BBA 45846

A NEW INTERPRETATION OF THE EVIDENCE FOR A FIRST-ORDER THERMAL REACTION IN THE PRODUCTION OF OXYGEN BY *CHLORELLA PYRENOIDOSA**

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(Received May 27th, 1969)

SUMMARY

1. A study has been made on the influence of modulation frequency on the size and phase of the signal from an oxygen electrode of the type devised by JOLIOT AND JOLIOT[3].

2. Using *Chlorella pyrenoidosa* as the material, the results of this study were in excellent agreement with the equations derived on the assumption that diffusion of O_2 from the cells to the electrode was the rate-limiting process.

3. These findings are in conflict with a similar study by JOLIOT *et al.*[2] who found that there were deviations from the predictions of the diffusion equations and who interpreted these deviations as evidence for a first-order thermal reaction leading to O_2 evolution.

4. The differences between the two sets of results can be resolved if it is accepted that JOLIOT *et al.* overestimated the thickness of the Chlorella layer on this electrode.

INTRODUCTION

In a series of papers, JOLIOT and co-workers[1-4] have described a new method for studying photosynthetic O_2 evolution and have named the apparatus a modulated oxygen electrode. This technique employs an intensity-modulated light beam to illuminate a layer of algae (or chloroplasts) situated on the surface of a platinum electrode. The O_2 production by the cells is correspondingly modulated, and the O_2 interacts with the platinum surface to produce an alternating current in the electrical circuit. By amplifying only the electrical current modulated at the correct frequency, it is possible to observe O_2 produced by photosynthesis independently of other types of O_2 production or consumption.

An electrode system which is essentially identical to that described by JOLIOT AND JOLIOT[3] has now been put into operation. With this electrode, the investigation of JOLIOT *et al.*[2,4] of the response of the steady-state signal from Chlorella to alterations in the modulation frequency has been repeated. In contrast to JOLIOT *et al.*[2,4], I have found no evidence to suggest that a first-order thermal reaction is influencing

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the results. The results reported here appear consistent with O_2 diffusion being the rate-limiting process.

METHODS

As mentioned above, the oxygen electrode employed was virtually identical to the one described by JOLIOT AND JOLIOT[3]. At the start of an experiment, the algal cells were layered on top of the platinum electrode. The thickness of the layer of Chlorella was determined after spinning a sample of the cell suspension for a few minutes in a Model CL International clinical centrifuge. Knowing the packed cell volume as a fraction of the total volume of the cell suspension and also the dimensions of the oxygen electrode, it was possible to calculate the thickness of the cell layer. A d.c. potential was applied to the electrodes of the reaction cell, the platinum electrode being — 0.74 V. This potential difference was continually monitored during the experiment. The solution flowing through the cell was 0.35 M NaCl *plus* 0.02 M Tris buffer (pH 7.4). The cells were illuminated with a chopped beam of white light (average intensity 4000 ergs·cm^{-2}·sec^{-1}) which had passed through 5 cm of water and a heat filter (CS 1-69, 2,65 mm) before reaching the cells. After approx. 20 min had elapsed, the signal from the electrodes became quite stable, and measurements could be made.

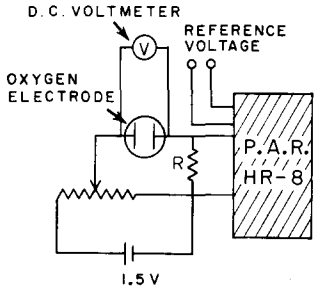

Fig. 1. Electrical circuit used to measure the alternating current from oxygen electrode. The HR-8 lock-in amplifier was used with a Type-A high impedance preamplifier.

The electrical circuit is shown in Fig. 1. The alternating current from the oxygen electrode flowed through the resistor R, producing a voltage which was measured with an HR-8 lock-in amplifier (Type-A preamplifier). A reference voltage for the lock-in amplifier was provided by a BZ-1 chopper which also chopped the light beam. The influence of the size of the resistor R on the results was investigated. It was found that when R was 50 Ω or lower, the value of the current and its phase remained constant for any frequency below 130 cycles/sec.

THEORETICAL CONSIDERATIONS

When algal cells are lying in a layer on the platinum electrode, O_2 molecules must diffuse from the interior of the cells to the platinum surface before they can be detected. JOLIOT *et al.*[2] presented a theoretical analysis of this process and concluded that it could be resolved into two parts: (a) diffusion within the cell layer and (b)

diffusion from the cell layer across a narrow layer of solution to the platinum surface. The equations which describe the influence of diffusion on the current size and phase take forms which are dependent on the experimental conditions. For a thin layer of cells of thickness Δp situated at a distance p from the platinum electrode,

$$A = \frac{\psi(p)\Delta p}{D} \exp\left(-\frac{p\sqrt{\omega}}{\sqrt{2D}}\right) \tag{1}$$

$$\phi = \frac{p\sqrt{\omega}}{\sqrt{2D}} \tag{2}$$

where A = amplitude of the alternating current; $\psi(p)\Delta p$ = rate of O_2 production in the layer of cells; ω = angular frequency; D = diffusion coefficient of O_2 in water $= 2.9 \cdot 10^{-5}$ cm$^2 \cdot$sec^{-1}; ϕ = phase difference between the electric current and the light modulation.

