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# HIGHLY CONCENTRATED CARBON DIOXIDE AS A CARBON SOURCE FOR CONTINUOUS ALGAE CULTURES

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## INTRODUCTION

The use of concentrated carbon dioxide as the carbon source for algae in a photosynthetic gas exchanger was first suggested by Bowman in the Journal of the British Interplanetary Society in 1953 (ref. 1). He did not, however, indicate how this was to be done. Several authors (refs. 2, 7, 8) have reported that concentrations of carbon dioxide much in excess of 5-10 percent are toxic to algae. It is important from an engineering point of view to use much higher concentrations in the development of a practical gas exchanger in order to reduce the area required for gas exchange. The purpose of this paper is to present some of the results of research into this problem.

The problem is best illustrated quantitatively by calculations based on an expression for the absorption of gas by a liquid with or without chemical reaction in the liquid phase (ref. 9).

$$K_g = \frac{N}{A \triangle y} \qquad (1)$$

Where

$K_g$ = overall absorption coefficient expressed in terms of gas phase compositions, lb moles/hr-ft$^2$-unit$\triangle y$

$N$ = absorption rate, lb moles/hr

$A$ = interfacial area, ft$^2$

$\triangle y$ = driving force in terms of gas phase compositions



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When a carbon dioxide consumption rate of 301 liters per day (corresponding to the production from one man) is used to define N and an empirically determined value of $1.00 \times 10^{-4}$ lb moles of carbon dioxide per hour-foot$^2$-$\Delta y$ is used for $K_g$, the interfacial area may be calculated to be 4500 ft$^2$ for a culture receiving carbon dioxide as one percent in air. This large area would be prohibitive for a practical photosynthetic gas exchanger. At the other end of the scale, the area required if pure carbon dioxide were used is 33 ft$^2$, a much more attractive figure. From this consideration alone, it is clear that the use of high concentrations of carbon dioxide may be expected to yield a significant reduction in the weight and volume required for a man-sized photosynthetic gas exchanger, and simplification of growth control as well.

## CULTURE SYSTEM AND METHODS

Realization of balance in carbon dioxide uptake and total growth of the algae is the essential experimental objective. This may be accomplished by employment of a chemostat in which constant dilution is maintained at a rate somewhat less than the maximum possible growth rate of the algae. The cells may grow at a rate greater or less than the dilution rate and increase or decrease in concentration until the point of limitation by the growth factor in least supply is attained (ref. 4). At this point, growth rate becomes equal to the dilution rate and cell concentration remains constant. A condition of constant growth rate and cell concentration is made to order for constant carbon dioxide addition at a rate corresponding to its utilization.

An important detail is that balance of these factors is easiest to effect when some factor other than carbon dioxide is limiting. This allows variation of the carbon dioxide addition rate from that resulting in carbon dioxide excess to a rate corresponding with carbon dioxide deficiency.

When carbon dioxide dissolves in water, pH falls to a point governed by the partial pressure of the gas, the concentration of cation available for combination with carbonate or bicarbonate ions, and equilibria between molecular and ionic species in solution. The data in Table 1 are illustrative of these relationships. If carbon dioxide is removed at a rate faster than that of its addition, pH will rise, and if the gas is removed and added at the same rate, pH will remain constant.

Differential anion and cation uptake or deliberate inclusion of bicarbonate salts in the diluting solution might dampen the effects of aberrations in the balance of growth and carbon dioxide supply. For example, in a continuous culture supplied with potassium nitrate, preferential uptake of nitrate ion by the algae should result in a constant excess of potassium ions. This should effectively increase the carbonate and bicarbonate concentration in the growth solution, and raise the minimum pH attainable with partial pressures of carbon dioxide equal to or less than 760 mm Hg. The data in Table 1 would apply if all of the potassium in double and quadruple strength Krauss and Thomas medium (ref. 6) were available for formation of potassium bicarbonate.

Carbon dioxide deficiency would, of course, result in a decline in cell concentration. However, if excess $K^+$ were present, the resulting increase in pH might be detrimental in itself, and by effects such as minor element precipitation.



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Table 1

Influence of $CO_2$ Partial Pressure and Potassium Bicarbonate Concentration on Minimum Theoretically Obtainable pH at 37°C. Computed According to Ref. 10.

| $CO_2$ Partial Pressure, mm Hg | No. $KHCO_3$ | Solution pH 0.023 M $KHCO_3$ | 0.046 M $KHCO_3$ |
|---|---|---|---|
| 760 | 4.0 | 6.3 | 6.6 |
| 570 | 4.05 | 6.4 | 6.75 |
| 380 | 4.1 | 6.6 | 6.9 |
| 190 | 4.25 | 6.85 | 7.2 |
| 76 | 4.5 | 7.3 | 7.6 |



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The manner in which these principles have been reduced to practice is best explained by reviewing the culture system shown diagrammatically in Figure 1. The configuration consists of two basic components, the culture vessel and the external circuit. More than 90 percent of the volume of the system is contained in the culture vessel surrounding the light source. Instruments actuating unit processes which contribute to or regulate continuous growth in the vessel are located in the external circuit. Three of these unit processes, culture recirculation, culture dilution, and gas addition, are of primary importance.

