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The Influence of Carbon Dioxide and Oxygen Partial Pressures on Chlorella Growth in Photosynthetic Steady-state Cultures

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The effects of O_2 and CO_2 partial pressures in the range 0 to 1 atm on the maximum growth rate, starch production and photosynthetic efficiencies of growing cultures of Chlorella were determined. Stepwise increases of about 0·2 atm in the partial pressure of either gas inhibited growth, often completely. In contrast, gradual increases (steps of less than 0·1 atm) in the partial pressure of either gas maintained growth, and the cultures became adapted to high partial pressures. Adapted cultures tolerated 0·6 atm CO_2 or 0·8 atm O_2 without any growth inhibition as measured by maximum specific growth rate. Also, starch production was little affected by the increases in O_2 or CO_2 partial pressures.

The photosynthetic efficiency, as measured by the growth yield from light (400 to 700 nm wavelength) absorbed, and the maintenance energy were the same in adapted cultures with the O_2 partial pressure at either 0·2 or 0·8 atm. The maximum growth yield from light was 0·0172 g kJ^{-1}, with 95 % confidence limits of 0·0156 to 0·0189. The maintenance energy was 1·27 kJ (g dry wt)$^{-1}$ h^{-1}, with 95 % confidence limits of 0·89 to 1·65. The photosynthetic efficiency in light-limited cultures was 36·5 %, with 95 % confidence limits of 33 to 40 %.

INTRODUCTION

Despite the central roles of CO_2 and O_2 in photosynthesis, knowledge of the effects of the partial pressures of these gases on photosynthesis is conflicting or vague. Warburg (1920) first reported that increasing O_2 partial pressure above 0·02 atm reversibly inhibited photosynthesis as measured by O_2 evolution by 'resting', that is, non-growing algal cell suspensions over a 30 min period. Björkman (1966) found with Chlorella that there was no measurable inhibition of CO_2 fixation by O_2. Turner & Brittain (1962) referred to the O_2 inhibition of photosynthesis as the 'Warburg effect', but these authors considered exclusively short term (about 60 min) effects in resting cell suspensions.

Myers (1953) reported that algal photosynthesis is inhibited by an increase in CO_2 partial pressure above 0·05 atm. In addition, CO_2 has been reported as antagonizing the Warburg effect (Turner & Brittain, 1962), although this effect seems highly variable.

In our experience, step changes of more than a few per cent in CO_2 or O_2 partial pressure inhibited growth of a continuous culture of Chlorella. The cells, however, became adapted to tolerate the increased partial pressures of the gases when the partial pressure was gradually increased over two or three generations of the Chlorella cells. This adaptation to the high partial pressures was greatly facilitated by application of chemostat continuous flow culture.

According to Tolbert (1974), 'photorespiration' is defined as 'respiration uncoupled to ATP production' in photosynthetic plants or algae. Tolbert stated that 'photorespiration increases with increase in p_{O_2}' but again the observations referred to step changes in gas partial pressure in resting cell suspensions. Since determination of the photorespiration rate

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involved decreasing the p_{CO_2} to the compensation point when the CO_2 uptake rate equals the production rate, the photorespiration could be an artefact. Also the decrease in the CO_2 partial pressure could have stimulated endogenous metabolism. 'Resting cells' are known to respond in this way to the withdrawal of a substrate (Pirt, 1975).

The specific rate of light absorption [q, kJ (g dry wt)$^{-1}$ h^{-1}] during growth of a light-limited culture is expected to accord with the bioenergetics equation (Pirt, 1975): $q = (\mu/Y_G)+m$, where μ is the specific growth rate (h^{-1}), Y_G is the true growth yield (g dry wt kJ^{-1}) and m is the maintenance energy [kJ (g dry wt)$^{-1}$ h^{-1}]. Any photorespiration term would affect m, and a change in m can be determined by a plot of q against μ (Pirt, 1975). This test is best applied by varying μ in a chemostat culture (Pirt, 1975). In energy conservation it is important to know not only whether the O_2 or CO_2 partial pressures inhibit growth, but also whether they have any effect on the maximum growth yield (Y_G) and the maintenance energy (m).

The present paper reports the growth inhibitory effect of high O_2 and CO_2 in partial pressures up to 0·95 atm for O_2 and 1 atm for CO_2, as measured by the maximum specific growth rate (μ_m) of adapted Chlorella cells in chemostat cultures. Also, the photosynthetic efficiencies and maintenance energies at low and high O_2 partial pressures are compared.