When the cells make up a thick layer, the equations take the following forms:

$$A = \frac{B\psi_{(0)}}{\sqrt{\omega D}} \exp\left(-\frac{\varepsilon\sqrt{\omega}}{\sqrt{2D}}\right) \tag{3}$$

$$\phi = \frac{\pi}{4} + \frac{\varepsilon\sqrt{\omega}}{\sqrt{2D}} \tag{4}$$

where B = a proportionality constant; ε = the effective width of the layer of solution between the platinum electrode and the cells closest to that electrode; $\psi_{(0)}$ = rate of O_2 production per unit volume among the cells closest to the electrode. Eqns. 3 and 4 apply only when the following relationship is true:

$$\frac{q\sqrt{\omega}}{\sqrt{2D}} \geqslant \pi \tag{5}$$

where q is the thickness of the cell layer. When $q = 30\ \mu$, the above ratio attains a value π for a frequency of 9 cycles/sec.

RESULTS AND DISCUSSION

The influence of the modulation frequency on the current phase and magnitude for thick (35 μ) and thin (monolayer) layers of Chlorella is shown in Figs. 2 and 3. For both situations, the phase angle is a linear function of the square root of the frequency. In Fig. 2, Line A is from the thick-layer data and extrapolates to 50° at zero frequency, while Line B is from the thin-layer data and passes through the origin. These findings are in good agreement with Eqns. 2 and 4. In Fig. 3, the plots are log $A\sqrt{\nu}$ (Line A, thick layer) and log A (Line B, thin layer) *versus* $\sqrt{\nu}$. Both are straight lines, in agreement with Eqns. 1 and 3. Thus the diffusion analysis developed by JOLIOT *et al.*[2] accurately describes the results of this work.

For a thick layer, the slopes of the phase and current magnitude plots are related to the values of ε (see Eqns. 3 and 4). The Lines A in Figs. 2 and 3 give values of 7 μ and 6.7 μ for ε, the effective width of the layer of solution between the platinum electrode and the closest cells. These values are as large as the diameters of the largest

Chlorella present in the solution and suggest that either (a) the cells touching the electrode are inactive, (b) the lowest cells are not in contact with the electrode or (c) O_2 is not released from the surface of the Chlorella facing the electrode. The data from the monolayer study rule out the first possibility, but (b) or (c) would explain an high value of ε.

In contrast with the findings described above, JOLIOT *et al.*[2] found that the Eqns. 3 and 4 did not fully describe the results of their work. Their analysis suggested that deviations from these equations began at frequencies as low as 20 cycles/sec for the phase angle and 40 cycles/sec for the current size. JOLIOT *et al.*[2] also estimated ε to be 2 μ or less, which is considerably smaller than the values mentioned previously.

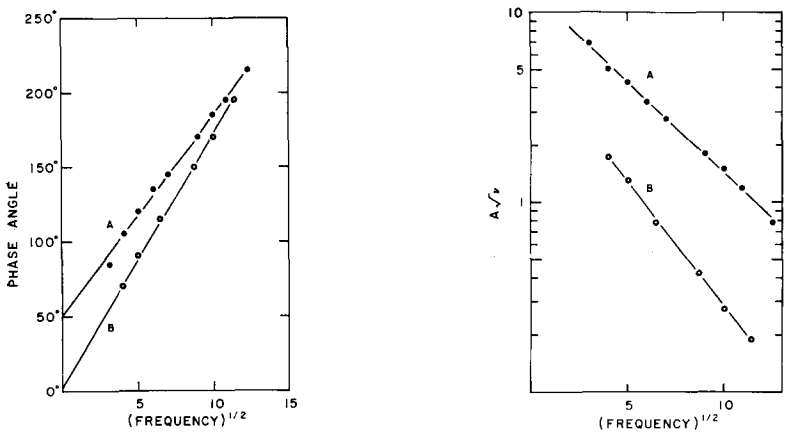

Fig. 2. The variation of phase angle with the square root of the modulation frequency. Line A, a 35-μ layer of Chlorella; Line B, a monolayer.

