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FEEDBACK CONTROLLING OXYGEN PRODUCTION IN A CROSS-REACTION BETWEEN TWO PHOTOSYSTEMS IN PHOTOSYNTHESIS

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SUMMARY

The non-linear light-saturation curve for oxygen production in both *Chlorella vulgaris* and *Phormidium luridium* at low light intensities, under anaerobic conditions is shown to be caused by the reduction of a pool of electron carriers coupled to both an endogenous reducing agent R, and to oxygen. The light dependence of oxygen production in these algae was studied by a repetitive-flash method, which allows a direct analysis of the steady-state kinetics of pool reduction. We propose a kinetic model which quantitatively accounts for these kinetics and several transient phenomena. This model centers on a novel cross reaction at the pool of photo and dark electron input and output, allowing a delicate poising of oxygen production by the environment. This model shows a positive feedback of oxygen on oxygen production.

INTRODUCTION

Inherent in the light-saturation curve of oxygen production in photosynthesis (oxygen rate *versus* light intensity) is considerable information on the organization and kinetics of the photosynthetic system. By detailed analysis of these curves it is possible to determine the extent of energy transfer between photosynthetic units[1] and to draw some mechanistic conclusions from the dependence of the oxygen rate on light intensity.

It has been generally observed that under aerobic conditions the light-saturation curve for oxygen production is linear at light intensities below saturation. Slight deviations from linearity, under these conditions, have been attributed to light-induced changes in the rate of respiration[2,3].

Franck *et al.*[4] observed that anaerobic conditions induce a non-linear dependence of oxygen production rate on light intensity. Franck *et al.*[4] also found that prolonged exposure to anaerobic conditions in the dark increased the length of the induction period for oxygen production and lowered the oxygen yield from single flashes of long duration (10 ms). These experiments were extensions of Gaffron's[5]

Abbreviations: PMS, phenazine methosulfate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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earlier observations that anaerobic conditions greatly prolonged the induction period but did not affect the final steady-state value of oxygen production. Herron and Mauzerall[6] showed that, at a 2 ppm background oxygen level, *Chlorella* cells displayed a light-saturation curve characterized by three distinct regions. These were a light intensity-independent rate of oxygen production at high light intensity, a linear dependence at moderate light levels, and a non-linear dependence (approximating second order) at low light intensity. These results were confirmed by Diner and Mauzerall[7] in the blue-green alga *Phormidium luridum* under anaerobic conditions; however, the non-linearity was not shown by a cell-free preparation isolated from this alga[7].

We will show, in this paper, that respiration effects are not the cause of the non-linearity observed under anaerobic conditions. We attribute this non-linear behavior to a reduction of photogenerated oxidizing equivalents, occurring between the two sequential photoreactions of photosynthesis. We will show how the background oxygen concentration offsets this reduction and regulates the ability of the photosynthetic system to make oxygen. Finally, we will present a kinetic model that incorporates these features and quantitatively explains our experimental results.

METHODS

The blue-green alga *Phormidium luridum* var. *Oliviceae* was obtained through the courtesy of Dr H. W. Siegelman and the green alga, *Chlorella vulgaris*, Beijerinck, Trelease strain was obtained through the courtesy of Dr S. Granick.

Phormidium was cultured at 23 °C in Kratz and Myers[8] Medium D at a continuous light intensity of $3 \cdot 10^3$ ergs/cm^2 per s, provided by cool-white fluorescent lamps. Cells were grown in 500-ml flasks placed on a rotary shaker and were used in the late stage of logarithmic growth (usually 5–7 days after inoculation).

Chlorella were grown on a mineral medium[9] and under the same conditions as *Phormidium*.

Oxygen measurements were performed with an oxygen luminometer[10,11], usually at a background oxygen concentration of 3 ppm O_2 in Ar. When necessary, the oxygen content of the flow gas was varied up to 412 ppm using calibrated gases (Matheson).

Algal cells were suspended in 2 ml of either 10 mM sodium succinate, 23 mM NaCl and 6.7 mM $CaCl_2$, pH 5.9, for coupling to Hill oxidants, or 50 mM $NaHCO_3$, pH 8.0, for coupling to CO_2. The cell suspension was placed in a horizontal cuvette which could be illuminated from above or below. The suspension, 2 mm in thickness and 3.6 by 2.2 cm, was rapidly equilibrated with the gas phase by means of a magnetic stirring bar. For greater sensitivity, a 6-ml circular cuvette (5.4 cm inner diameter) was used, which allowed for a suspension thickness of 2.4 mm. The equilibration times of the oxygen-detecting apparatus required that the cells remained under anaerobic conditions for 20 min prior to illumination. Longer incubation times lengthened slightly the induction time required to reach steady-state oxygen production rates, but had no effect on their absolute values.

Oxygen measurements were also performed using an oxygen polarograph, containing a large surface (0.785 cm^2) platinum electrode[12]. The polarograph was machined from lucite and contained a circular chamber 2.0 cm in diameter and 0.75

cm deep. The chamber was covered with a 1/8 inch lucite plate that, when clamped in place, made an air-tight seal with silicone grease. The chamber had O-ring fittings for gas inlet and outlet so that gases of known oxygen concentration might be interchangeably allowed to flow through it. The outflow could be monitored by the luminometer as an absolute check on the O_2 level. The floor of the chamber consisted of a 1-cm diameter platinum foil disc, countersunk 0.003 inch below the floor surface. This platinum electrode was cemented in place with epoxy glue and electrically connected to an external jack by a thin copper wire soldered to the underside of the platinum. A thin silver wire, the Ag/AgCl electrode, extended around the periphery of the chamber and was also connected to an external jack. The algal cells, suspended in 0.1 M KCl and 0.05 M $NaHCO_3$, pH 8.0, were usually placed directly on the platinum surface and covered by a cellophane dialysis membrane (Union Carbide) moistened with the same solution. This membrane provided electrolytic contact between the platinum disc and the Ag/AgCl electrode. The platinum electrode was polarized -0.60 *V* relative to the Ag/AgCl electrode.

Oxygen–argon mixtures, ranging from 3 ppm to 19500 ppm O_2 (10% of air), were prepared and analyzed by the Matheson Co. This gas was humidified by passing through a water bubbler. The gas flow rate was maintained at 30 ml/min using the flow controller valve of the luminometer.

Our oxygen polarograph cannot be used as an absolute measure of oxygen production, owing to difficulties of calibration and is thus usable only as a relative measure of oxygen rate. Nonetheless, the oxygen polarograph and the oxygen luminometer show a 1:1 correspondence throughout the five orders of magnitude range of oxygen rates encountered in our experiments. Because the luminometer response is clearly linear over this range of oxygen rates[10], we conclude that the response of the polarograph is also linear as well. As with the luminometer, the oxygen signal measured by the polarograph is the difference between the light-on and light-off currents. The total current in the polarograph, however, is not linear with oxygen at low partial pressures.

The oxygen concentration, observed by the algal cells on the platinum surface is probably within 20% of the oxygen concentration of the flow gas. Experiments performed on the oxygen polarograph at various oxygen concentrations (3–412 ppm O_2) were faithfully reproduced by the luminometer, indicating that in both methods the algal cells experience the same oxygen concentration despite the oxygen sink represented by the platinum surface of the former method. Our explanation is that the platinum electrode is an inefficient oxygen sink, taking up only a small fraction per s of the oxygen in the volume above it. At aerobic levels the current measured in the polarograph is $8 \cdot 10^{-6}$ A, which corresponds to an oxygen uptake by the electrode of $2 \cdot 10^{-11}$ mole O_2/s for the electrode surface area. The oxygen concentration in water equilibrated with air is $2.5 \cdot 10^{-4}$ M. If the volume above the electrode surface is 2 μl (corresponding to a 25-μm-thick layer set by the position of the dialysis membrane), then that volume contains $5 \cdot 10^{-10}$ mole O_2. Thus the oxygen consumption by the electrode is only 1/25 of the total oxygen content of this volume in 1 s. Because of rapid diffusion in the liquid phase (diffusion of oxygen in water at 25 °C about 1 ms/μm or 0.6 s/25 μm) the electrode must negligibly deplete the oxygen concentration of the solution above it. These arguments are in direct opposition to the usual picture of a linear O_2 gradient extending from the oxygen concentration at the gas–liquid interface (established by Henry's law) to a zero concentration at the platinum surface.

The continuous light source used in our experiments was a Leitz Prado projector, containing a 750-W tungsten lamp (Sylvania DDB) and an efficient heat filter. The light was filtered by Corning sharp cut-off filters 2-59 (> 635 nm) or 2-64 (> 665 nm) for red light and 3-66 (> 570 nm), 3-67 (> 555 nm) or yellow cellophane (> 480 nm) for yellow light, and where necessary in combination with 5 cm of water. For light-saturation curves, which required variation of the light intensities, calibrated Schott neutral density filters (ND-419) were used.

In flashing experiments, a Strobrite flash lamp (Type 3015, U.S. Scientific Instruments) delivered saturating flashes that were 5 μs duration at half height and 3% of peak height at 25 μs. In repetitive double-flash experiments, a second flash was provided by a Stroboslave (Type 1539-A General Radio) which delivered saturating 4-μs flashes (half height). Both flash lamps were filtered with the above-mentioned red or yellow filters. Light intensities were measured using a Radiometer (Model 65, Yellow Springs Instrument Co.) for continuous light and a Lite-Mike (Model 560B, EG and G Inc.) for flashing light.