Whereas previous studies on the effects of O_2 and CO_2 on photosynthetic Chlorella cells have used almost entirely 'resting cells', the work this paper presents is concerned with growing cells. A preliminary account of the results has been presented (Watts Pirt & Pirt, 1979).

METHODS

Organism. A pure culture of *Chlorella* 211/8k from the Culture Centre for Algae and Protozoa, Cambridge, was grown at 37 °C and with the pH controlled at 6·5 to 6·7. Stock cultures were maintained on a urea/mineral salts medium under 5 % CO_2 in air as described previously (Watts Pirt & Pirt, 1977).

Chemostat culture. The culture vessel was a stirred illuminated chemostat, as previously outlined (Watts Pirt & Pirt, 1977) with a 67 mm inside diameter and 65 mm culture depth at the side-arm overflow, and a working volume of 180 ml.

Gas supply. The chosen gas composition was supplied to the chemostat culture at a flow rate of 40 ml min^{-1}. The required mixtures in terms of CO_2 and O_2, in air or N_2, were achieved by mixing the appropriate components from a range of supply cylinders containing the following gases: pure CO_2, 5 % CO_2 in air, 95 % O_2 plus 5 % CO_2, 80 % O_2 plus 20 % CO_2, and nitrogen. Flow controllers (G. A. Platon) and flowmeters (Rotameter Co.) were used to regulate individual gas feed rates, before combining gases to pass into the culture vessel.

Media. For growth under nitrogen limitation and for the determination of the effect of CO_2 and O_2 on growth, medium A8 was used, with composition as described by Watts Pirt & Pirt (1977), except that $NaVO_3$ was included at 0·05 mg l^{-1}. For light-limited growth in the chemostat, medium A19 of the following composition was used (g l^{-1}): urea, 1·87; KH_2PO_4, 1·45; $MgSO_4.7H_2O$, 0·675; $CaCl_2.2H_2O$, 0·02; and trace element solution at double the level of A8, and including $NaVO_3$ at 0·1 mg l^{-1} and $NiSO_4.H_2O$ at 0·0125 mg l^{-1}.

Analytical methods. The methods of measurement of total cell dry weight, starch and urea were as described by Watts Pirt & Pirt (1977). Biomass was determined as total cell dry weight, designated $\bar{x}$ in steady-state conditions. From the measurement of starch in the biomass ($\bar{s}$), it was possible to calculate the value of the real biomass, designated ($\bar{x}-\bar{s}$).

Determination of maximum specific growth rate (μ_m). Both the 'washout method' in the chemostat culture (Pirt, 1975) and batch culture were applied to determine the maximum specific growth rate (μ_m). The washout method was found to cause an underestimation when CO_2 in the gas phase exceeded 0·2 atm, and similarly when O_2 approached 0·6 atm. This may have been due to transient inhibitory effects at the high CO_2 and O_2 levels. At these high levels, the μ_m was checked by batch culture immediately after the end of the washout period when apparently the transient inhibition was overcome.

Measurement of incident light on the chemostat, and calorific value of the biomass. The methods of Pirt *et al.* (1980) were used in the determination of light-limited biomass production parameters and calorific value of the biomass. From the evaluation of light input and energy content of the biomass as its calorific value, it was possible to determine the photosynthetic efficiency of the light-limited cultures.

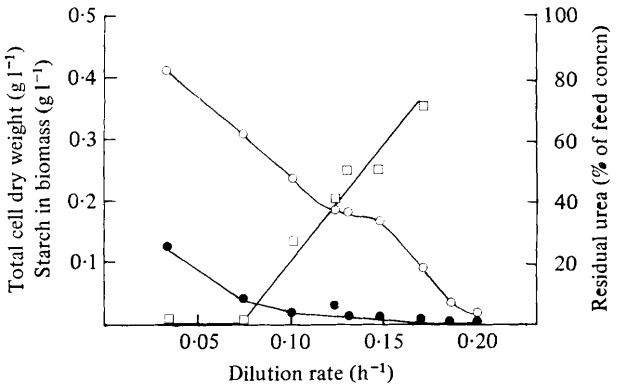

Fig. 1. CO_2 limitation of growth of Chlorella in steady-state chemostat culture in medium A8 with a feed of 0·005 atm CO_2 in air at 40 ml min^{-1} in a 180 ml culture: ○, total cell dry weight; ●, starch content of biomass, □, residual urea.

