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Further Improvements in Stationary Platinum Electrode of Haxo & Blinks [1, 2]

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Polarographic methods in a wide variety of technical designs have been applied to measurement of oxygen production in photosynthesis. One line of development, measuring oxygen concentration in solution, led from the dropping mercury electrode of Petering and Daniels (12) to the pulse-polarized platinum electrode of Olson et al. (11) and its further development by Brackett et al. (3) for recording both oxygen concentration and rate of exchange. A second line of development stems from the stationary platinum electrode of Blinks and Skow (2). A thin layer of cells was pressed tightly against a platinum cathode in order to obtain rapid response to oxygen production. Haxo and Blinks (8) introduced the important improvement of an adjacent large reservoir of nutrient solution. Their arrangement has a special advantage. Oxygen exchange of a thin layer of cells is imposed upon steady state diffusion of oxygen from a large solution reservoir to the surface of the platinum cathode. Hence a change in electrode current from one steady value to another becomes a measure of the change in rate of net oxygen evolution between corresponding steady states in the algal cell layer.

The Haxo and Blinks electrode offers three important advantages: A, it provides a current signal which directly measures net rate of oxygen exchange, B, it has excellent time resolution as evidenced by ability to detect transients of the induction period (14, 15) and chromatic transients (1), and C, it has a sensitivity so high that it can be applied to study of 0.1 μl or 25 μg dry weight of algae in a light beam of 25 mm^2 cross section. The Haxo and Blinks electrode also has distinct limitations: A, it is subject to the known drifts and instabilities of the stationary platinum electrode and B, it lacks an effective means of calibration and can measure only relative values of change in rate in net oxygen evolution.

Further modification of the Haxo and Blinks electrode has been made by Haxo (7, cf. also Setlik, 14) to permit use with unicellular algae. The horizontal flat platinum cathode is the floor of a chamber 0.1 to 1.0 mm deep. A drop of algal suspension is placed in the chamber and covered with a cellophane membrane. The cells settle out to produce a film on the platinum surface. The arrangement permits use of a wide variety of unicellular algae. It is also possible to vary at will the thickness of the deposited algal film. For convenience the modified arrangement will be referred to as the Haxo electrode. Its form, as currently used in this laboratory, is shown in figure 1B.

Although the Haxo electrode has been used in a number of laboratories and cited in several publications (5, 6, 7, 10), details of design and operating characteristics have not been published. In spite of its recognized limitations, we sought to take advantage of its high sensitivity for study of the Emerson enhancement effect. The present paper describes developments which increase precision of measurement and presents some of its operating characteristics.

As originally used, the Haxo electrode was immersed in a large, unstirred reservoir of nutrient solution. A calomel anode was connected to the solution reservoir by an agarized salt bridge. An EMF of 0.5 to 0.7 volts was imposed across the electrodes and current flow was measured as the potential drop across a series resistance. In practice this arrangement is subject to instability and drifts in current flow even without any algal film on the electrode. Two major sources of instability and drift have been minimized by changes in design.

Flowing Electrode Arrangement. When used with a thin (5–10 μ) film of algae and small depth of chamber (0.25 mm) the Haxo electrode is sensitive