For steady-state flashing experiments, oxygen production rate was measured by apparatus with a long time response to improve signal/noise. Where a fast time response was required, as when the magnitude of oxygen release by individual flashes was determined (Fig. 9) the polarograph was operated in a voltage clamp mode using a feedback amplifier circuit (Philbrick/Nexus Model 1021 operational amplifier). The output voltage signal was fed into a Sanborn recorder (Hewlett–Packard 7702A) with a response time of 10 ms. In this way, oxygen pulses with a half fall time of 50–100 ms could be observed without appreciable distortion.

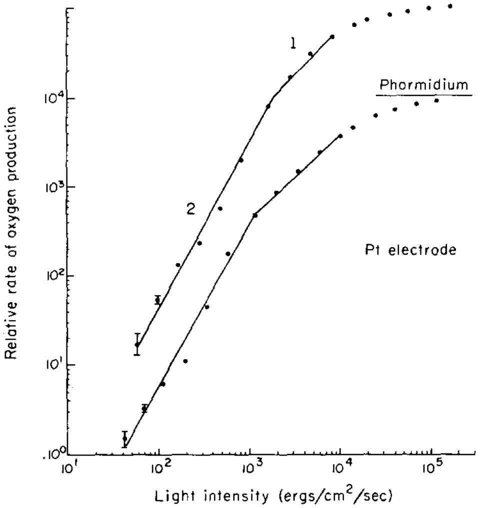
The chlorophyll content of *Chlorella* and *Phormidium* cells was determined by extraction according to the method of Vernon[13]. Extracted chlorophyll extinction coefficients were related to *in vivo* absorption, determined with opal glass windows[14], so that we could make rapid estimates of cell chlorophyll content. These relations are as follows: *Phormidium* cell-free preparation 1.0 A (*in vivo* at 680 nm) = 12.5 μg chlorophyll a/ml; *Phormidium* whole cells 1.0 A (*in vivo* at 678 nm) = 11.3 μg chlorophyll a/ml, and *Chlorella* whole cells 1.0 A (*in vivo* at 677 nm) = 29.4 μg chlorophyll $a+b$/ml.

RESULTS AND DISCUSSION

Light-saturation curves

Light-saturation curves for oxygen production in *Chlorella* and *Phormidium* under anaerobic conditions (3 ppm O_2) are shown in Fig. 1. They show the same light-saturated rate as under aerobic conditions and a first-order light intensity dependence for oxygen production. The difference between the aerobic and anaerobic curves (Fig. 2) occurs at low light intensity, where the latter show a break to non-linear intensity dependence while the more aerobic curves remain linear. This non-linearity is probably a common photosynthetic trait, as it is observed for the prokaryotic alga, *Phormidium* and for the eukaryotic alga, *Chlorella* which are separated by 1–2 billion years on the evolutionary time scale[15].

Respiration. Early explanations for non-linearities observed in the light saturation curves have involved light-dependent depression of dark respiration[2,3]. We offer



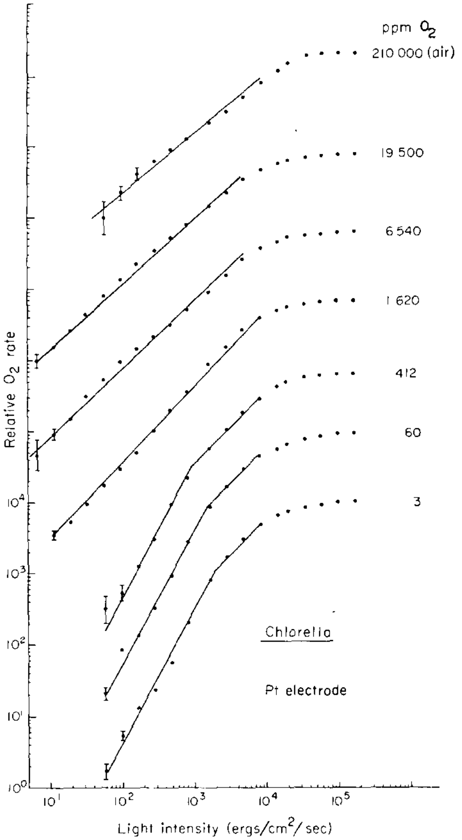


Fig. 1. Light-saturation curves for *Chlorella* and *Phormidium* coupled to CO_2 at 3 ppm O_2. The cells were illuminated with continuous red light (> 665 nm). These curves have been displaced with respect to each other to facilitate comparison.

Fig. 2. Light-saturation curves for *Chlorella*, coupled to CO_2, at the indicated oxygen concentrations (in ppm O_2). The gas-flow oxygen polarograph could only be used up to 19500 ppm O_2 because of alkaline conditions at the electrode surface for concentrations higher than this. The 210000 ppm O_2 run was performed in an oxygen polarograph similar to the one described by Joliot and Joliot[16]. Continuous red light (> 665 nm) was used. These curves have been displaced with respect to each other and the light-saturated rates are all the same.

the following arguments to make absolutely clear that, under anaerobic conditions, respiration effects play no role in the non-linear light dependence.

(1) A constant level of dark respiration cannot effect the shape of the light-saturation curve, but rather gives the oxygen production rate a constant negative offset.

(2) To alter the shape of our light-saturation curves, the respiration rate must represent an appreciable fraction (at least 5%) of the light-saturated oxygen rate and must vary as a function of the light intensity. That this light-dependent respiration is negligible in our experiments is shown as follows:

(a) A modulated light-saturation curve at 3 ppm O_2 (Fig. 3) shows the same

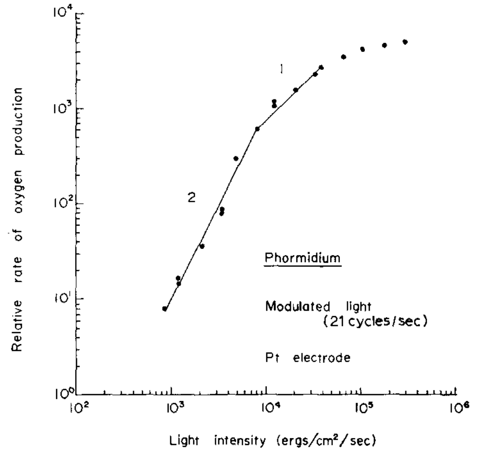


Fig. 3. Modulated light saturation curve with *Phormidium* cells coupled to CO_2 at 3 ppm O_2. Red light (> 665 nm) was modulated at 21 cycles/s. The oxygen signal was amplified by a picoammeter (Keithley Model 414S) and tuned amplifier (Type 1232-A, General Radio Co.) and detected by a phase-sensitive amplifier (HR-8, Princeton Applied Res.), locked to a reference voltage from the light chopper.

non-linear light intensity dependence as the continuous light curve. Joliot and Joliot[16] have demonstrated that light, modulated at > 5 cycles/s, is sufficient to exclude light-related O_2 uptake effects, which are slow, from oxygen production measurements. The identity of the modulated and continuous curves indicate that such respiration is negligible at 3 ppm O_2.

(b) Under fully aerobic conditions, *Chlorella* and *Phormidium* show a respiration rate 2–3% of the light-saturated oxygen rate. Under the 3 ppm O_2 atmosphere used for the most anaerobic light-saturation curve, respiration is greatly depressed. Cells placed in the oxygen luminometer in the dark caused no decrease in the oxygen concentration of calibrated gases of low oxygen content. Analysis of the data shows that dark respiration, at close to 3 ppm O_2 background, must have been depressed at least 100-fold from the aerobic level. Any variation in this level of respiration can only contribute negligibly to the region of non-linear light dependence which begins at approx. 10% of the light-saturated O_2 production rate (Figs 1 and 3). Kok[2,3] reported light-dependent changes in respiration rate that led to small non-linearities in the aerobic light saturation curves (Kok effect). Because of the extremely low level of respiration here, the Kok effect must be negligible. In fact, Healy and Myers[17] have also found that, in *Chlamydomonas*, the Kok effect is absent under anaerobic conditions.

We conclude from these arguments that light-induced respiration effects are not the cause of the non-linearity we observe under anaerobic conditions, but are instead due to a reversible decrease of active oxygen-producing centers at low light intensity.

Repetitive single-flash analysis

The analysis of this system is highly simplified by using repetitive short ($5 \cdot 10^{-5}$ s) saturating light flashes, during which every photosynthetic unit is excited only once.

By varying the frequency of pulsing, the relaxation of the photosynthetic system between flashes may be studied, each flash acting as a monitor of the activity of the photoreactions. This flash method is identical to that of Emerson and Arnold[18], except that the phenomenon under consideration here is slower than the turnover time of photosynthesis that they studied.