RESULTS

CO_2 limitation of growth

With a feed of 0·005 atm CO_2 in air at 40 ml min^{-1} to a chemostat culture of Chlorella, CO_2 limitation of growth was observed at dilution rates above 0·07 h^{-1}, in a 180 ml culture vessel with a shallow vortex (Fig. 1). Residual urea was detected at dilution rates above 0·07 h^{-1}. At dilution rates of 0·03 h^{-1} and 0·07 h^{-1} the starch content of the biomass was lower than under CO_2-sufficient conditions.

The calculation of the output rate of total biomass ($D\bar{x}$) confirmed CO_2 limitation of growth over the dilution rate (D) range 0·07 to 0·15 h^{-1} (Fig. 2), where the biomass output rate was constant at about 0·024 g biomass l^{-1} h^{-1}, under a constant flow rate of CO_2. Above 0·15 h^{-1}, washout of the culture was approached.

A carbon balance was calculated to compare total carbon input, as CO_2 plus the carbon input in the urea feed, with the output of carbon in the biomass. The CO_2 dissolved in the culture feed was calculated and deduced to be negligible. Over the dilution rate range of 0·07 to 0·146 h^{-1}, the carbon input rate (CO_2+urea carbon) ranged from 0·555 to 0·564 mmol h^{-1}, and the carbon in biomass output rate ranged from 0·16 to 0·175 mmol h^{-1}. Therefore, in this culture system, when growth was carbon limited, around 30 to 31 % of the supplied carbon was utilized.

Growth of Chlorella in CO_2 up to 1 atm

Steady-state cultures of Chlorella were obtained in the chemostat with CO_2 partial pressures up to 1 atm (in air) under a gas flow rate of 40 ml min^{-1} in the 180 ml vessel. In order to reach steady-state conditions with CO_2 above 0·3 atm it was essential to allow a period of adaptation by waiting for at least four to five volumes of culture to pass through the system. When the CO_2 partial pressure was raised between steady states, there was a maximum stepwise increase of 0·1 atm in order to avoid inhibition of the culture before it had become adapted to the new CO_2 level. The culture was then allowed to adapt to this level for 0·75 to 1 volume changes in the chemostat before the CO_2 partial pressure was further increased.

The maximum specific growth rate of the Chlorella cultures was measured by the washout method applied to a culture that had been adapted to the particular CO_2 level, and confirmed consecutively by batch culture. Above 0·2 atm CO_2, the maximum growth rate measured by washout was less than in the consecutive batch culture, due to transient

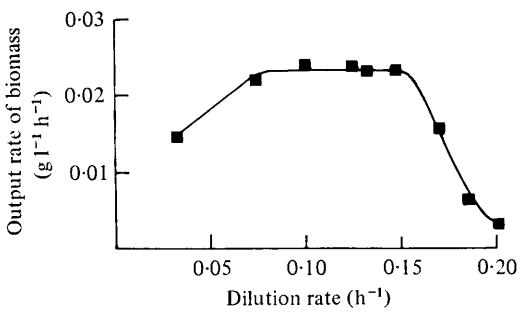

Fig. 2

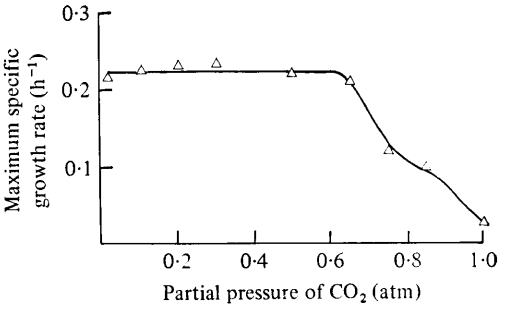

Fig. 3

Fig. 2. Output rate of Chlorella biomass (total g cell dry wt l^{-1} h^{-1}) in a steady-state chemostat culture under 0·005 atm CO_2 in air.

Fig. 3. Effect of partial pressure of CO_2 up to 1 atm on the maximum specific growth rate of adapted Chlorella cells, with a CO_2 in air flow of 40 ml min^{-1} in a 180 ml chemostat.