Culture recirculation helps to bring about a functional separation of the gas stream from heat exchange and also to equalize irradiance. It also results in the independence of liquid composition from its position within the culture system, and in the equilibrium of the liquid phase with both influent and effluent gas phases. In the configuration considered here, the recirculating stream enters the top of the culture vessel and proceeds helically downward countercurrent to the direction of the flow of gas. Mixing and recirculation rates in this device were one minute and eighteen seconds, respectively.

The culture was continuously diluted with double strength Krauss and Thomas nutrient solution (ref. 6) using a Beckman solution metering pump with a 0-10 ml/min capacity. Under steady-state conditions growth rate (k) and dilution rate (the ratio of flow rate, f to culture volume, v) are equal. The resulting relationship,

$$f = kv\ (.693) \qquad (2)$$

where k in $\log_2$ units is the number of doublings per day, has proven useful for predicting nutrient and gas feed rates.

The culture vessel itself was an annular glass cylinder containing a single General Electric 150 watt T-10 fluorescent lamp of 1.25 inches diameter. The culture chamber surrounds the lamp at a distance of 4 mm, and averages 3 mm in thickness over 55 inches of its total 68 inch length. The remainder of the total length was occupied by a bellows seal and terminal expansions for glass joints, which initiated and terminated the external circuit. An adapter for the uppermost joint contained a common outlet for effluent gas and culture, as illustrated in Figure 1.

Pilot studies showed that this culture system was capable of producing six grams of dry algae per day, of which about half were assumed to be carbon. The corresponding pure carbon dioxide addition rate of 3.3 ml/min was in the lowest part of the accurate range of the most sensitive commercially available flowmeter. It is interesting to note that this flow rate is roughly one-fifth that which might be predicted from the power required by the lamp (ref. 5).

Evaluation and control of the performance of the culture system was largely dependent on the results of gas analyses and on determinations of dry weight. Therefore, these methods deserve attention in some detail.

The gases used for the experiments which yielded almost all of the data quoted in this paper were pure carbon dioxide and two artificial mixtures



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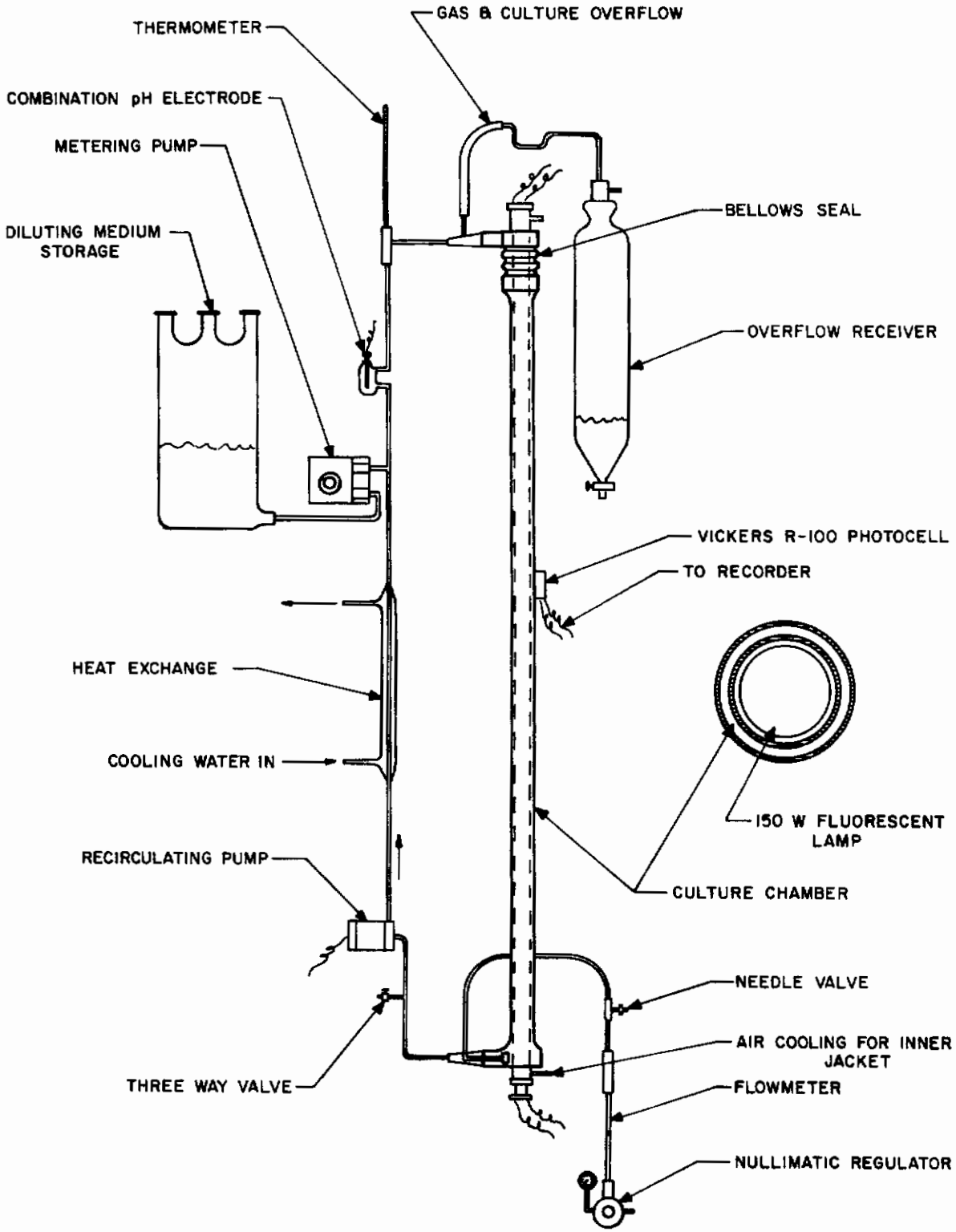


FIGURE 1: RECIRCULATING ALGAE SYSTEM



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containing 71 and 41 percent carbon dioxide in nitrogen. The exact compositions of these gases, determined by gas chromatography, were (1) highly concentrated or pure $CO_2$: 98.68% $CO_2$, with the remainder air ($\sigma$ = 0.098), (2) 71.45% $CO_2$ and 28.54% $N_2$ ($\sigma$ = 0.45), and (3) 41.15% $CO_2$ and 58.84% $N_2$ ($\sigma$ = 1.80).

