in the medium containing urea grew faster, almost 20 percent more oxygen was evolved when nitrate was present. The O_2/CO_2 ratio for the seven readings for nitrate-grown cells had an average value of 1.10; that for the urea-grown cells was 0.96. The rate of carbon dioxide consumed per unit volume of cells was the same whether urea or nitrate was used. This indicates that the extra amount of oxygen evolved by the nitrate culture is not directly associated with photosynthesis and that the overall metabolism of the cell is not changed by the nitrogen source. The most obvious explanation for these observations is that the nitrate must be reduced before it can be metabolized by the cell. Urea however is already at the reduction level required for cell reactions and as a result can be more readily assimilated, leading to faster growth.

Support for this conclusion has been obtained by adding urea to cells growing in the nitrate medium. There was an immediate drop in the O_2/CO_2 ratio, showing that urea is absorbed preferentially by the cells.

The higher oxygen yield and photosynthetic quotient observed with nitrate-grown cultures may be explained by assuming that growing algae can photochemically reduce the nitrate, releasing oxygen in the process; i.e., nitrate can substitute for carbon dioxide in photosynthesis:

$$HNO_3 + H_2O \xrightarrow{\text{light}} NH_3 + 2O_2.$$

This is not simply a theoretical reaction, for it is well known that algae can produce oxygen in the absence of carbon dioxide if nitrate is present, the so-called Hill reaction (4). This reaction would account for the fact that higher O_2/CO_2 ratios are observed with nitrate than with urea and that similar carbon dioxide absorption rates occurred regardless of which nitrogen source was used.

It is noteworthy that each oxygen production rate reaches its maximum value at a cell concentration of about 0.55 percent. At this point the cultures must be entering the linear phase of growth. The production of oxygen by the control culture at the beginning of the experiment was probably due to the assimilation of nitrogen already present in the cell.

Factors Limiting Oxygen Production

Light Limitation - To confirm and extend earlier findings, parallel experiments were made with dilute suspensions at two light intensities using both nitrate and urea as nitrogen sources. The data obtained are shown in Fig. 4 in which the rate of oxygen evolved per cubic centimeter of cells is plotted against cell density.