CO_2 inhibition. Steady-state cultures were obtained in 1 atm CO_2 with dilution rates up to 0·03 h^{-1}.

Figure 3 shows that the maximum specific growth rate of Chlorella did not decrease until the CO_2 pressure exceeded 0·65 atm, and fell by 85 % at 1 atm.

Influence of partial pressure of CO_2 on starch production

The specific rate of starch production in steady-state culture was measured at a dilution rate of 0·11 h^{-1} in a range of CO_2 partial pressures. At this dilution rate, starch production was not affected until the CO_2 pressure exceeded 0·6 atm (Fig. 4).

Influence of partial pressure of O_2 on maximum specific growth rate

If pure CO_2 were supplied to a photosynthesizing culture, the O_2 partial pressure in the culture could approach 1 atm. Hence, it was necessary to determine whether such increased O_2 levels are inhibitory to Chlorella growth.

Chlorella cultures were adapted to increased levels of O_2 in continuous flow culture by gradually raising the partial pressure of O_2 in steps of no more than 0·2 atm, and allowing the culture to stabilize at the new level with the passage of at least three to four volumes of nutrient through the chemostat to ensure steady-state conditions had been reached. Gas flow was controlled at 40 ml min^{-1} with CO_2 at 0·05 atm; the pressure was made up to 1 atm with N_2.

There was no reduction in maximum specific growth rate until the O_2 pressure exceeded 0·8 atm (Fig. 5). The maximum specific growth rate was measured by washout and batch culture, and the two measurements corresponded until the partial pressure of O_2 reached 0·65 atm, when the washout value was 30 % lower than in the consecutive batch culture. As the measurements were run consecutively, it was assumed that the O_2 transiently became inhibitory during washout.

Growth of Chlorella in 0·2 atm CO_2 plus 0·8 atm O_2

In the photosynthetic production of biomass and starch by *in vitro* cultures of Chlorella, it would be feasible to control the supply rate of pure CO_2 feed to the culture such that the exit gas would be significantly enriched in the photosynthetically produced O_2. For example, if the CO_2 were supplied at a rate such that 80 % was used, then the exit gas would contain 0·2 atm residual CO_2 and 0·8 atm O_2. As well as reducing the gas flow rate required, this would constitute a means of photosynthetic production of pure O_2 if the residual CO_2 were scrubbed out.

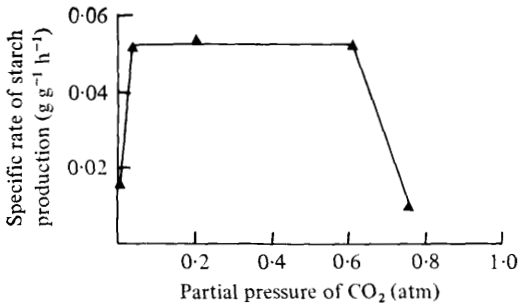

Fig. 4

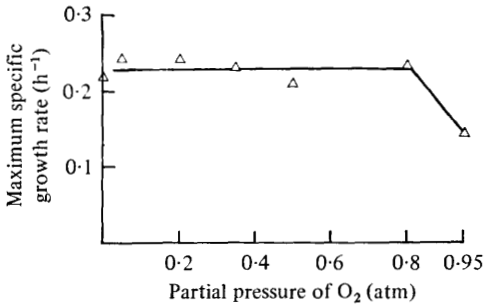

Fig. 5

Fig. 4. Effect of CO_2 partial pressure on the specific rate of starch production [g starch (g cell real biomass)$^{-1}$ h^{-1}] in a chemostat culture of Chlorella under nitrogen-limited growth at a dilution rate of 0·11 h^{-1}. The CO_2 was diluted with air; the gas flow rate was 40 ml min^{-1}.

Fig. 5. Maximum specific growth rate of adapted cultures of Chlorella in O_2 partial pressures up to 0·95 atm, with CO_2 maintained at 0·05 atm. The gas pressure was made up to 1 atm with N_2; the gas flow rate was 40 ml min^{-1}.