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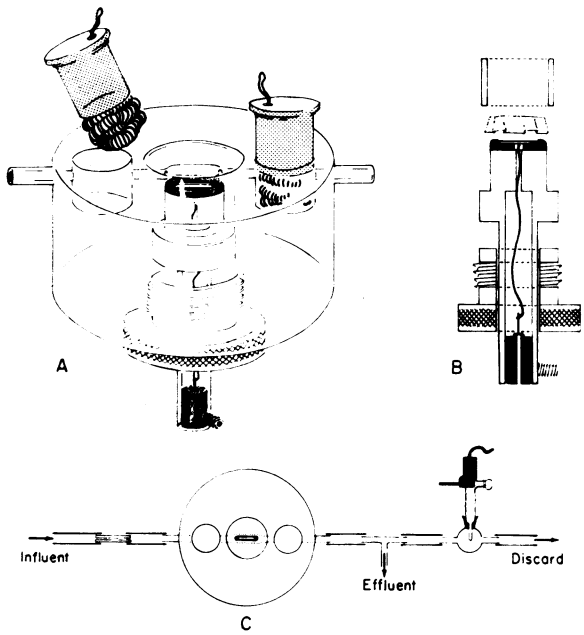
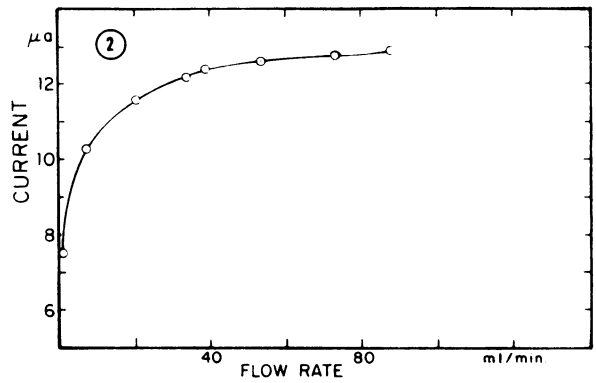


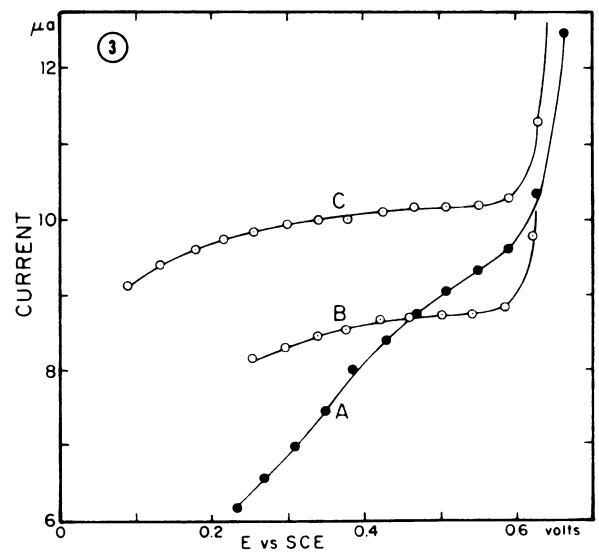
Fig. 1. Flowing electrode arrangement. A, electrode chamber and lucite block housing showing the central platinum strip cathode and two platinum wire anodes mounted in teflon plugs. B, exploded view of platinum strip cathode and mounting post. C, top view showing the flow stream and reference calomel electrode.

to turbulence in the external reservoir of solution. Adequate approach to stagnant conditions in the external reservoir can be obtained only by exacting temperature control and freedom from vibration. In our own laboratory we could not obtain adequate stability by using an unstirred reservoir. Nor could we devise any means of stirring, such as used by Setlik (14), which would give adequate stability. Finally we reduced the reservoir to a small volume (0.4 cc) and then were able to obtain good stability in current signal by rapid flow of nutrient solution through the reservoir.

Our flowing electrode arrangement is shown in figure 1 and its response to flow rate is described by figure 2. In practice a constant flow of about 45 cc/minute is obtained by a constant head of 60 cm against a capillary orifice. The nutrient solution (Knops) is previously aerated with 5 % carbon dioxide in air and may be thermostatted if desired.

Reference Electrode. A second problem with the Haxo electrode lies in the selection of a desirable anode. The essential difficulty is that, at least for common algal media, the polarogram of current vs. EMF has no perfectly flat plateau (cf. fig 3). Any small variation in potential at the platinum cathode changes its sensitivity to oxygen. For simple operation it is desirable to hold the source EMF constant. With any large resistance in the electrical circuit, any change in current flow accompanying change in oxygen evolution by the algal film will also give rise to significant change in potential at the platinum cathode. Hence the reference electrode, the ion conductance path, and the measuring circuit must have minimum resistance.