Samples of effluent gas taken by piercing the pressure hose between the culture outlet and the gas trap (Figure 1) were analyzed for carbon dioxide, nitrogen, and oxygen. During the experiment with pure carbon dioxide, the nitrogen concentration in the effluent gas varied from almost none to 2 percent, or about the same percentage as was observed in the pure gas added to the culture. This indicates that gas could only have leaked out of, and not into the culture system, and that air contamination occurred during sampling or analysis.

Gas addition rates were determined by comparison of flowmeter readings with a calibration curve. Values between 6 to 7 ml per minute were only approximate because of the changing slope of the curve. The lowest flow rate which could be determined with any assurance of accuracy was 4 ml/min.

Dry weight determinations were performed on 25 ml aliquots according to Gerloff, et al (ref. 3). Flocculation with $Al_2(SO_4)_3$ was unnecessary. The data cited in Tables and Figures are means for three separate determinations per sample.

No attempt was made to exclude bacterial contaminants in any of the experiments. The qualitative composition of the bacterial flora, and the proportions of the various species remained fairly constant throughout. These results will be reported separately.

## EXPERIMENTAL RESULTS

Two experiments will be discussed in detail to demonstrate the importance of achieving balance in total growth and carbon dioxide addition rates. One of these experiments was a 28-day run with pure carbon dioxide. Seventy-one percent carbon dioxide in nitrogen served as the carbon source for the other.

Figures 2 and 3 contain basic productivity data for these experiments, which, in general, establish the practicality of administering highly-concentrated carbon dioxide to continuous algae cultures supplied with nitrate as the nitrogen source. These and additional results obtained during four specific intervals within these experiments substantiate the balance concept.

Interval A, Figures 2 and 4, was a six day period of constancy both of arbitrarily variable factors such as dilution and gas addition rates, and of growth dependent factors such as total algae produced, effluent gas composition, and pH. The gas analysis data signifies not only the continuity of the conversion of carbon dioxide to oxygen, but also its completeness. Production of a gas containing more than 96 percent oxygen from pure carbon dioxide is a rather significant result in itself.



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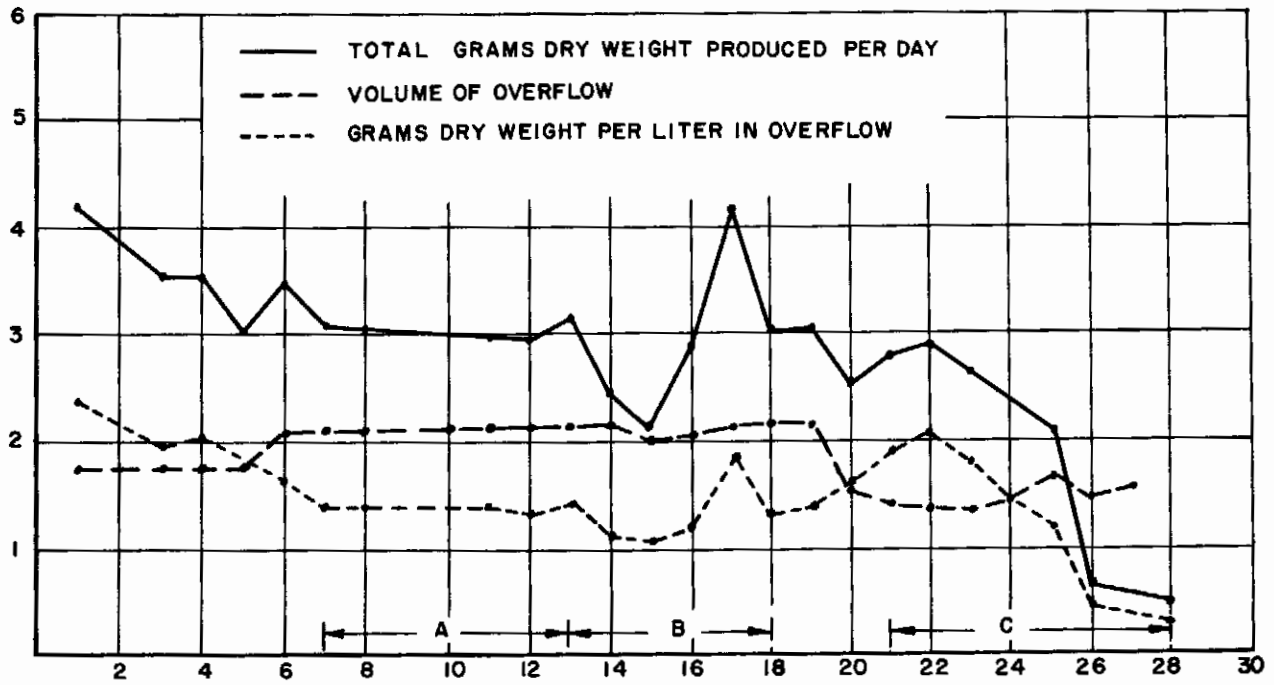


CULTURE VOLUME 1220 ml.