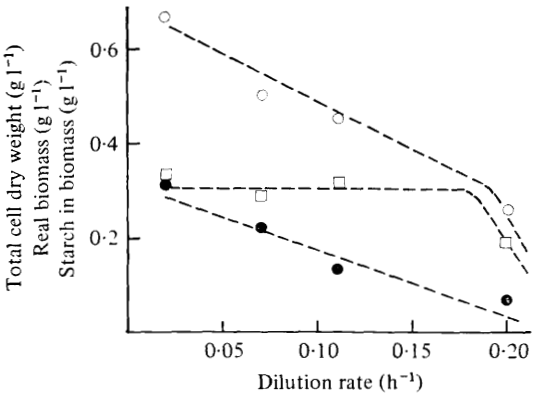

Fig. 6. Biomass and starch production by Chlorella in 0·2 atm CO_2 plus 0·8 atm O_2: ○, total cell dry weight; ●, starch in biomass; □, real biomass. The measurements are compared with results previously obtained (Watts Pirt & Pirt, 1977) which are shown by the dashed lines and represent biomass and starch production in 0·05 atm CO_2 in air.

Therefore, the behaviour of Chlorella was followed in a 180 ml chemostat culture supplied with 0·2 atm CO_2 and 0·8 atm O_2 at 40 ml min^{-1}, over a range of dilution rates to compare steady-state levels of biomass and starch with those under 0·05 atm CO_2 in air. As seen in Fig. 6, there was no inhibition of growth of Chlorella in 0·2 atm CO_2 plus 0·8 atm O_2.

Determination of maximum photosynthetic efficiency of Chlorella cultures in 0·2 atm CO_2 plus 0·8 atm O_2

Although it was confirmed that photosynthetic Chlorella cultures could be grown in an atmosphere of 0·2 atm CO_2 plus 0·8 atm O_2, it was necessary to measure the photosynthetic light uptake rates by steady-state cultures under light-limited growth and to calculate the maximum photosynthetic efficiency for comparison with that in cultures with 0·1 atm CO_2 in air. With the intention of producing biomass, starch and, in addition, pure O_2 from a pure CO_2 feed to a photosynthesizing algal culture, a decrease in the photosynthetic efficiency would have reduced the economic potential of the system.

The specific rate of light uptake by Chlorella was determined over a range of dilution rates. In each light-limited steady-state the biomass was measured and the specific rate of light uptake (q) was calculated as $I.a/v.x$, where I was the incident light intensity, a was the illuminated area and v the culture volume. The graph of q against μ gave a straight line relationship. Linear regression analysis of the results both with 0·2 atm CO_2 plus 0·8 atm O_2, and with 0·1 atm CO_2 in air, gave straight line relationships, with no significant differences calculated at 95 % confidence limits. From the slope of the lines, the maximum growth yield on light (Y_G) was found to be 0·0172 g kJ^{-1} (95 % confidence limits 0·0156 to 0·0189 g kJ^{-1}). From the intercept, the value of the maintenance coefficient on light was 1·27 kJ g^{-1} h^{-1} (95 % confidence interval 0·89 to 1·65 kJ g^{-1} h^{-1}).

The calorific value of light-limited Chlorella biomass was found to be 21·15 kJ g^{-1}, corrected for the nitrogen content (Kersting, 1972). Therefore, the maximum photosynthetic efficiency of light uptake by Chlorella, which was the product of Y_G and the calorific value, was calculated to be 36·5 % (95 % confidence limits 33 to 40 %).

DISCUSSION

The outstanding feature of the experiments reported here is the ability of growing Chlorella cells to adapt to high partial pressures of CO_2 or O_2 which, the literature suggests, can be totally inhibitory to resting cell photosynthesis. It is clear that almost total inhibition will occur transiently in the growing culture when the CO_2 or O_2 partial pressures are increased in large steps. The adaptation in the growing culture is assumed to be a phenotypic change since it could occur in three to four generations which would be inconsistent with selection of mutant cells. The nature of the adaptation is unknown.

It has been suggested that inhibition of ribulose bisphosphate carboxylase may be responsible for the O_2 and CO_2 effects (Laing *et al.*, 1974). The activity of ribulose bisphosphate carboxylase in the growing culture could be relevant to elucidation of the molecular basis of the adaptation.

The constancy of the maximum growth yield (Y_G) and the maintenance energy (m) with an increase in the O_2 partial pressure to 0·8 atm indicates that Chlorella does not contain any metabolic site which is made inefficient by oxidation at the high O_2 partial pressures. Since photorespiration would be included in the maintenance energy, it follows that photorespiration, if it existed, was unaffected by the increased O_2 pressure in the adapted cell.

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