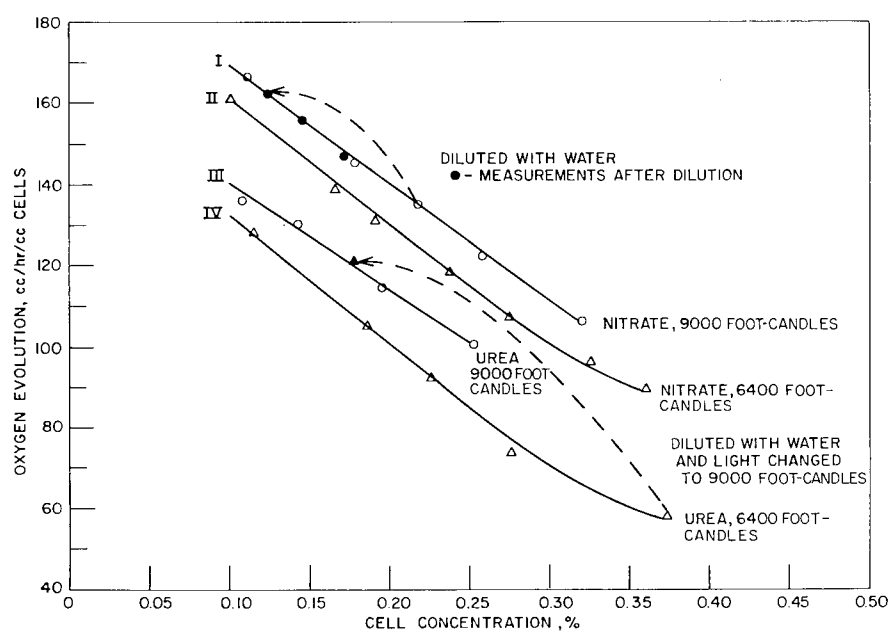

Fig. 4 - Oxygen output of cells as a function of population density

As expected, more oxygen was produced by the culture containing nitrate at both light intensities. It may be seen that the oxygen evolution per unit volume cells apparently decreases linearly with cell concentration in the density range of 0.1 to about 0.3 percent in every case. Two reasons may be offered for this decrease in the activity of the cells. As the culture becomes more dense, the cells shade each other, thereby reducing the light energy received by each cell. Alternatively it is conceivable that there is a linear depletion of an essential ingredient in the nutrient medium causing a lowering of the oxygen output, since there was no dilution with the medium during this experiment. The first alternative appeared most likely, since runs at the same light intensity (I and III, II and IV) gave lines of very nearly the same slope. More direct evidence was provided by repeating run I and diluting the growing culture with water in a batch addition until the cell density was halved, as shown in Fig. 4. If the decrease in oxygen production were due to a nutrient deficiency, a drop in the oxygen output of the cells would be expected as the culture grew. However, the graph shows that the oxygen output was higher after the dilution, and subsequent values continued to fall on the original line. This strongly suggests that a light limitation accounts for the progressively smaller oxygen output as the culture became more dense. Supporting evidence was obtained by diluting another culture (line IV) with water and increasing the light intensity at the same time. The rate of oxygen output was raised to the level expected at that light intensity and density (line III).

According to Eq. (3), for a culture in the linear phase of growth, a plot of the rate of oxygen output per unit volume of cells versus cell density should give a rectangular hyperbola. On the other hand, for a culture in the exponential phase, the rate of oxygen evolution per unit volume of cells should be constant and independent of cell concentration according to Eq. (8). Since neither Eq. (3) nor Eq. (8) was found to hold in the cultures tested, the cultures must be in the transition stage between the two phases.

Nutrient Limitation - The foregoing series of tests have shown that the limiting factor in the growth of the cultures tested was light absorption and not a deficiency in the nutrient medium. The oxygen production curves therefore represent base lines, and any lower departure from these curves is indicative of secondary changes, e.g., a depletion of an essential component in the medium. Advantage has been taken of this fact in the study of the nutritional requirement of the cells.

Preliminary work has been directed toward determining which component in the medium becomes depleted first. In these experiments, the cultures were not diluted continuously with fresh medium, and the cultures were grown under the conditions used in run I, Fig. 4; the curve for this run represents a base line for the comparisons shown in Fig. 5. The bottom curve of Fig. 5 represents the results obtained when all of the components were reduced to one-fifth the normal value. This caused an abrupt drop from the control line, indicating at least one nutrient was not present in sufficient quantity to sustain normal growth. In view of the fact that algae assimilate relatively large amounts of nitrogen in their metabolism, nitrate might be expected to become deficient before any other nutrient. In the next run nitrate was raised to its normal concentration while the other components were kept at one-fifth the normal concentration. The oxygen output of the cells grown in this medium was found to be equal to that of cells grown in the complete medium, matching the base line curve. It is evident that whenever the culture suffers from a nutrient lack, nitrogen becomes deficient before any other nutrient. Corroborative evidence has been obtained in another run in which the nitrogen level was 0.4 of the normal concentration and the other components were kept at the same reduced concentrations. The oxygen yield was approximately that expected if a deficiency of nitrate was limiting growth. By reducing the concentration of other components in the medium one at a time, further information on the nutritional requirements should be obtained, and perhaps the limiting concentration of each nutrient could be determined. Moreover the scheme may be useful in screening or evaluating substances which may have a beneficial effect on growth.