PERIODS A, B AND C ARE TREATED IN GREATER DETAIL IN THE TEXT, AND IN FIGURES 4, 5 AND 6

FIGURE 2: PRODUCTIVITY OF A CHLORELLA CHEMOSTAT ADMINISTERED PURE CARBON DIOXIDE



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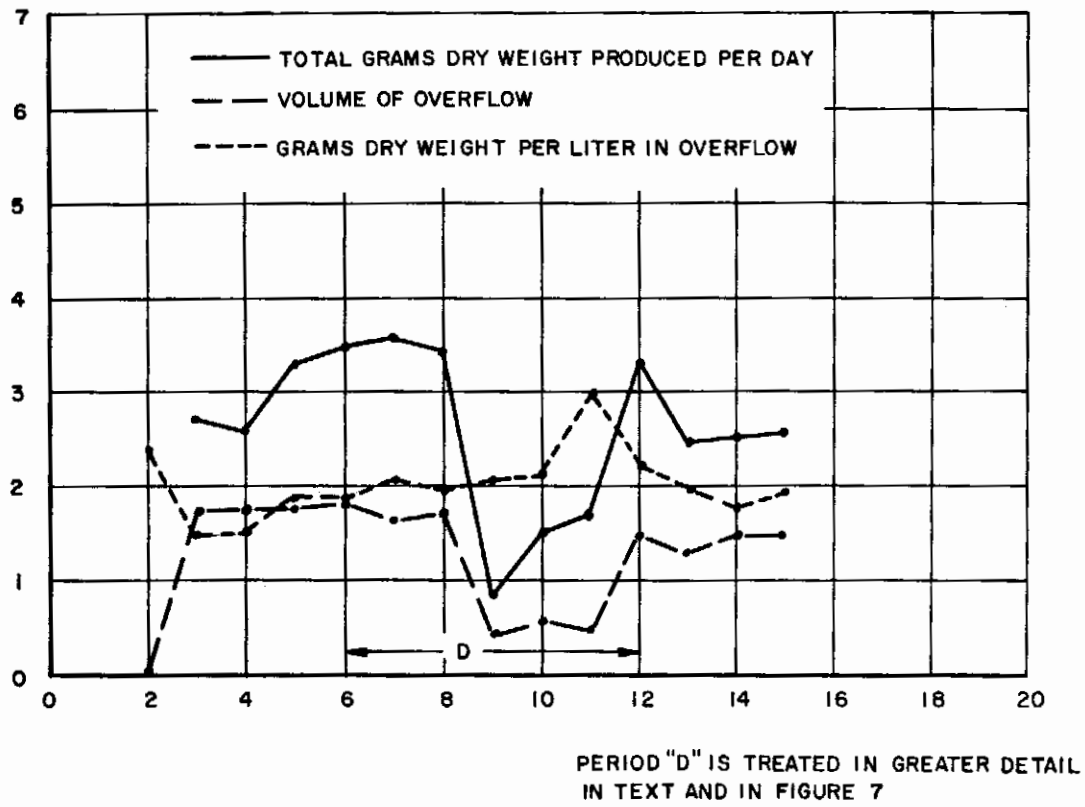