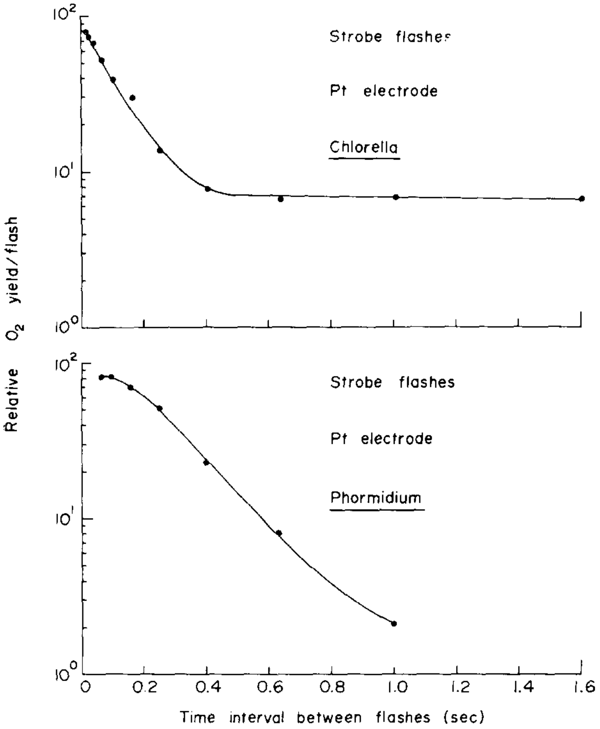


Fig. 4. Repetitive saturating single-flash curves for *Chlorella* and *Phormidium*, coupled to CO_2, at 3 ppm O_2. Red flashes (>635 nm) were used in the *Chlorella* experiment and yellow flashes (>555 nm) in the *Phormidium* experiment.

Typical periodic flash experiments are shown in Figs 4 and 6 for *Chlorella* and *Phormidium* coupled to CO_2. Saturating flashes were separated by the time intervals indicated and at each time point the steady-state oxygen yield per flash was determined. This type of plot is essentially a derivative of the continuous light-saturation curves of Fig. 1 and allows the loss kinetics of the non-linear region to be observed directly. The curve consists of a region of constant and maximal flash yield (between 25 and 175 ms flash separation for *Phormidium* and, less obvious, for *Chlorella* at this time scale <50 ms) corresponding to the linear region of the light-saturation curve. In this region, all oxygen-producing centers are active and are effectively excited in a saturating light flash.

As the time interval between flashes is increased (>175 ms for *Phormidium*, >50 ms for *Chlorella*) the oxygen yield per flash decreases, showing an initial exponential decay of 160 ms. Because each saturating flash determines how many oxygen-producing centers remain active, their relative concentration at each time point may be determined. This region of the flash curve corresponds to the non-linear region of the light-saturation curve.

As the time interval between flashes is shortened from the region of constant flash yield (<25 ms for both algae), the oxygen yield per flash again decreases (Fig. 2, ref. 19). This region of the flash curve corresponds to the saturated region of the light-saturation curve and is a measure of the turnover time of photosynthesis. We will discuss this matter of turnover in more detail in the following paper.

Temperature dependence of flash yield. We have found that this decrease of the oxygen flash yield is a strongly temperature-dependent process, as shown in Fig. 5. Repetitive single-flash experiments performed at 5.4, 15 and 24 °C at 3 ppm O_2 show an increase in the oxygen yield per flash at long time intervals as the temperature is decreased. In fact, the flash yield at 1 s between flashes is 10 times greater at 5.4 than at 24 °C. The temperature dependence of the initial decay rate corresponds to a $Q_{10} = 3.3$ which is even greater than that measured for the turnover time of photosynthesis ($Q_{10} = 2.2$, as described in the following paper[19]). Significant too, is the temperature independence of the maximum flash yield, an observation in agreement with the results of Emerson and Arnold[18]. We also calculate from the maximum flash yield the classical Emerson and Arnold unit size of 2500 chlorophyll molecules[20] per O_2 released.

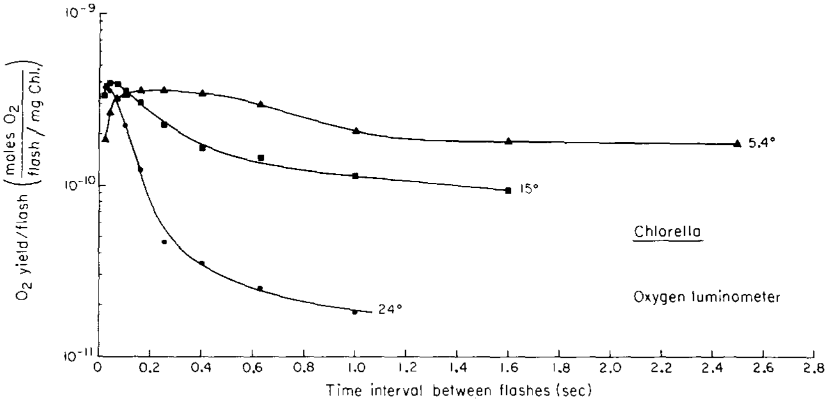


Fig. 5. Repetitive saturating single-flash curves for *Chlorella*, coupled to CO_2, at various temperatures. The luminometer cuvette was immersed in a circulating water bath that maintained the temperature at the desired level. Red flashes (>635 nm) were used. Total chlorophyll content of the 2-ml cuvette was 5.1, 5.9 and 12.1 μg for the 5.4, 15 and 24 °C runs, respectively.

A reductive reaction controls the flash yield. Having shown that kinetics of decreasing oxygen flash yield may be determined quantitatively, we will now provide arguments to show that this loss occurs by reduction. We will also attempt to localize the site of reduction.

(1) Replacing CO_2 as the electron acceptor, with benzoquinone, in *Phormidium*, abolishes the loss reaction (Fig. 6). The turnover time with benzoquinone (29 ms) is also markedly increased over that for CO_2 (6 ms, see following paper). That *Chlorella* responds similarly to benzoquinone was shown by Burr[9], who found that the anaerobic light saturation curve for these cells, coupled to $1.25 \cdot 10^{-3}$ M benzoquinone, was strictly linear below light saturation.

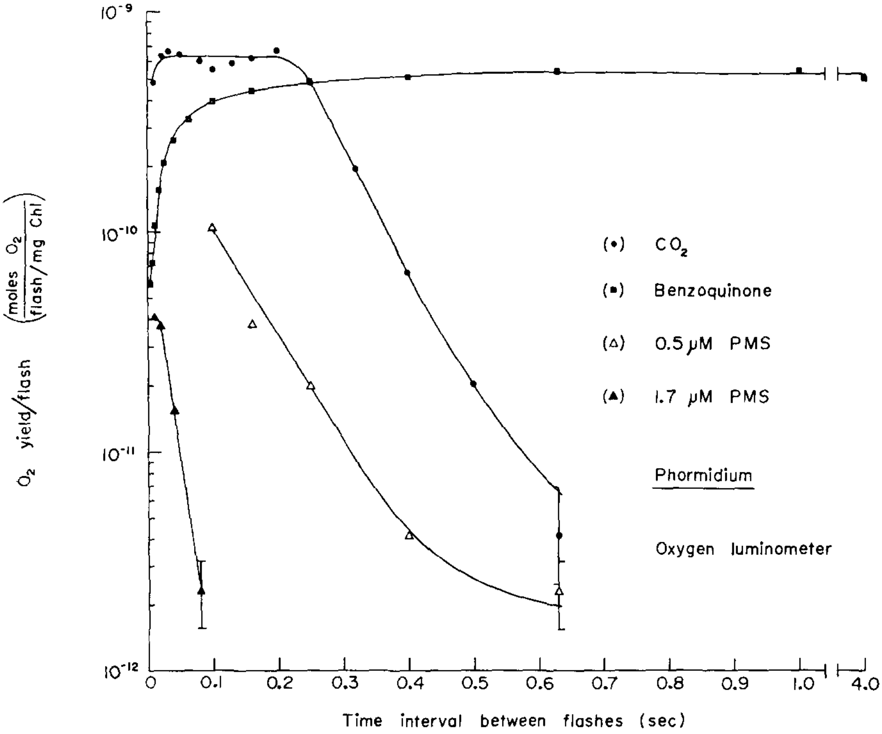


Fig. 6. Repetitive saturating single flashes, given to *Phormidium* at 3 ppm O_2, in the presence of CO_2, benzoquinone or phenazine methosulfate (PMS). Cells (8.9 μg chlorophyll *a*), suspended in 2 ml of the usual succinate buffer (pH 5.9) *plus* 0.25 mM benzoquinone, were given yellow flashes, (> 570 nm) (■). Cells (52 μg chlorophyll *a*), suspended in 6 ml of 0.01 M $NaHCO_3$ (pH 8.4), were given yellow flashes (> 480 nm) in the presence (▲) or absence (●) of 1.67 μM PMS. Cells (66 μg chlorophyll *a*), suspended in 6 ml of 0.01 M $NaHCO_3$ (pH 8.4) and 0.5 μM PMS (△), were given yellow flashes (> 480 nm).

Two other repetitive single-flash curves (Fig. 6) were performed in the presence of phenazine methosulfate (PMS) ($E_m = +0.08$ V) which gets into algal cells and is readily reduced by NADPH. This redox reagent has been commonly used, in photosynthetic organisms, for increasing the rate of reduction of electron transport components in the dark following their light-induced oxidation and in promoting cyclic electron transport. We find that PMS increases the loss rate and decreases the maximum attainable flash yield at concentrations of 0.5 and 1.7 μM. In fact 25 μM PMS completely inhibits O_2 production.

(2) Similar results are observed for the powerful oxidant, molecular oxygen. As the background oxygen is increased (Fig. 12) the O_2 yield per flash, at long time intervals between flashes, also increases.