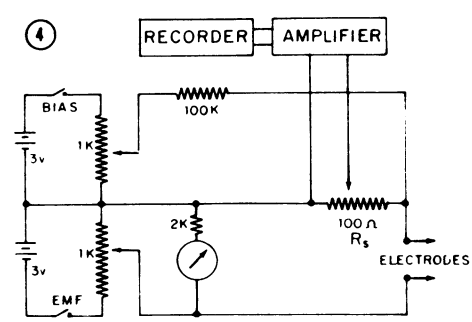


Fig. 2. Effect of flow rate on current signal.

Fig. 3. Current as a function of cathodic potential measured vs. the saturated calomel electrode (SCE). Curve A obtained with an algal film immediately after installation. Curve B, obtained on the same preparation 40 hours after curve A. Curve C, obtained with a different algal film preparation.

Fig. 4. Electrical circuit.

Difficulty with the calomel electrode lies in the necessary salt bridges. With saturated KCl to minimize resistance they experience continuous loss of salt by diffusion to the nutrient solution. There results a slowly drifting cathode potential and a drifting sensitivity. In order to eliminate the salt bridges we tried large area silver anodes and addition of KCl to the nutrient solution. This provides the most stable system yet found. However, in the course of several days operation with Chlorella we observed decreasing photosynthetic response. We could confirm from growth experiments that silver is toxic to Chlorella even in the presence of 0.05 M KCl. Hence we abandoned the silver anodes.

Finally we introduced large area platinum anodes together with a small saturated calomel reference electrode which permits measurement of the cathodic potential with a vacuum tube voltmeter. In practice an applied EMF (about 1.4 v) is chosen to give a cathode potential of 0.50 volts vs. the saturated calomel electrode (SCE). Two platinum anodes, each a coil of 5 ft of 24 gauge wire, are positioned (fig 1A). The reference calomel electrode is placed in the effluent by-pass line (fig 2C) so that it cannot contaminate the nutrient solution to be reused. The total resistance between the two platinum anodes and the working platinum cathode is 800 ohms.

The electrical circuit shown in figure 4 provides a constant EMF and permits bias of any fraction of the potential across the variable resistance, R_s. The net potential drop across R_s is amplified by a Keithley Model 150 amplifier and recorded as the current signal.

Working Characteristics. In practical working use with Chlorella a cell suspension is placed upon the electrode, covered with a cellophane membrane, and inserted in the system. During the first 12 hours the preparation shows a dark current drifting upward from about seven to about ten microamps. We attribute the drift principally to a decrease in respiratory rate known to occur during the same period (4). When not in use an EMF is not applied and the preparation is illuminated, usually at 680 mμ with 30 μwatts/cm², so that response to a standard light signal remains approximately constant day after day. Cells thus maintained under low illumination are judged to be similar to active dark cells in the sense of Tamiya et al. (16) or cells as trained for quantum yield measurements. A given preparation of Chlorella can be used for 5 to 10 days.

The current vs. voltage polarogram is subject to two kinds of variation shown in figure 3. Curves A and B show the change with time observed in one preparation, although this is the most severe change ever observed. Comparison of curves B and C, obtained on different preparations, illustrates experience that sensitivity to oxygen is not constant from one preparation to the next.

In spite of the variations in electrode response noted above, it is possible to state some practical working characteristics. These are provided by the following figures obtained at 24 C with an electrode chamber 2 × 13 × 0.25 mm deep covered with a 0.001 inch cellophane membrane, a potential of 0.50 volts vs. SCE, with Knops solution saturated with 5 % carbon dioxide in air, and with and without a film of Chlorella.