FIGURE 3: PRODUCTIVITY OF A CHLORELLA CHEMOSTAT ADMINISTERED 71% CARBON DIOXIDE IN NITROGEN



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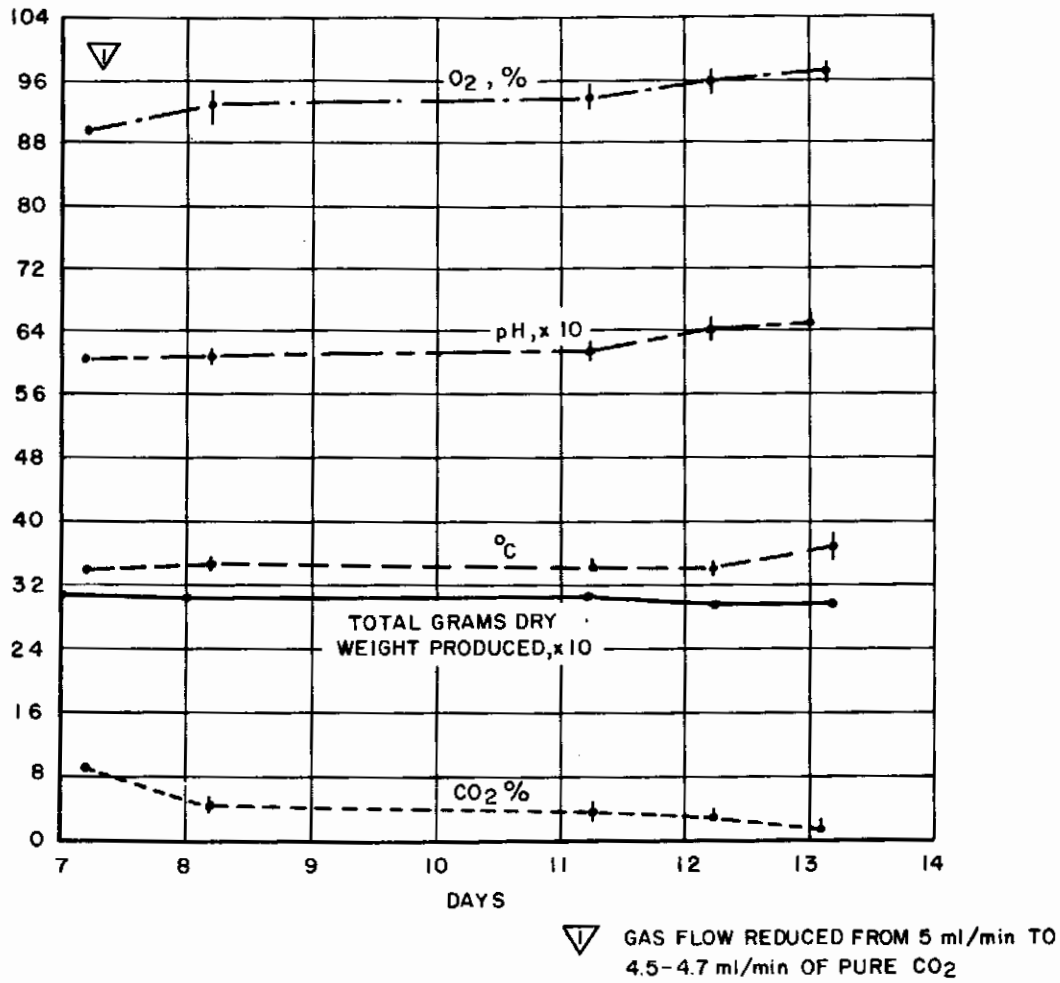


FIGURE 4: EFFLUENT GAS COMPOSITION AND pH UNDER CONDITIONS OF CONSTANT PRODUCTIVITY



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The maximum and minimum carbon dioxide addition rates (corrected for 5% carbon dioxide in the effluent gas) and rates of dry weight production during this interval have been used to compute the culture volumes necessary for removal of the 301 liters of carbon dioxide produced per day by one human. The results of these computations, which are summarized in Table 2, are in surprising disagreement and require an accounting for 2.8 liters of carbon dioxide per day. If this much carbon dioxide were lost as 0.056 M bicarbonate, more potassium ion would have been required to preserve electrical neutrality than was supplied. Also, the pH of a 0.056 M $KHCO_3$ solution in equilibrium with carbon dioxide at a partial pressure of approximately 380 mm Hg would have been in excess of 6.9, although the pH range actually found was 6.2 to 6.4. Roughly one-sixth of the excess carbon dioxide could be accounted for as bicarbonate, the remainder must have escaped through leaks. However, it is significant that the potassium ion in 0.01 M $KHCO_3$ represented approximately one-half of that added as potassium nitrate and phosphate in the diluting solution. This indicates the extent of differential absorption of the nitrate and phosphate anions.

On the afternoon of the twelfth day after inoculation, the temperature of the culture was increased to 39° from 37°, where it was allowed to remain until the morning of the following day. This action initiated the series of events which is illustrated in Interval B of Figure 2, and in more detail in Figure 5.

Increases in the carbon dioxide content in the effluent gas and corresponding decreases in oxygen production were noted on the day following the increase in temperature. These continued until on the morning of the third day after inoculation, a concentration of 32% carbon dioxide was recorded. The apparent lack of an immediate correlation between the decline in photosynthetic rate and dry weight produced was probably a result of disparity in the time intervals represented by the samples. The overflow sample contained algae produced immediately after the temperature increase when an accelerated growth rate may have been in effect. The effluent gas samples were taken after 8:00 a.m. the following morning when growth rate of the algae had decreased.

The action taken to correct the effects of excessive carbon dioxide was merely to turn off the gas flow for three and one-half hours. The effectiveness of this measure was apparent from the rapid increase in the oxygen content in the effluent gas. One-half hour after resumption of carbon dioxide addition, the effluent gas contained 97.8 percent oxygen. Subsequently, growth recovered to a level first exceeding then equalling that achieved during the steady-state of Interval A.

During the last six days of the experiment (Interval C) progressive accumulation of carbon dioxide was allowed to proceed without correction. The result was the end of the culture as an effective gas exchange unit (Figure 6). Wall growth, which became noticeable after the carbon dioxide content of the effluent gas reached 18 percent, increased to an overwhelming extent two days later and was an important factor in the decision to terminate the experiment.



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Table 2

Culture Volumes Required for Removal of 301 Liters of Carbon Dioxide per Day Computed From $CO_2$ Addition Rates and Culture Productivity Data

| | From Pure $CO_2$ Addition Rates (Corrected for Effluent Gas Composition) | | From Productivity Data | |
|---|---|---|---|---|
| | Addition Rate liters/day | Liters of Culture Required | gm dry Weight/Day | Liters of Culture Required |
| Minimum | 6.45 | 59 | 3.12 | 126 |
| Maximum | 6.45 | 57 | 2.97 | 132 |