(3) *Effect of far-red light on loss.* According to the traditional "Z scheme"[21], selective excitation of Photosystem I with far-red light should result in the oxidation of electron transport components on the reducing side of Photosystem II. We have found (Fig. 7) that continuous far-red light (730 nm) superimposed on red flashes, in a repetitive single-flash experiment, increases the oxygen flash yield observed at long

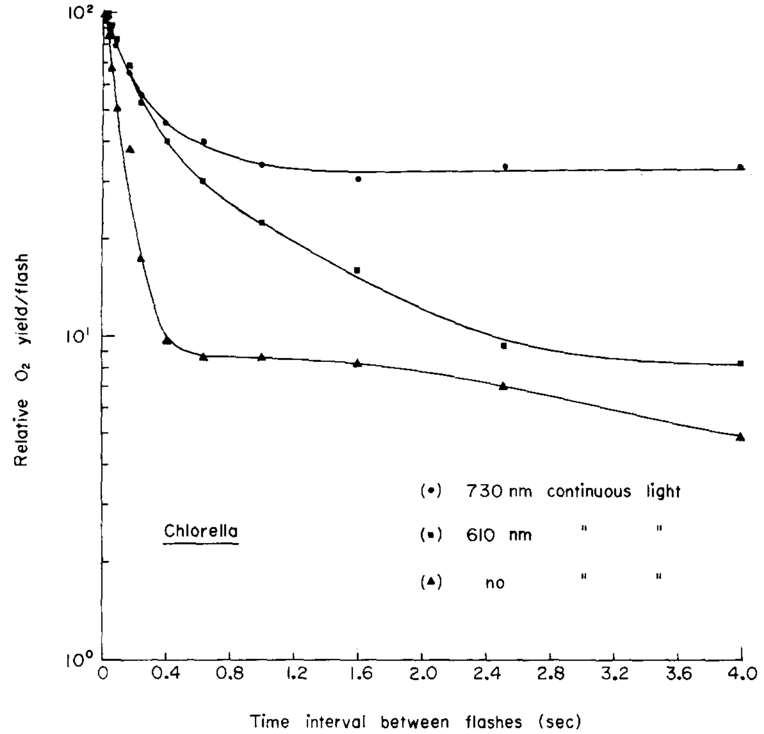


Fig. 7. Effect of continuous background illumination, superimposed on saturating repetitive flashes in *Chlorella*, coupled to CO_2, at 3 ppm O_2. Red flashes (>635 nm) were used. For far-red background illumination (●) continuous light was filtered through a sharp cutoff filter (>710 nm) *plus* a 730-nm Bausch and Lomb interference filter (half-width $\simeq 20$ nm). For near-red illumination (■), a sharp cutoff yellow filter (>570 nm) *plus* a 610-nm Bausch and Lomb interference filter (half-width$\simeq 20$ nm) were used. Intensities of far-red light ($2.1 \cdot 10^4$ ergs/cm² per s) and near-red light ($4.0 \cdot 10^2$ ergs/cm² per s) were adjusted to give the same low level rate of oxygen production (0.5%) of the O_2 rate obtained at one flash/16 ms).

times (1 flash/4 s) 7-fold over the yield without background illumination. Continuous near-red light (610 nm), adjusted in intensity so that the low level continuous O_2 rate produced by this illumination matches that of continuous far-red light, gives nearly the same O_2 yield/flash at long time as the no-background illumination experiment.

(4) *Phormidium cell-free preparation.* We have isolated from *Phormidium* a preparation that evolves oxygen in the light, when coupled to high potential one-electron oxidants like $K_3Fe(CN)_6$ (ref. 7). Because oxygen evolution in this preparation is insensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), we believe that $K_3Fe(CN)_6$ couples between the DCMU-sensitive site and Photosystem II, thus by passing the electron transport chain and Photosystem I. As shown in Fig. 8, a repetitive single-flash experiment with this preparation, at 3 ppm O_2, shows essentially no loss. The conditions for this experiment assure the presence of an oxidized acceptor to Photosystem II, as long as the turnover time is not exceeded.

In sum, these experiments show that low concentrations of reductant (PMS)

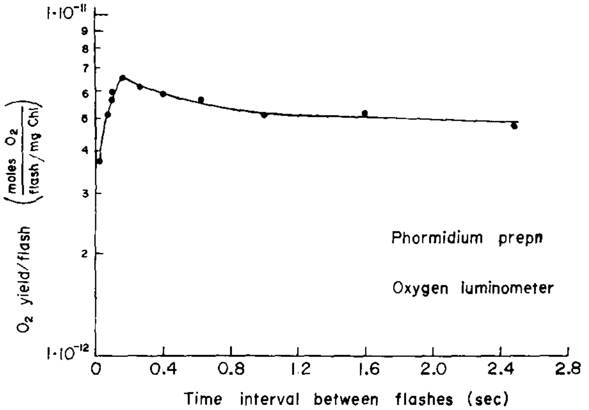


Fig. 8. Repetitive saturating single-flash curve for the *Phormidium* cell-free preparation coupled to $K_3Fe(CN)_6$ at 3 ppm O_2. The preparation (0.14 mg chlorophyll *a*) was suspended in 6 ml of the usual succinate buffer (pH 5.9) containing 0.67 mM $K_3Fe(CN)_6$. Yellow flashes (> 480 nm) were provided by three flash lamps firing simultaneously.

enhance the loss rate, while oxidants (O_2, benzoquinone or far-red light) diminish or obliterate it. We conclude that a reduction is responsible for the loss in oxygen yield per flash for coupling to CO_2 under anaerobic conditions.

The reduction site on the reducing side of Photosystem II

The far-red light and cell-free preparation experiments suggest further that the site of reduction lies on the reducing side of Photosystem II.

There is additional support for this claim:

(1) Duysens and Sweers[22] suggested that a high quantum yield of fluorescence results from the reduction of the electron carriers linking the two photosystems of photosynthesis. Their argument was that excitation of Photosystem II (the predominant source of fluorescence emission) would result in a greater fluorescence yield if the primary electron transfer were blocked by a reduced Photosystem II electron acceptor.

Franck *et al.*[4] and later Malkin[23] have reported a rapid rise to a high quantum yield of fluorescence for *Chlorella* cells and spinach chloroplasts, respectively, following incubation in the dark, under anaerobic conditions. Because of the disappearance of the usual fluorescence induction phase under these conditions the pool must have become reduced prior to illumination, this reduction occurring between the two photoreactions.

(2) Butler[24] has reported that, for a leaf that is placed in the high fluorescence state by strong white illumination, the return to the low fluorescence state (oxidation of Q) is retarded by anaerobic conditions. Admission of air rapidly returned the photosynthetic system to the low fluorescence state.

It seems clear that the presence of oxygen is required for the rapid reoxidation of the Photosystem II acceptor in the dark.

The localisation of the reduction to the reducing side of Photosystem II further implies that the oxidative action of O_2 and benzoquinone occurs either at this site or directly with the reductant. Further discussion in this and the following paper, as well as the above-cited experiment of Butler's, support the former of these two possibilities.

Deactivation not responsible for decreasing flash yield. While we have shown that the primary target site for the reduction lies between the photoreactions, there remain two explanations for the decreasing oxygen flash yield in anaerobic repetitive single-flash experiments. The first of these is the deactivation of oxygen precursor states (particularly S_3)[25,26,27] on the oxidizing side of Photosystem II. As demonstrated by Lemasson and Barbieri[28], this deactivation is appreciably accelerated by the reduction of the pool between the photoreactions. The second possibility is that the reduction of the electron carriers and in turn the Photosystem II electron acceptor are solely responsible for the decreasing flash yield. We will demonstrate by the following arguments that the first of these two possibilities is inconsistent with our results.

(1) Lemasson and Barbieri[28] used continuous light preillumination, followed at various time intervals by one or two flashes, to determine the effect of the redox state of the pool on the rate of deactivation of states S_2 and S_3 in *Chlorella*. The most rapid deactivation ($t_{\frac{1}{2}} = 1.5$ s) was observed for S_3 under preilluminating conditions that nearly fully reduced the pool. This decay time is 10 times too slow to account for the loss kinetics observed for *Chlorella* in the anaerobic repetitive single-flash curves.

(2) We considered the possibility that the lifetimes of the states were shortened further under anaerobic conditions. A train of 4 saturating flashes was given to *Chlorella*, following 5 min of anaerobic dark adaptation (Fig. 9). The yields of the first four flashes were compared for flash sequences of 1, 10 and 20 flashes/s and were found to be constant despite the change in flash frequency. No oxygen was evolved from the first two flashes. The steady-state flash yields, however, were quite different and

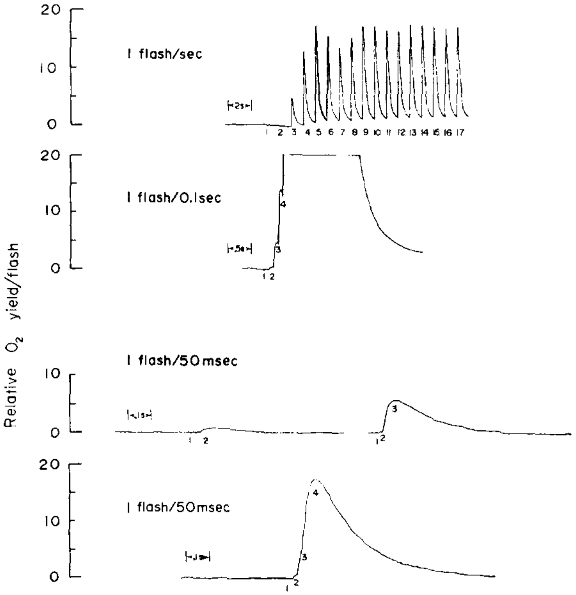


Fig. 9. Transient flashing of *Chlorella* at 3 ppm O_2, following 5 min of dark adaptation. Cells were suspended in the usual bicarbonate buffer (pH 8.0) and assayed in the oxygen polarograph. The polarograph was operated in a voltage clamp mode using a fast feedback amplifier (see Methods). Saturating flashes (> 635 nm) were given at the indicated time intervals. All measurements are to the same vertical scale and are directly comparable.

are given in Fig. 4. If S_2+S_3 were to have decayed to S_1 and S_2, respectively, in 160 ms, then one would have expected a much higher oxygen yield for the third and fourth flashes for the 20 flashes/s train than for that at 1 flash/s. This was clearly not the case and S_2 and S_3 must live for appreciably longer than 1 s.