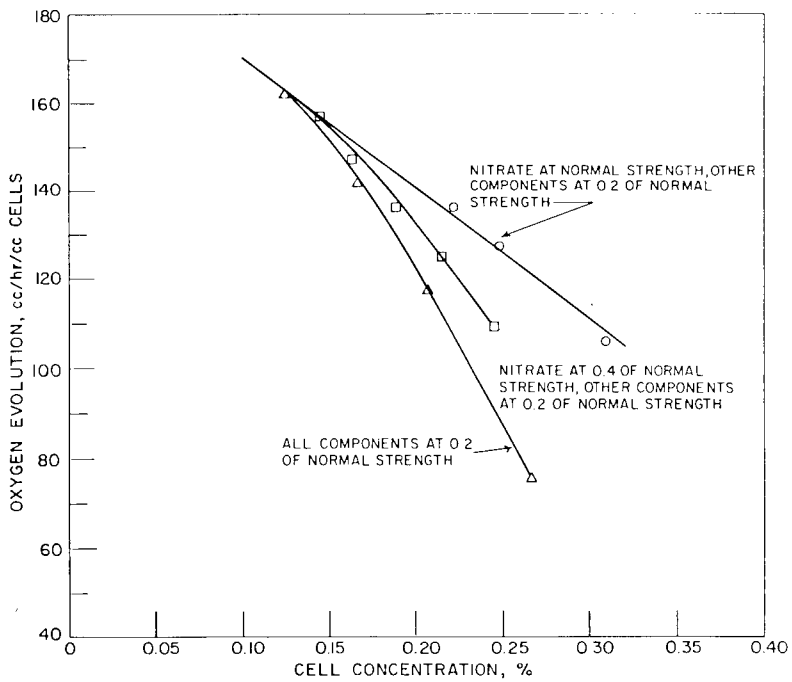

Fig. 5 - Effect of nitrate concentration on oxygen output of cells. Top curve (O) represents normal oxygen output as determined by previous experiments (Curve I, Fig. 4).

FUTURE PLANS

An objective of future work is to raise the oxygen productivity per unit volume of suspension by more efficient utilization of light by the culture. Undoubtedly the most effective way to accomplish this is to reduce the length of the light path, for, as pointed out earlier, evidence has been obtained here and elsewhere that growth rate is inversely proportional to the thickness of the culture. A culture unit similar to the one described in this report has been built in this laboratory to determine the effect of culture thickness on oxygen productivity. Although shorter light paths may markedly raise the oxygen yield, the design of a large gas exchanger in which the lights are placed so that the light has to penetrate only a short distance may pose a formidable engineering problem, and a compromise would have to be reached in the choice of the most practicable culture thickness.

Another approach seems to be more vigorous stirring of the suspension, although this would probably be less effective than changing the depth of the culture. This should permit the use of denser cultures and stronger light since a cell would be exposed to short flashes of intense light followed by a period of darkness when the cell could utilize the light in the latter stages of photosynthesis. This conclusion has been borne out by test-tube studies at this laboratory for the growth of dense cultures (4 percent) was increased two- to threefold by rapid mechanical stirring (1). It remains to be seen, however, whether increased stirring can appreciably improve the oxygen yield obtainable with thinner suspensions when more light is available to the cells.

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SUMMARY

1. The oxygen production of cultures in the linear phase of growth has been found to be a logarithmic function of light intensity. At the highest light intensity tested, 17,000 foot-candles, the oxygen output was approximately 385 cubic centimeters per hour per liter of suspension. However, a significant number of cells may be solarized at this intensity, and an oxygen rate of 370 cubic centimeters per hour per liter of suspension may be a more realistic value.

2. The oxygen production of nitrate-grown cells was almost 20 percent higher than that of urea-grown cells. The reduction of the nitrate by the algae in the presence of light probably accounts for the extra amount of oxygen evolved.

3. The rate of oxygen evolved per unit volume of cells was found to decrease linearly with cell concentration in the density range of 0.1 to 0.3 percent. The results indicated that a decrease in light energy received by each cell was responsible for the decrease in oxygen yield.

4. Preliminary experiments concerning the nutritional requirements of the cells have shown that the nitrogen source is the first nutrient to become deficient in the medium.

ACKNOWLEDGMENT

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