Fig. 3. The effect of frequency on current magnitude. Line A, a 35-μ layer of Chlorella; log $A\sqrt{\bar{\nu}}$ *vs.* $\sqrt{\bar{\nu}}$. Line B, a monolayer of Chlorella; log A *vs.* $\sqrt{\bar{\nu}}$.

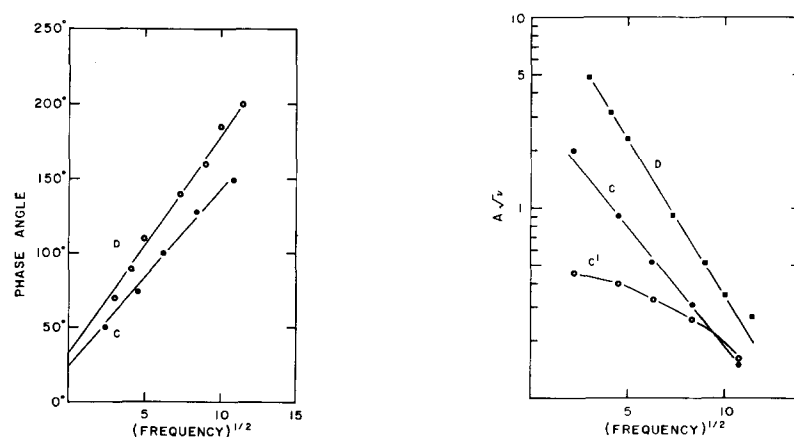

Fig. 4. The variation of phase angle with the square root of the modulation frequency. Line C, results obtained by JOLIOT *et al.*[2] (Fig. 10); Line D, results from the present study for a 12-μ layer of cells.

Fig. 5. Line C', log $A\sqrt{\bar{\nu}}$ *vs.* $\sqrt{\bar{\nu}}$; and Line C, log A *vs.* $\sqrt{\bar{\nu}}$ from results for a 30-μ layer in Fig. 10 of the paper by JOLIOT *et al.*[2]. Line D, log A *vs.* $\sqrt{\bar{\nu}}$ for a 12-μ layer of cells in this study.

To understand the source of these conflicting results, the data from Fig. 10 in the paper by JOLIOT *et al.*[2] have been replotted in Figs. 4 and 5. These results were obtained from a 30-μ layer. The phase data have been plotted in Fig. 4 giving a straight line (C) which intercepts the ordinate at 24°. When the current-size data were plotted according to Eqn. 3, the curved Line C' in Fig. 5 was obtained. If the same data were plotted according to Eqn. 1, a straight line was found (Line C). This behaviour is very much like that found in the present study with cell layers of intermediate thickness, *e.g.* Lines D in Figs. 4 and 5. Thus the results obtained by JOLIOT *et al.*[2] would tally with the present work if there were fewer Chlorella cells on the platinum electrode than they had calculated. It is therefore interesting that JOLIOT *et al.*[2] placed the Chlorella cells on the platinum electrode while the latter was withdrawn from the apparatus. The electrode was then pushed into position against the membrane. In the present work, the Chlorella were introduced into the completed apparatus. A hole at one end of the rectangular platinum electrode permitted the cell suspension to be injected. The suspension could be seen moving over the platinum surface and out *via* a second hole on the other side of the electrode.

The 2-μ value of ε which JOLIOT *et al.*[2] quote is obtained by procedures involving extrapolation to zero frequency. If instead we follow the procedure described above for the phase data, a value of 6.3 μ is obtained. This is certainly an overestimate of the correct value if it is accepted that the layer was of intermediate thickness. The smaller value probably arises because the Chlorella of JOLIOT *et al.*[2] were grown in weak light to produce small cells. However, the difference between 6.3 μ and 7 μ is not nearly so startling as that between 2 and 7 μ.

JOLIOT *et al.*[2] also investigated the effect of temperature on the phase of the signal and found a $Q_{10} = 1.65$. The interpretation of such a result depends on the other information available. JOLIOT *et al.*[2] claimed that the Q_{10} referred to the rate-limiting thermal reaction which they had discovered. In the present study there is no evidence for such a thermal reaction, and so an explanation of the Q_{10} must await further experiments.

In conclusion it is proper to consider the nature of the rate-limiting thermal reaction in the O_2 production. JOLIOT *et al.*[2] claimed that it was first order and had a rate constant of 820 sec^{-1}. On the basis of the work presented here, it is not possible to say that the rate-limiting step is indeed first order. However, if it is assumed that this is the case, then the minimum value of k would be about 5000 sec^{-1}.

ACKNOWLEDGMENTS

The author would like to thank Dr. Leo P. Vernon for his help and advice during the work and Drs. B. Mayne and D. Fleischman for their criticism of the manuscript. The assistance of Mr. J. Hostens on electronic problems is also gratefully acknowledged.

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