(3) The final proof against the oxidizing side deactivation comes from repetitive steady-state double-flash experiments. In one typical experiment with *Chlorella* (Fig. 3b of the following paper[19]) flash pairs were given each second while the time interval between the members of flashes pair was varied. If S_3 were to have decayed in 160 ms, then a second flash following the first by a time shorter than this, should convert S_3 to S_4 producing oxygen and preventing the reductive loss. What we observe, instead, is an oxygen yield for the second flash no larger than the first.

Thus, at best, the second flash converts the same number of S_3 states to S_4 as the first, despite the fact that the first flash was preceded by a time interval of 1 s and the second flash by a time interval of 50–100 ms.

We conclude that deactivation on the oxidizing side of Photosystem II is not responsible for the reductive loss observed in anaerobic repetitive single-flash experiments.

Pool of electron carrier. The double-flash experiment also establishes certain criteria for the reducing side model. Apparently, the oxidizing equivalent generated by Photosystem I during the flash is not expressed as an increase in the oxygen yield of the second flash. One would have predicted from the *Chlorella* repetitive single-flash curve of Fig. 4 that the oxygen yield of the second flash should approach the maximum of this curve or 10–20 times the flash yield at low pulse rate. This experiment implies the existence of a large number of reducing equivalents between the two photoreactions, which minimize the effect of a single oxidizing equivalent on the electron acceptor to Photosystem II.

This argument is strengthened by repetitive multiple-flash experiments, in which a train of up to five saturating flashes were given each second. In an experiment in which the members of the train were separated by 20 ms, the oxygen yield of the fifth flash was only double the first. Thus the number of equivalents between the two photoreactions must be appreciably greater than 5.

An alternate explanation for these observations is that the acceptor to Photosystem I, X, is present in limiting concentrations. This means that the Photosystem II acceptor, Q, could not be reoxidized until X slowly recovered from an earlier flash. This explanation is unlikely, in that the turnover time of photosynthesis (*i.e.* the rate-limiting step that determines the maximum photosynthetic rate) is 5 ms at 24 °C, as determined by repetitive single flashes (see following paper[19]). The restoration of X must be at least this fast, if not faster, while the shortest time interval between flashes in the repetitive multiple-flash experiments was at least several times this 5-ms time. It is clear that X is fully functional for each flash in these experiments. The problem was resolved with an experiment in which a long train of intense far-red flashes (20/s), more effectively exciting Photosystem II then I, were given to *Chlorella* following a period of anaerobic dark adaptation. The half-maximum yield was attained after approx. 20 flashes. This last experiment and the apparent inability to observe an enhanced flash yield (to oxidize Q) in repetitive double- and multiple-flash experiments all imply the presence of a pool of electron carriers between the two reaction centers, which requires many flashes to empty. Only when the pool is emptied of electrons (*i.e.* oxidized) is the maximum flash yield obtained. The ability to oxidize the pool by rapid flashing, in repetitive single-flash experiments, suggests that when the pool is reduced, Photosystem I removes more reducing equivalents per red flash than Photosystem II contributes. It will be shown in the kinetic discussion to follow that a pool of appreciable size is required to satisfy not only transient flash experiments, but for a kinetic description of the steady state as well.

Other photosynthesis workers have also found evidence for a pool located close to Photosystem II. Joliot[29] estimated a pool size of 10–15 equivalents in *Chlorella*. He arrived at this number by measuring the size of the oxygen gush on transient illumination. Forbush and Kok[30] estimated a pool size of about 20 equivalents from measurements of the fluorescence induction curve in spinach chloroplasts. Finally, Stiehl and Witt[31] have identified a pool of 7 plastoquinones (14 equivalents) and 5 plastoquinones (10 equivalents) in spinach chloroplasts and *Chlorella*, respectively, using optical absorbance change measurements.

A further conclusion from the inability to save Q in repetitive double-flash experiments is that the pool (A) and Q are related by a small equilibrium constant. If this were not the case and electron transfer to the pool were strongly favored (large K) then the contribution of an oxidizing equivalent to the pool by Photosystem I on the first flash would oxidize Q and give a large enhancement of the oxygen yield on the second flash. Because there is no such enhancement Q and A must have fairly close redox potentials (small K).

Kinetic models. We propose two kinetic models that incorporate some of the kinetic features that we have discussed. These schemes differ in that they postulate two different roles for oxygen:

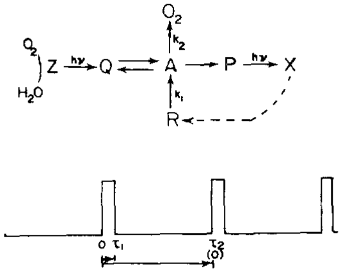
(1) In which oxygen interacts directly with the electron transport chain *via* the pool A (Fig. 10).

(2) In which oxygen interacts directly with reductant R, instead of A, controlling the concentration of reductant available to reduce the pool.

We will discuss the first scheme (Fig. 10) in some detail, followed by a brief discussion of the disagreement of the second scheme with our results. These schemes contain two photocenters, *i.e.* donor–acceptor couples Z–Q and P–X. The electron transport chain consists of a pool A of $n-1$ members in equilibrium with Q. A and Q are related by a redox equilibrium constant, K, equal to 1. The justification for this low equilibrium constant derives from our inability to observe an enhanced second flash in repetitive double-flash experiments and from the work of others[30,32]. P is maintained in a reduced state by A[33–35].

In both of these schemes the ability of photocenter Z–Q to respond to a saturating flash is determined by the oxidation state of the acceptor Q. If Q is fully reduced then excitation of the photocenter cannot result in a net throughput of an electron from water to Q and no oxygen is produced. When Q is oxidized, excitation results in a transfer of one equivalent from water to Q. In light of earlier results we can safely assume that oxidizing equivalents on the oxidizing side of the photocenter are stable in our time scale and that once four of these are accumulated an oxygen molecule is produced. A single successful excitation of Z–Q thus produces 1/4 of an O_2 molecule.

A is indirectly affected by the operation of the two photoreactions (*i.e. via* Q and P). It is reduced by Q and oxidized by P. There are two non-photochemical phenomena that affect the redox state of A. One is the reduction of A by reductant R.



$$(AQ)_f = -(AQ)_i\frac{b}{n} + (AQ)_i + (AQ)_0\frac{b}{n} \tag{1}$$

$$(AQ)_0 = Q + Q^- + A + A^- \tag{2}$$

$$b = \sum_{x=x'}^{\infty}\frac{e^{-a}a^x}{x!} \tag{3}$$

$$\frac{d(AQ)}{dt} = k_2O_2(AQ)^- - k_1R(AQ) \tag{4}$$

$$\frac{O_2}{\text{flash}} = \frac{1}{4}\frac{(AQ)_i}{(AQ)_0} = \frac{1}{4}\left[\frac{bk_2O_2(1-e^{-m\tau_2})}{m(1+(\frac{b}{n}-1)e^{-m\tau_2})} + \frac{b^2e^{-m\tau_2}}{n(1+(\frac{b}{n}-1)e^{-m\tau_2})}\right] \tag{5}$$

$$m = k_1R + k_2O_2 \tag{6}$$

Fig. 10. Kinetic model and derived expression for O_2 per flash.

The other, in the first model, is the oxidation of A by O_2. In the second model it is the concentration of R that is affected by O_2 and thus indirectly the redox state of A.

We have derived a kinetic expression from the first scheme that is based on repetitive single-flash excitation (Fig. 10), where long trains of light flashes establish a steady state. Flash duration, τ_1, is much shorter than the time interval between flashes, τ_2. Because the system is driven into a steady state, the concentrations of Q, A, and P are perturbed by a flash, but then return to their respective pre-flash levels.

Because A is the focal point of the scheme, we will be concerned, first with the effect of dark and light events on its redox state and, second, with how it affects the redox state of Q, which in turn determines the oxygen yield in a light flash. Because A and Q are related by redox equilibrium constant $K = 1$ they may be combined into one pool (AQ) with n members. The total concentration of (AQ) in the sample, $(AQ)_0$, is related to the total concentration of Q in the sample, Q_0, by $Q_0/(AQ)_0 = 1/n$.

If (AQ) represents the concentration of oxidized pool members immediately prior to a light flash, and $Q/Q_0 = (AQ)/(AQ)_0$ resulting from the assumed redox equilibrium constant, $K = 1$, then the concentration of oxidized Q before a flash $Q_i = (AQ)_i/n$. When photoreaction Z–Q is excited by a saturating flash, all Q_i is reduced and the concentration of reducing equivalents donated by Z to (AQ) is $(AQ)_i/n$.

For light flashes of less than saturating intensity, $(AQ)_i/n$ must be multiplied by the probability, b, that a reaction center will be effectively hit during that light flash. The distribution of photon hits on reaction centers, from which this probability is calculated, is expressed by a Poisson distribution.