Without algal film: Total current flow, ca. 10 μamps. Stability at R_s 100 ohms, biased: short time variations, <0.005 μamps drift, <0.01 μamps/hr. With a 5μ film of Chlorella: Total current flow: dark, ca. 10 μamps; at light saturation, ca. 25 μamps. Response to 120 μwatts/cm² at 680 mμ, 5 μamps. Light absorption by film at 680 mμ, 50 %. A typical record obtained at high sensitivity is shown in figure 5.

Relation Between Respiration & Photosynthesis. Since response to light is measured only as a current difference, the Haxo electrode offers no way to measure rates of respiration and photosynthesis independently. Blinks (1) has estimated the compensation point by comparing the currents obtained in the dark with and without an algal thallus in place. However, the added diffusion resistance of the algal thallus and the necessary disturbances at the platinum

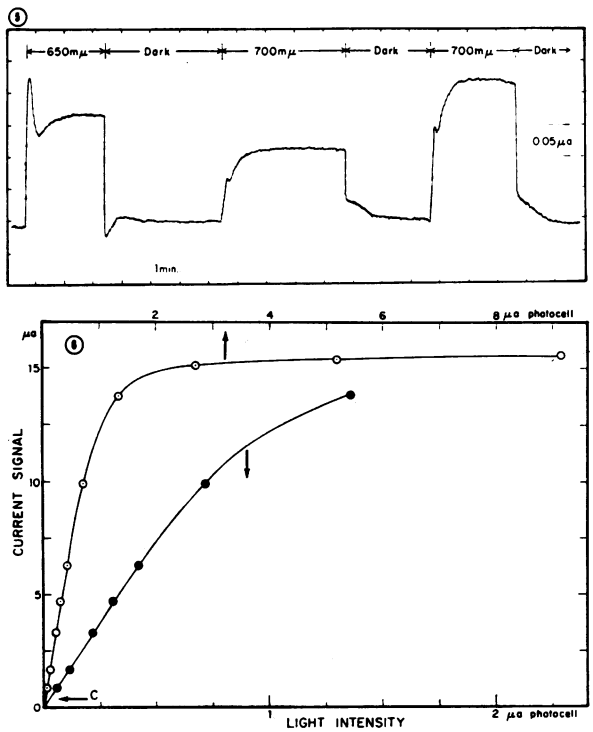


Fig. 5. Typical record obtained at high sensitivity. The three light signals, left to right, were about 9 μwatts/cm² of 650 mμ, 19 μwatts/cm² of 700 mμ, and 34 μwatts/cm² of 700 mμ.

Fig. 6. A light intensity curve obtained with a monochromator set on zero order (white light). Relative light intensity was measured as the current from a selenium photocell. C designates the estimated level of compensation taken as 1/40 of the current at light-saturation.

surface give rise to uncertainties. The latter difficulty is even more severe with the Haxo electrode arrangement.

A second possible method for estimating compensation can be based upon determination of the light intensity curve and the use of a ratio between compensation and saturation obtained from other measurements. From measurements by Warburg manometry we estimate that respiration rate of *Chlorella pyrenoidosa* is about 1/40 (1/80–1/20) the rate of photosynthesis at light saturation. A light intensity curve for one preparation is shown in figure 6. Here we estimate compensation as a difference current of 0.4 μamps and certainly in the range of 0.2 to 0.8 μamps. Thus the rates of photosynthesis shown in figure 5 are judged to be below compensation.

Thickness of Algal Film. The thickness of the algal film is readily calculated from the packed cell volume and the depth of the chamber. Experience leads us to believe that the film thickness obtained in repetitive preparations is reproducible to about ± 10 %. A film thickness of choice for Chlorella is 5 μ, giving a layer about one cell thick. On an electrode of 26 mm^2 this represents a total cell quantity of 0.13 mm^3 of cells. For work in spectral regions of low absorption, as in the far red, we have used films up to 30 μ thick without apparent change in photosynthetic behavior. Such films do show a lower dark signal, attributed to increased respiration and diffusion resistance of the film, of about five microamps as compared to the 10 μamps commonly observed with thin films or with the bare electrode. Attempts to use very thick films for complete light absorption ended in failure. Films 100 μ thick gave a very low dark current of about one microamp and only small photosynthetic response. It appears that such a thick layer leads to anaerobic conditions.