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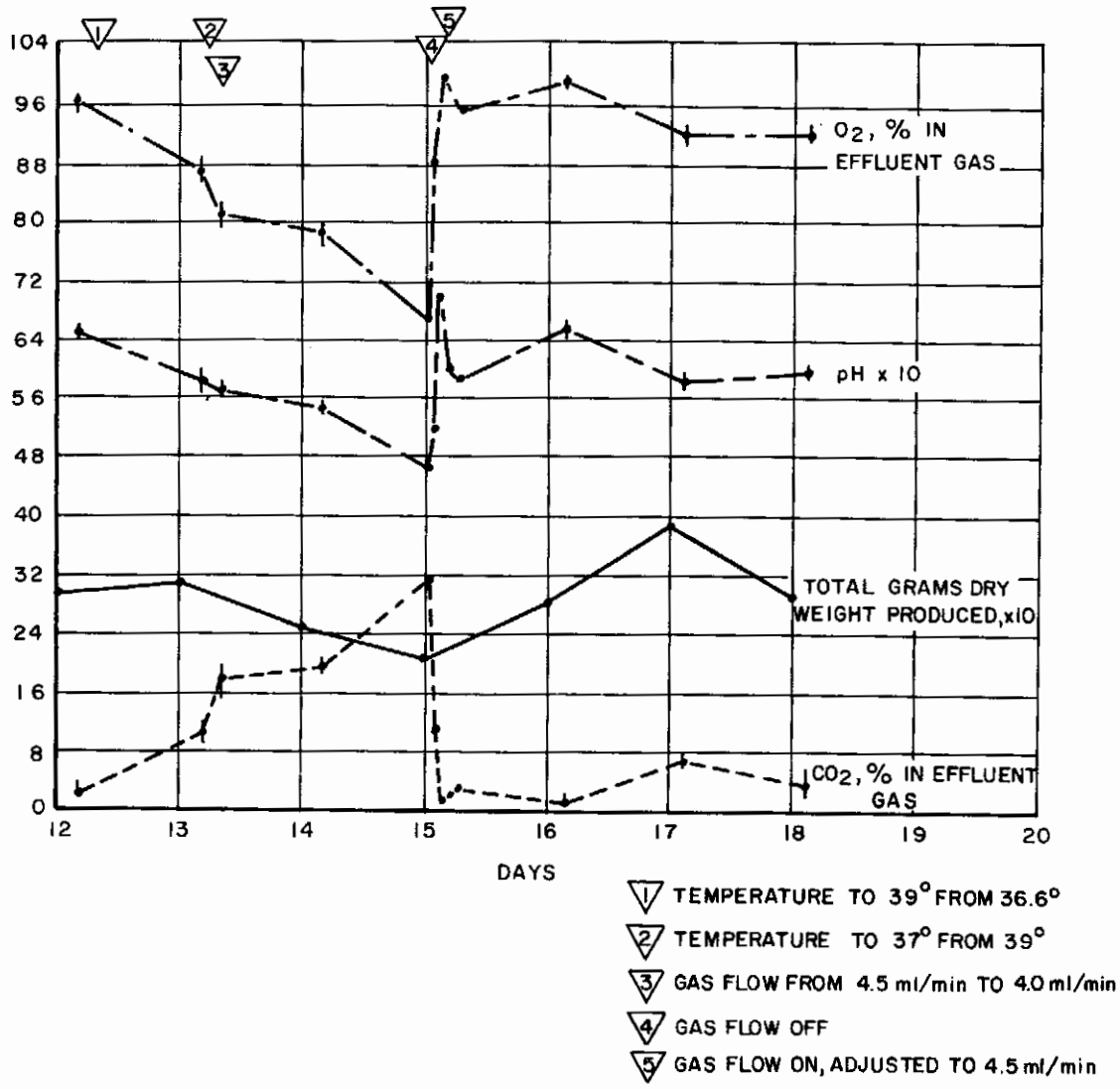


FIGURE 5: CORRECTION OF THE EFFECTS OF EXCESSIVE PURE CARBON DIOXIDE ADDITION RATES WITHOUT ALTERATION OF THE CULTURE DILUTION RATE