This probability factor b may be calculated from the cumulative Poisson ratio Eqn 3 (Fig. 10) where b is the probability that a reaction center will receive x' or more hits during a light flash. a is the average number of hits per reaction center per light flash and is equal to σI, where σ is the optical cross section for an effective hit and I is the photons per unit area per flash (*i.e.* integrated intensity). $b = 1$ for saturating flashes. By varying a (*i.e.* I) it is possible to determine how many hits per flash, x', are required to operate the reaction centers. We will discuss this analysis in a later section.

Returning to the kinetic scheme, the concentration of reducing equivalents contributed by Z to Q may be expressed as $(AQ)_ib/n$ for a flash of any intensity. This term is also 4 times the amount of oxygen produced by a light flash. At the other end of the chain, P, following excitation by a saturating flash, oxidizes A. Because P is maintained in a fully reduced state by A, it removes a full reducing equivalent from A in each saturating flash. This full equivalent may be expressed as $(AQ)_0/n$. Again, for a flash of any intensity $(AQ)_0 b/n$ reducing equivalents are removed by P.

Thus, after a light flash the concentration of oxidized pool in the sample, $(AQ)_f$, may be expressed by Eqn 1 (Fig. 10). $(AQ)_i$ is the concentration of oxidized (AQ) before the flash, $(AQ)_0 b/n$ are the oxidizing equivalents contributed (or reducing equivalents removed) by P, and $(AQ)_i b/n$ are the reducing equivalents contributed (or oxidizing equivalents removed) by Z.

By using the same probability parameter b for the terms contributed by both P and Z, we have tacitly assumed that the optical cross sections of the two photocenters are the same. As a first approximation in our theory, and considering that our experimental flashes are broad band red (>635 nm), our assumption of equivalent cross-sections is not an unreasonable one to make. In addition, differences in optical cross-section are of no concern where saturating flashes are used.

The kinetic analysis of this scheme is based on recurrence analysis. That is, before each flash, the system relaxes to the situation that existed immediately before the previous flash. If the flash frequency is kept constant, then, in the steady state, each flash sees the system in the same state. The relaxation of the system between flashes is determined by the opposing reduction of (AQ) by R and oxidation of $(AQ)^-$ by O_2. This relaxation following a flash is expressed by Eqn 4 (Fig. 10).

If we assume R and O_2 constant (*i.e.* pseudo-first-order approximation) then we can derive the final expression Eqn 5 (Fig. 10), where τ_2 = time interval between flashes.

Oxygen uptake. Because the reduced members of pool (AQ) consume a small amount of oxygen, the above equation must be corrected by subtracting from the O_2 yield per flash the term

$$\frac{1}{4}\left[\left(1-\frac{(AQ)_i}{(AQ)_0}\right)-\frac{k_1 R}{m}\right]k_2 O_2 \tau_2$$

where $(AQ)_i/(AQ)_0$ is the fraction of pool members in the oxidized state, a term that is readily calculated (Eqn 5). At low background O_2, $k_2 O_2$ is small and the contribution of this O_2 uptake term to the calculated theoretical curve is negligible. At high O_2 the difference between the two terms in brackets is small and the entire term is again negligible. Only between certain oxygen concentrations (*i.e.* 6540 and 19500 ppm O_2) is the term of any significance. Here too, however, the correction is only about 3% of the points on the curve. This again confirms our earlier claim that the effect of light on O_2 uptake is only a second-order correction in the steady state and we will not refer to it again.

Determination of $k_1 R$ and n. By allowing the experimental data to fix the oxygen yield per flash at long times (*e.g.* 1.55 s) a family of curves may be generated giving identical yield at this time but with different combinations of pool size and decay constant. Fig. 11 shows such a family of curves for pool sizes from 1 to 500. In this manner the best fit at 3 ppm O_2 was obtained with a pool size of 50 and a pseudo-first-order decay constant $k_1 R = 0.40\ s^{-1}$.

Determination of k_2. In order to determine the rate constant k_2 for oxygen oxidation of the pool, we refer to Fig. 12 which shows repetitive single-flash experi-

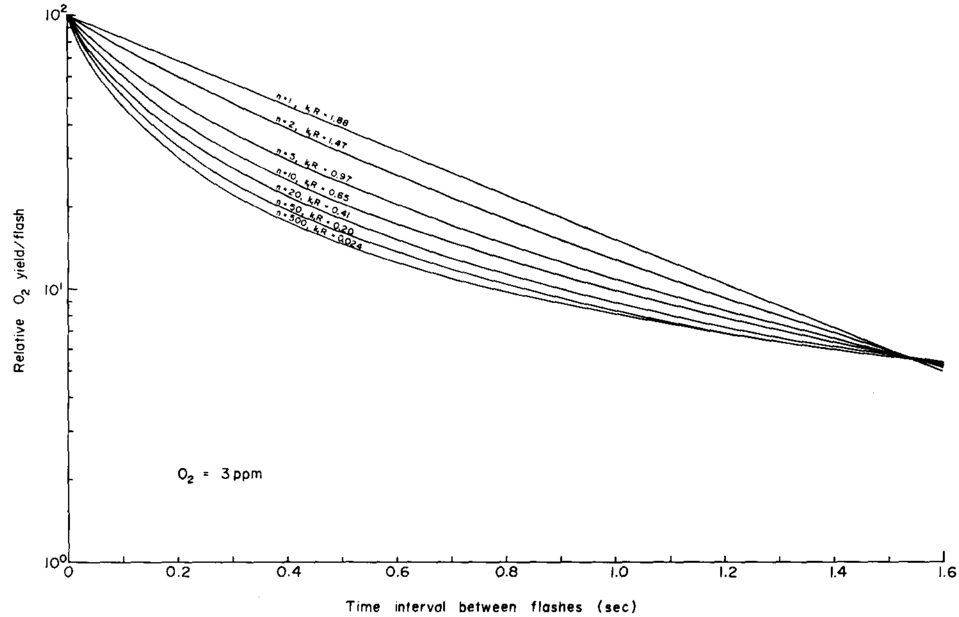


Fig. 11. Theoretical repetitive single-flash curves calculated from the kinetic scheme (Fig. 10) for a constant flash yield at 1.55 s and various pool sizes. If the flash yield is specified at long time, then the value of k_1R is fixed by the pool size.

ments for *Chlorella* performed at the indicated oxygen concentrations. The curves at 60 ppm O_2 and above show flash yields that become independent of time at long time intervals between flashes. These constant flash yields indicate that at long times the rate of reduction of the pool by R is balanced by the rate of oxidation by O_2. Therefore, at long time, d(AQ)/dt=0 in Eqn 4 (Fig. 10). Knowing k_1R, AQ and O_2, k_2 may be calculated from this equation.

Best agreement between theoretical repetitive single-flash curves and the experimental data of Fig. 12 was obtained with $k_2 = 1 \cdot 10^4\ s^{-1} \cdot M^{-1}$ ($1 \cdot 10^{-5}\ s^{-1} \cdot ppm^{-1}$)[11].

Theoretical flash curves. Theoretical curves were calculated for repetitive single-flash experiments from 3 to 19500 ppm O_2, using the rate parameters discussed above for *Chlorella*. These curves are shown in Fig. 12 (solid lines) and are compared to the experimental data. Reasonably good agreement is obtained. For the *Phormidium* repetitive single-flash curve, rate parameter $k_1R = 3.5\ s^{-1}$ and a pool size $n=2$ give a good fit to the data points (Fig. 13). This small pool size is consistent with the considerable (3–4 times) enhancement of the second flash relative to the first in repetitive double-flash experiments with *Phormidium* (Fig. 3C following paper[19]). The constant flash yield at short time intervals (Fig. 13) is accounted for in the following paper.

Poising of flash yield by R and O_2. It is apparent from the theoretical curves of Fig. 12 that our cross reaction theory (Fig. 10) results in a steady-state redox poising of pool (AQ) (*i.e.* constant flash yield at long times). This poising occurs because of the opposing actions of R and O_2 on the redox state of pool (AQ). An examination of the derived kinetic equation shows that this poising ability arises from the ratio $k_2O_2/k_2R+k_2O_2$ appearing in the first term of that equation.

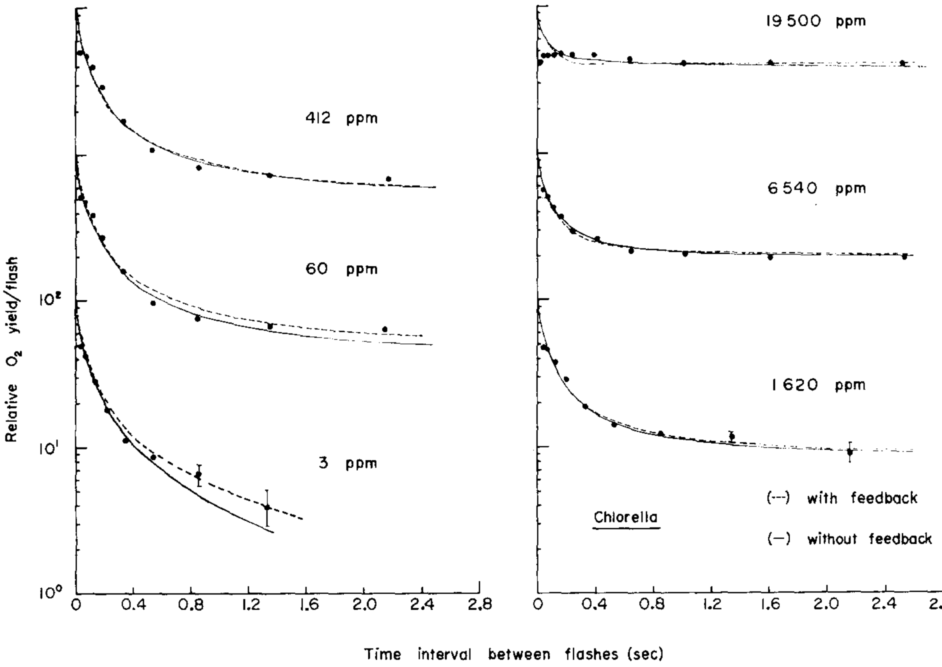


Fig. 12. Repetitive saturating single-flash experiments with *Chlorella*, coupled to CO_2, at various background oxygen concentrations (in ppm O_2). Cells were assayed on the oxygen polarograph in the usual manner (see Methods). Red flashes (> 635 nm) were used. These curves have been displaced with respect to each other and the maximum flash yields at short time are all the same. The data are compared to the theoretical repetitive single-flash curve calculated from the feedback model (– – – – –) (Fig. 14) and the model without feedback (———) (Eqn 5, Fig. 10).