Conditions Within Electrode Chamber. Perhaps the most serious hazard in using the Haxo electrode lies in the inability to specify the micro-environment of the algal cells. The electrode chamber contains an algal film held at some position between the platinum cathode and a dialyzing membrane. At the outside of the dialyzing membrane the oxygen concentration is held constant. At the platinum surface the oxygen concentration approaches zero.

There is some uncertainty as to the detailed mechanism of reduction of oxygen at a platinum cathode (9, 13). At the same time it appears that the net reaction under our conditions can be represented as

$$O_2 + 2\ H_2O + 4\ e \rightarrow 4\ OH^-$$

There are several consequences of the electrode reaction.

For a thin film in our present electrode chamber the respiratory demand is small compared to oxygen consumption by the electrode. A typical current flow of 10 μamps corresponds to 26×10^{-6} μmoles/second of oxygen consumed by the electrode. For a 5 μ layer of Chlorella at the low respiration rate of starved cells (4) the respiratory oxygen consumption is estimated at only 0.8×10^{-6} μmoles/second. Note that hydroxyl ion is also being produced at the electrode at about 104×10^{-6} μmoles/second. When the algal film rests upon the platinum surface the cells may be exposed to unusual, and possibly unfavorable, conditions of low oxygen concentration and high pH.

We are now engaged in attempts to determine profiles of oxygen and ion concentrations across the depth of the electrode chamber and to discover the parameters for optimum design. In the meantime we have proceeded, as in other laboratories, with resort to practical checks. The relationship between current signal and light intensity provides evidence of linear response (fig 6). Variations in film thickness and variations in oxygen concentration in the external solution provide empirical checks against anomalies due to anaerobiosis. Our experience leads to belief that the Haxo electrode provides a reliable and very sensitive tool for studying steady-state rates of net photosynthetic oxygen evolution in Chlorella.

Summary

The stationary platinum electrode of Haxo and Blinks, modified by Haxo, provides high sensitivity and speed of response in measuring net photosynthesis of unicellular algae. Two further modifications, the rapid flow of external solution and the use of massive platinum anodes, increase stability and precision of measurement. Performance characteristics of such an arrangement provide evidence of its advantages and restrictions.

Acknowledgments

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Effects of Non-Ionizing Radiation on Expansion of Disks From Leaves of Dark-Grown Bean Plants [1, 2]

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In recent years, the number and variety of responses noted following exposure of plants to red and far red light threatens to exceed those attributed to auxins. It has been assumed that the same receptor pigment, phytochrome, is involved but, writes Liverman, ". . . it would be difficult to explain all these different responses on the basis of a series of identical reactions." (10). Relatively few of the test objects used, however, have been subjected to detailed study of the physical parameters of the response to visible radiation. Indeed, the quantitative data necessary to judge if even the physical factors are identical are woefully lacking. Such is the case with the light-controlled expansion of the primary leaves of dark-grown bean plants. Liverman (10) has summarized the extensive researches of many workers on the use of growth factors in leaf expansion and some attention has been given to the responses to light in relation to chemical stimulation and repression of expansion.

The work reported here was, in fact, initiated to confirm and to extend previous studies on the biophysics of leaf disk expansion. It was found, however, that for these contemplated studies, a more detailed picture of the effects of light was necessary.

Materials & Methods

Seed of *Phaseolus vulgaris* L., Burpee Dwarf Stringless Greenpod, were soaked for 6 hours in tap water and planted in plastic pans containing moist, well-washed Terralite vermiculite. The pans were covered to exclude all light and were incubated at 23 C for 9 days. Additional water was added at day 2 to replace that imbibed by the seeds. Two disks were punched from each of the primary leaves with a No. 1 cork borer (3.60 mm) with a main side vein

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