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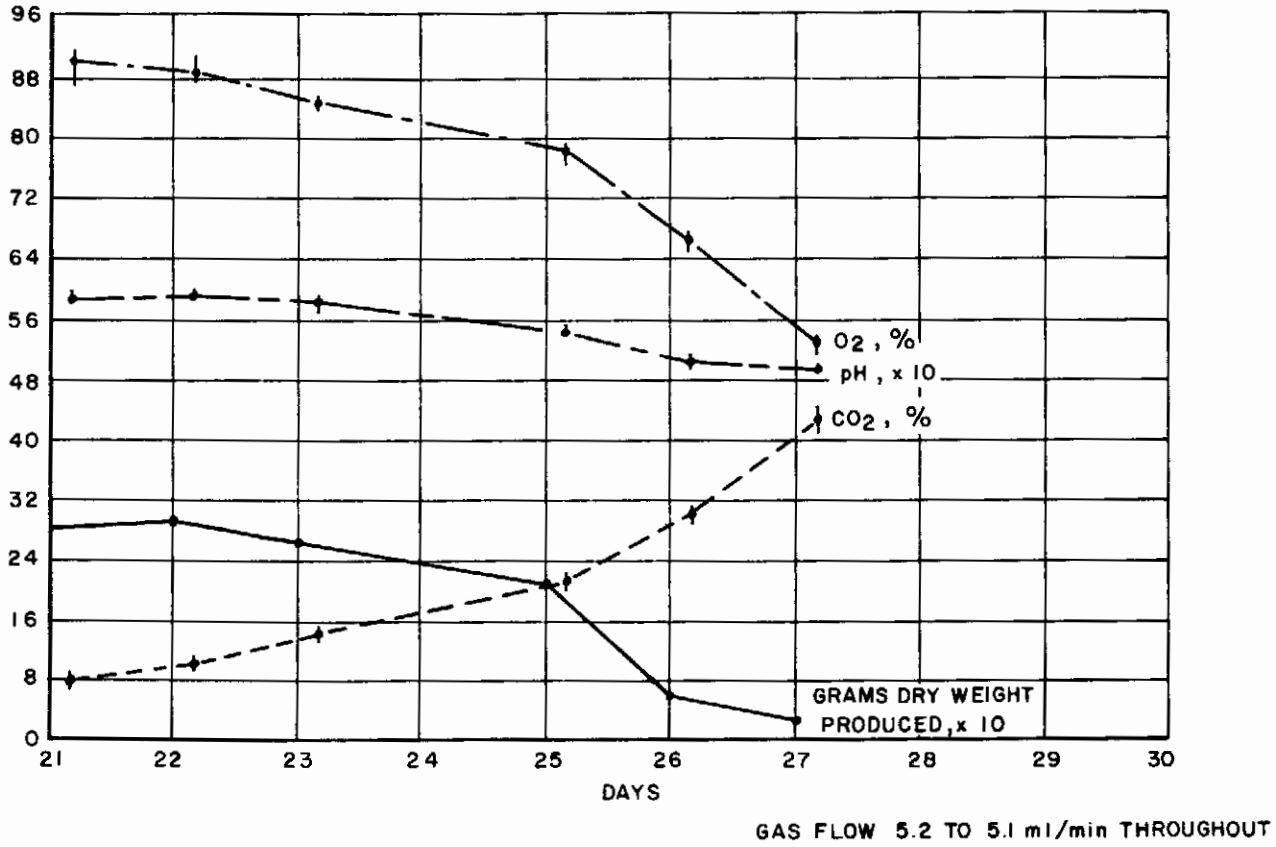


FIGURE 6: EFFECTS OF UNCORRECTED IMBALANCE IN RATES OF PURE CARBON DIOXIDE ADDITION AND CELL PRODUCTION



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It seems noteworthy that pH did not vary in direct inverse proportion either to the changes in partial pressure in the effluent gas or in the gas phase in the culture itself. This mitigates against control of carbon dioxide addition rates by direct coupling to changes in pH.

The expanded treatment of events occurring during Interval D, Figure 3, which is illustrated in Figure 7, shows how control of both gas addition and culture dilution rates is essential. During this interval of the experiment with 71 percent carbon dioxide in nitrogen, a decline in total growth followed a reduction in the dilution rate from the equivalent of an 11 hour generation time to that corresponding with a generation time of 48 hours. Just prior to alteration of the dilution rate, the gas addition rate was reduced in anticipation of lessening culture productivity.

After a new gas exchange equilibrium had been established, the carbon dioxide flow was increased to approximately the original rate to test the balance concept. Within 16 hours the effluent gas carbon dioxide content had increased to 32.5 percent. Again, restoration of gas exchange efficiency was effected by lessening the carbon dioxide addition rate. Recovery of the initial rate of cell production followed resumption of dilution at a rate corresponding to a generation time of 13.5 hours.

The rates of productivity and culture volumes in effect during periods of steady-state growth have been used to compute the culture volumes necessary for support of one human. These data appear in Table 3. The dry weight results represent days 7, 8, 11, 12, and 13 of the experiment with pure carbon dioxide (Figure 2); days 3, 4, 5, 6, 7, and 8 of the continuous culture receiving 71 percent carbon dioxide in nitrogen, and days 2, 5, and 6 of a similar experiment with 41 percent carbon dioxide in nitrogen.

The culture volumes derived from these results are all of the same order of magnitude. The tendency toward decreased volumes which follows decreased percentages of carbon dioxide probably represents the contribution of a number of factors, for example, oxygen toxicity, increased light available with decreasing liquid volume and increased gas ullage, and unforeseen factors such as nutrient limitation or excess which could easily accompany the variation in dilution rate from one culture to another.

The results quoted so far have been derived from experiments in which an almost identical recirculation rate was maintained throughout. Comparison of these data with those obtained with other recirculating pumps implies that productivity may be dependent on the design of this equipment and on the recirculation rate which it effects.

For example, when a March centrifugal pump was substituted for a Gelber pump, and with a regimen of pure carbon dioxide, 4.10 grams ($\sigma$ = 1.30) dry weight were produced per day. The March pump has an impeller of greater diameter than the Gelber. With a Sigma pump and 50 percent carbon dioxide in nitrogen, 4.8 grams of dry algae were produced per day. The recirculation rates brought about by the centrifugal pumps were approximately two times faster than that accomplished by the digital pump.