Let us return briefly to a consideration of the second kinetic scheme, mentioned at the start of this section, in which O_2 oxidizes R instead of A. We assume here, as we did for the first scheme, that R is large with respect to A and that reduction by R can be treated ae pseudo-first order. This assumption is reasonable in that neither rapid pulsing nor high oxygen concentration appear to exhaust the supply of R. We have derived a kinetic expression from the O_2 oxidizing R scheme which contains only a single term resembling the second term of Eqn 5 (Fig. 10) (*i.e.* a continuous monotonic decrease in the ratio $(AQ)/(AQ)_0$). Because the kinetic expression for the second scheme is unable to demonstrate the poising of pool (AQ) we reject direct oxygen-mediated oxidation of R, as postulated by this model.

Feedback model. Theoretical curves calculated from Eqn 5 and compared to the repetitive single-flash data (Fig. 12) are somewhat shallower, for a pool size of 50, than the experimental data. An improvement in this fit would be obtained by increasing the pool size, however, even a pool of 50 has probably exceeded the size of the largest electron transport pool.

The problem is resolved if X or some diffusible species reduced by X could make a small contribution to R. The concentration of reductant would then become dependent on the pulse rate, *i.e.* on the rate of excitation of P–X. This feedback leakage of reductant back into the pool is not an unreasonable assumption in that this is essentially what occurs in cyclic electron transport around Photosystem I. We

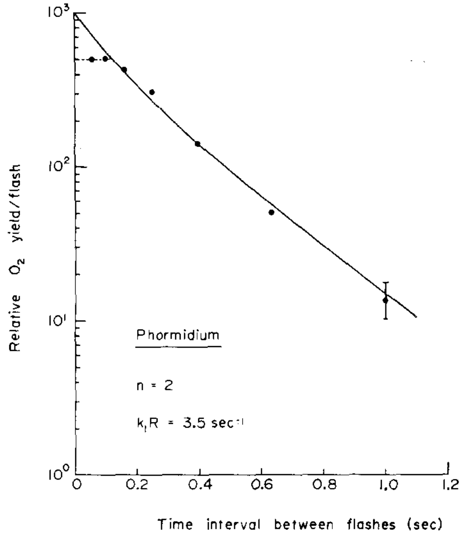


Fig. 13. Repetitive saturating single-flash curve for *Phormidium*, coupled to CO_2, at 3ppm O_2 compared to a curve calculated from the scheme without feedback (Fig. 10). The data are fit by a pool size of 2 and a loss rate of 3.5 s^{-1}. We will discuss in the following paper the redox state of the pool at maximum flash yield (dotted line).

note that it also provides symmetry to our scheme, both photoreactions providing feedback control over oxygen production, X by contributing to R, Z by contributing to O_2. A kinetic expression was derived for this revised model and is given below (Fig. 14), where a reduced species R_0, reduced by X, decays away with exponential rate constant k and k_1, k_2 and m are as before.

$$\frac{O_2}{\text{flash}} = \frac{1}{4(e^{m\tau_2 - Z} + (\frac{b}{n} - 1)e^{-k_1R_0/k})}\left[k_2 O_2 b \Gamma_z + \frac{b^2 e^{-k_1R_0/k}}{n}\right]$$

$$\Gamma_z = -\frac{1}{k}\left(\frac{k}{k_1R_0}\right)^{-m/k} \int_{\frac{k_1R_0}{k}}^{z} z^{-(m/k+1)} e^{-z}\, dz \qquad z = \frac{k_1R_0}{k} e^{-k\tau_2}$$

Fig. 14. Equation for model with feedback.

A good fit is obtained between the theoretical curves and the data if the concentration of R_0 is allowed to decay away with a first-order rate k of 3 s^{-1}. If this reductant were trapped within the photosynthetic membrane (thylakoids) and could interact with many electron transport chains before being used for CO_2 reduction then this slow rate constant might be accounted for. In any case no more than 20% of the reductant is recycled. The other kinetic parameters in this scheme are similar to the simpler model: $k_1R = 0.5$ s^{-1}, $k_2 = 1 \cdot 10^4$ s$^{-1} \cdot$M^{-1}, and, $k_1R_0 = 0.4$ s^{-1}. The pool size, however, has been appreciably reduced to 20 members. Theoretical curves computed for this scheme (dashed lines) are compared to the experimental repetitive flash data in Fig. 12 and show an improved fit to the data over the simpler scheme. We have left out of our model any consideration of the turnover time of photosynthesis. Because the turnover times for *Chlorella* and *Phormidium* are approximately 5 ms at 24 °C, as measured by repetitive single flashes[19] they are then too rapid to be of much significance in a discussion of the slower decay phenomena. The kinetics of turnover, however, will be considered in the following paper[19].

Number of photons required to operate photoreactions. Returning to a consideration of the probability parameter *b*, we pointed out that we could determine how many photons were required to operate photoreactions P–X and Z–Q. Using our theory (feedback scheme) we can calculate pulsed light-saturation curves for either one or two photons required to produce a stable intermediate in the production of an O_2 molecule. These curves were calculated for 3 and 19500 ppm O_2 background and 1 flash every 250 and 16 ms, and were compared to actual pulsed light saturation curves for *Chlorella* (Fig. 15). Best agreement is obtained between experiment and the one-hit curve (solid line) for all four conditions. The two-hit curve (dashed line) shows only a poor fit at 3 ppm O_2, 1 flash/16 ms and clearly does not fit in any of the other conditions. We conclude, therefore, that in the steady state, the photoreactions of photosynthesis require only a single photon hit per reaction center per flash for successful operation. We note that our theory shows a slight S-shape for the 3 ppm O_2 curves even though a single photon is required per center. This S-shape at low light intensity, apparent in both theory and experiment, is indicative of the non-linear light dependence that has concerned us in this paper.

Oxygen rate transients during illumination. There is another pool adjustment phenomenon that we have observed in light-saturation curves. Fig. 16 shows the raw data for a light-saturation curve for *Phormidium* at 3 ppm O_2 and the relative quantum yield (R/*I*) *versus* intensity (*I*) curve derived from this data. At high light intensity (sat-

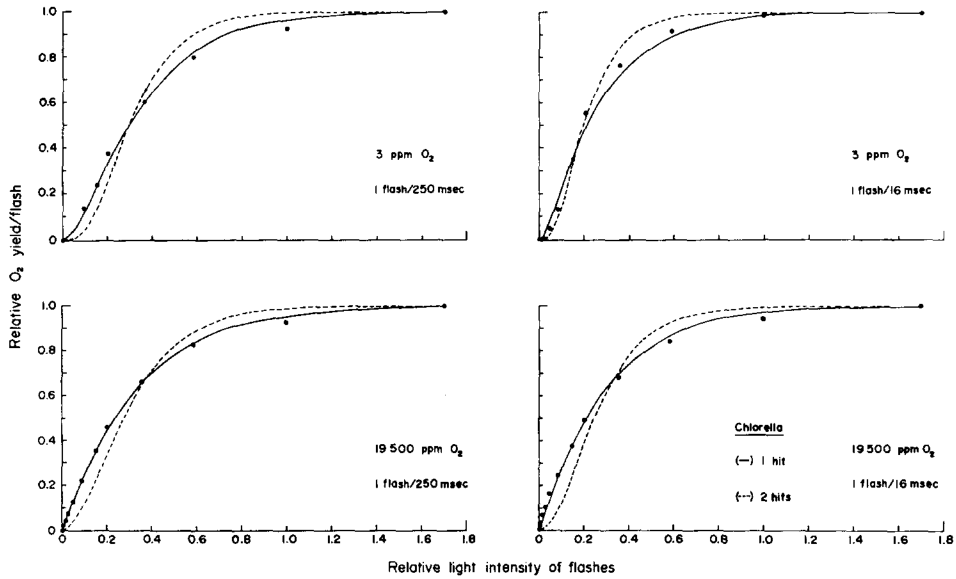


Fig. 15. Pulsed light-saturation curves for *Chlorella*, coupled to CO_2, at the indicated background oxygen concentrations and flash rates. Cells were assayed on the oxygen polarograph in the usual manner (see Methods). The intensity of the red flashes (> 635 nm) was varied with neutral density filters. Data points (●) are compared to single (———) and double (— — —) hit curves calculated using the feedback scheme (Fig. 14).

urated region of the light saturation curve) the pool is reduced because the rate of pool reduction has exceeded its rate of oxidation (see following paper[19]). As the light intensity is decreased the oxygen production rate undergoes a transient overshoot to below the steady-state rate followed by a gradual increase to the steady-state rate. The reason for this overshoot is that, after a small decrease in light intensity, the quantum yield for O_2 production remains the same until the pool accommodates ($(AQ)/(AQ)_0$ increases) to the new lower light intensity. This process is represented by

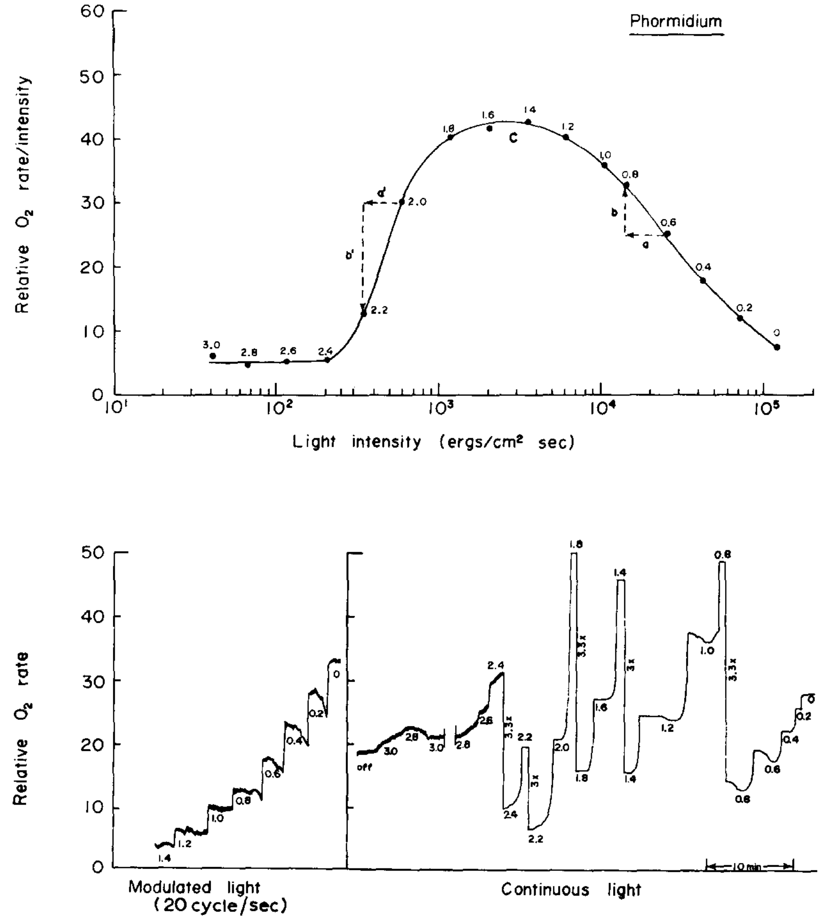


Fig. 16. Relative quantum yield of oxygen production (R/*I*) *versus* light intensity, from a light saturation curve at 3 ppm O_2, is compared to the raw data of the same experiment (*Phormidium*, coupled to CO_2). In this experiment the light intensity was decreased from the saturating light level. The arrows a to *b* and *a'* to *b'* indicate that following a small decrease in the light intensity there is an appreciable lag in the accommodation of the quantum yield to the new light level. This lag results in an overshoot at high light intensity and an undershoot at low light intensity. Comparison to modulated (20 cycles/s) light-saturation curve data indicates that these transients are not caused by transient oxygen uptake. Cells were assayed in the oxygen polarograph in the usual manner (see Methods). For both the modulated and continuous light-saturation experiments red light (> 665 nm) was used.

arrows *a* and *b* in the R/*I versus I* curve. As the pool accommodates, the quantum yield increases and the new light intensity and quantum yield determine the new steady-state rate. As the light intensity is further decreased the R/*I versus I* curve enters a region (indicated by *c*) of constant and maximum quantum yield. In this region the pool has achieved equal to or greater than half oxidation (see following paper[19]), so that the O_2 quantum yield has become insensitive to the pool oxidation state. This is reflected in the very rapid accommodation to any small change in the light intensity without over or undershooting. As the light intensity is decreased further still, and the pool becomes reduced, the O_2 quantum yield decreases. In this region, transients are the reverse of those observed in the region of rising quantum yield. As the light intensity undergoes a small decrease, the quantum yield momentarily remains the same (*a'* then *b'*) giving a higher than steady state oxygen rate (an undershoot). The pool then accommodates slowly ($(AQ)/(AQ)_0$ decreases) to the new intensity and the quantum yield decreases until the new quantum yield and intensity establish a new steady state.

That these transients represent true fluctuations in the pool oxidation state is shown in Fig. 16, which shows some raw data from a modulated (20 cycles/s) light-saturation curve. If these transients were solely due to variations in O_2 uptake, then they would not be observed under modulated light conditions. The modulated electrode response is sensitive only to light-induced O_2 production and not to the slower O_2 uptake. As previously argued, it is also likely that under the low O_2 conditions of this experiment, any transient O_2 uptake would tend to be smaller than the transients observed.

CONCLUSION

The novel contribution of this model is the cross reaction specified by R and O_2 with the pool A. We predict that variation of the background oxygen level should induce a concomitant change in optical absorbance of A, permitting its identity to be established. A kinetic study of these absorbance changes might indicate whether the interaction with oxygen is a simple autooxidation of the pool or a more indirect one, occurring by way of an electron transport chain acting across the traditional photosynthetic chain. It would also be of interest to determine whether the free energy of this cross reaction is conserved by the synthesis of ATP.

It is likely, however, that the only electron transport component that could comprise the 20 pool equivalents as required by the kinetic scheme is plastoquinone. As many as 5–7 plastoquinones per 500 chlorophylls are found in *Chlorella* and have been shown to be necessary for electron transport[36]. The plastoquinones have been shown to undergo reversible oxidation and reduction in the light[31,37]. In the next paper we will discuss the very close correlation between the kinetics of oxidation and reduction of plastoquinone and the turnover times of photosynthesis.

The reductant R is quite possibly NADPH because our kinetics require that it is present in a concentration per cell appreciably higher than that of A. In addition, the feedback scheme suggests that the concentration of reductant may even be enhanced by rapid pulse rate. Both of these requirements are satisfied by NADPH. If R is NADPH, we predict that its addition to broken chloroplast preparations permeable to NADPH would cause enhancement of oxygen uptake.

Reid[38] has reported a short-lived enhancement of O_2 uptake observed immediately after cells of *Chlorella fusca* were illuminated with intense red light in the absence of CO_2. He reported the greatest O_2 uptake following illumination with light preferentially absorbed by Photosystem II. This observation is consistent with our model, in that intense red light should result in the reduction of pool A, particularly if oxidized acceptor is limiting. Therefore, at the end of the illumination period, the oxidation of A by molecular oxygen should result in a transient O_2 uptake until the pool is oxidized back to its poised level. The same explanation might be applied to an observation of Joliot[29], who noted a slow oxygen uptake, following intense 2-s illumination of *Chlorella*, that was coincidental with pool oxidation.

Observations not explained by our scheme, but not inconsistent with it, are those of rapid oxygen consumption stimulated by selective excitation of Photosystem I[39,40].

Vidaver[41] reported that anaerobic conditions render the spectral region, specific for System II far less efficient for O_2 production than that close to System I. This action spectrum, markedly resembling System I, is readily explained by our model. We claim that under anaerobic conditions there are two points of electron input into the photosynthetic electron transport chain, Photosystem II (or Z–Q) and R. A high quantum yield for oxygen production, under anaerobic conditions, is then observed only when Photosystem I (or P–X) is able to remove reducing equivalents contributed by Photosystem II and R. To satisfy this requirement, more photons must be absorbed by Photosystem I than II, thus the enhancement of the action spectrum of Photosystem I relative to the rest of the red region.

The kinetic model contains a positive feedback of oxygen produced on oxygen production in photosynthesis. This positive feedback on oxygen production coupled with a positive feedback on the plant biomass predict an explosive rise in the oxygen concentration of the early atmosphere in accordance with the model of Berkner and Marshall[42]. This will be discussed in a forthcoming paper[43].

NOTE ADDED IN PROOF (Received April 26th, 1973)

There is an additional argument that the O_2 concentration above the Pt electrode, in the O_2 polarograph, is close to that at the gas–water interphase. Because the polarograph is operated in the steady state, a linear O_2 gradient ($\partial c/\partial x$=constant, Fick's second law) exists between the interphase and the electrode surface, provided the cellophane membrane is not a barrier to diffusion. The latter point was demonstrated in the polarograph by showing that the time response, of the electrode current, to a pulse of O_2 was consistent with diffusion in water alone, despite the presence of the membrane.

Estimating the diffusion coefficient, D, of O_2 in water to be $1\cdot 10^{-5}$ cm²/s at 25 °C, we can calculate the gradient from Fick's first law, $J = -D\,(\partial c/\partial x)$. Under aerobic conditions, the flux, J, of O_2 toward the electrode is $2.5\cdot 10^{-11}$ mole O_2/cm² per s (Methods). Thus, the gradient from the gas–water interphase to the electrode surface is $-2.5\cdot 10^{-6}$ M/cm². This corresponds to a negligible difference between the $2.5\cdot 10^{-4}$ M O_2 concentration at the interphase and the concentration at the electrode surface, about 25 μm away.

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