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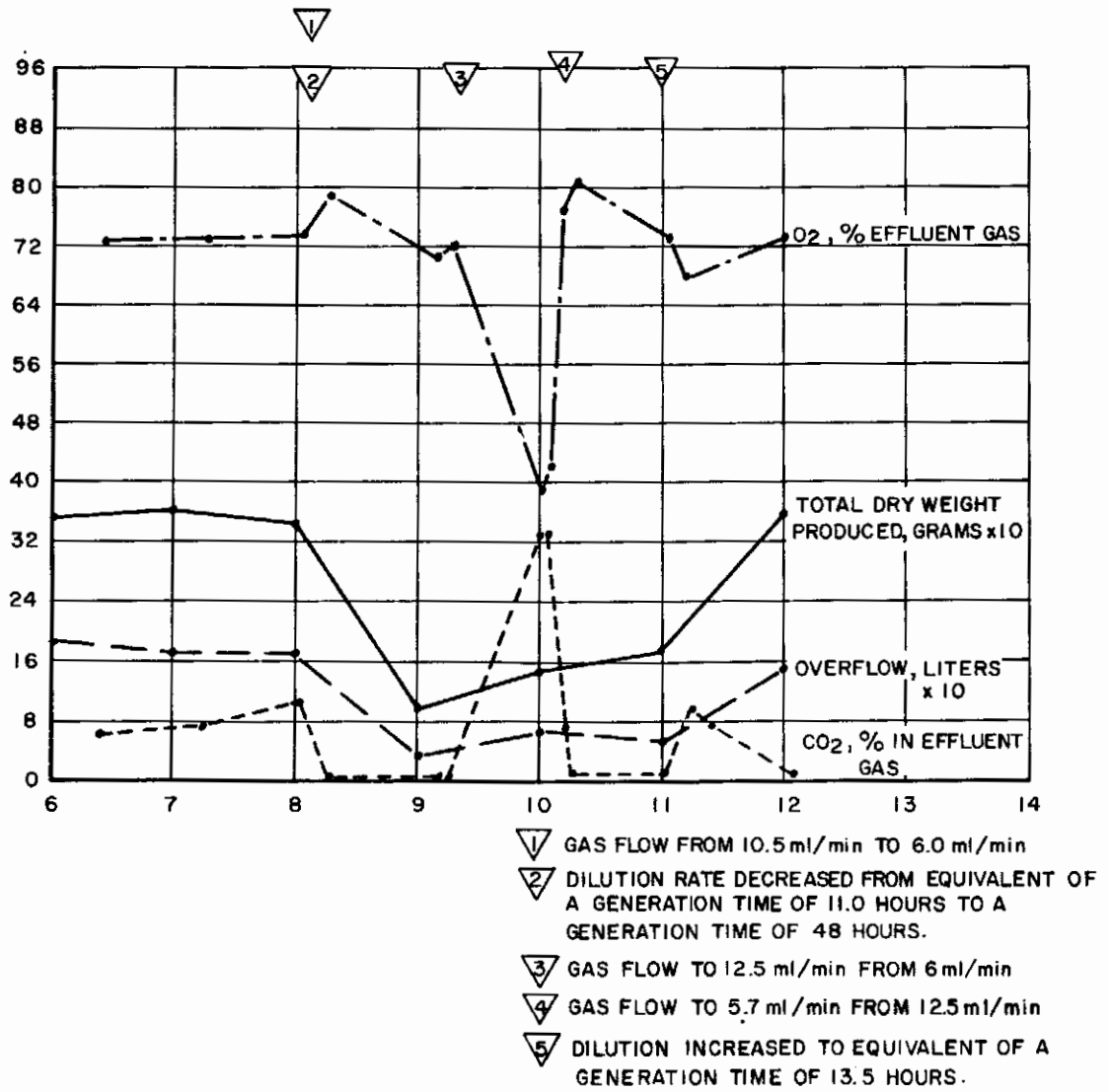


FIGURE 7: RELATIONSHIP OF EFFLUENT GAS COMPOSITION, DILUTION RATE, AND RATE OF ADDITION OF 71% CARBON DIOXIDE IN NITROGEN



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Table 3

Calculated Culture Volumes Necessary to Support One Man

| % $CO_2$ | Culture Volume, ml | Grams (dry) Produced per day | | Volume For One Man | |
|---|---|---|---|---|---|
| | | | | liters | gallons |
| | | $\bar{X}$ | $\sigma$ | | |
| 100 | 1220 | 3.04 | 0.06 | 129 | 34 |
| 71 | 1170 | 3.17 | 0.37 | 120 | 32 |
| 41 | 1150 | 3.62 | 0.16 | 103 | 27 |

## CONCLUSIONS

The results of the experiments reported here establish the feasibility of using highly concentrated carbon dioxide for continuous algae growth. This has been demonstrated in a continuous culture of Chlorella Tx 71105 in which algae growth rates were balanced against carbon dioxide addition rates, and in which potassium nitrate served as the nitrogen source.

The continuous culture was a chemostat, which is well-suited for studies involving constant gas addition rates, and for operation with nearly growth limiting carbon dioxide concentrations. This characteristic is particularly important in achieving balance of rates and, hence, practical control of the system. The degree of allowable excess of carbon dioxide over limiting amounts is a function of the concentration of cation available. Supply of a nitrate salt as nitrogen source may be mandatory as a means of continuous supply of cation at a rate proportional to total growth. For this reason studies of differential ion absorption are probably of considerable importance.

Functional separation of gas addition from heat exchange and turbulence, and independence of liquid composition from its position within the system are important characteristics of the culture configuration. Currently, these objectives have been accomplished by recirculation. In view of the dependence of results on design of the culture system and on components used, the man-sized culture volumes indicated in this paper should not be considered as optimum.

Future work toward the objective of minimizing these volumes could well be expended in achieving better control of gas addition rates, better definition of the contribution of buffering to control, determining optimum carbon dioxide concentrations, and finally, in determining the best characteristics of pumps and other system components.



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