THE HAXO AND BLINKS ELECTRODE

A MATHEMATICAL MODEL

G. GINGRAS *and* J. P. SAMSON

From the Département de Biochimie, Université de Montréal, Montréal, Québec, Canada

ABSTRACT Fick's diffusion equations have been solved for the Haxo and Blinks electrochemical determination of photosynthetic O_2 evolution. The model shows the method to be quantitative in the case of a "long" chamber measuring constant rates of O^2 evolution. Transients are amplitude distorted by an amount depending on the space distribution of the O_2 sources. A correction equation applied to the raw data permits the recovery of the original signal.

INTRODUCTION

The study of O_2 evolution by photosynthetic tissues has been carried out until now mainly with the help of the manometric and amperometric techniques.

A version of the latter, making use of the O_2 platinum cathode to measure photosynthetic O_2 evolution is due to Blinks and Skow (1). After improvements by Haxo and Blinks, this technique has found great favor in the measurement of action spectra and the kinetics of O_2 evolution in thin layers of algae and of chloroplasts. Although it has yielded much fundamental information, especially in the hands of Haxo and Blinks (2) and of French and coworkers (3, 4), the method has never been thoroughly described mathematically. For this reason, its quantitative use is limited to a single case (5).

It is the aim of the present article to propose a mathematical model providing the researchers in the field of photosynthesis with a much better defined instrument than has been available.

GENERAL DESCRIPTION

Several variations of the Haxo and Blinks design have been in use but they are all similar in principle. In essence, the instrument consists of a hollow chamber terminated at one end ($x = 0$) by a platinum cathode and at the other end ($x = l$), by a dialysis membrane, these two planes being parallel to one another. The photosynthetic tissue is imprisoned between these two ends and rests on the platinum cathode (Fig. 1). The walls are inert and considered impermeable to O_2. The anode, a nonpolarisable electrode such as Ag/Ag Cl, is not represented in the drawing. The

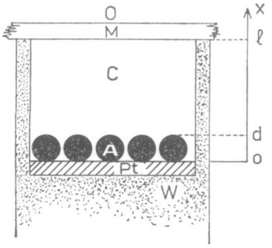


FIGURE 1 Schematics of the Haxo and Blinks electrochemical chamber. *O*: outside medium; *M*: dialysis membrane; *C*: chamber of length *l*; *A*: algae; *Pt*: platinum cathode; *W*: inert walls.

purpose of the dialysis membrane is to retain the cells while permitting free diffusion of small molecules such as O_2. The chamber is to be considered as an open system since the liquid medium bathing the membrane is continuously stirred and maintained at a constant O_2 concentration.

MATHEMATICAL MODEL

Basic Assumptions

(*a*) Dissolved O_2 behaves as an ideal gas: its diffusion coefficient is concentration independent. This is probably true under the operating conditions of the method.

(*b*) (i) The geometry of the chamber is precisely known.

(ii) There is no edge effect since there is no O_2 influx towards the edges. As a consequence we are dealing with a linear diffusion problem.

(*c*) (i) The spatial distribution of the O_2 sources is known and is assumed to be uniform within the chloroplast. For our purpose, the net distribution depends only on the shape of the latter.

(ii) The heterogeneity of the sources within any plane parallel to the platinum cathode is inconsequential. Their density can therefore be assumed uniform in any such plane.

(*d*) The diffusion coefficient for O_2 in the algae is the same as that in pure water.

Boundary Conditions

(*e*) At the membrane, the O_2 concentration is constant.

(*f*) At the platinum, the O_2 concentration is zero when working at the plateau of the polarogram.

N.B.: the following assumes the dialysis membrane to be nonexistent. We shall see however that this assumption is justified if the chamber is sufficiently long.

Fundamental Equations

The electrical current is proportional to the O_2 influx to the electrode which from Fick's first law is proportional to the concentration gradient at the electrode:

$$I(t) = Z\mathrm{F}AJ(0, t) = Z\mathrm{F}AD \frac{\partial C}{\partial x}(0, t) \qquad (1)$$

where I = electrical current measured at the electrode at time t, Z = the number of electrical equivalents per mole of O_2, F = one Faraday (96,500 coulombs), A = area of the electrode, $J(0, t)$ = the number of moles of O_2 per second and per unit area traversing the plane located at $x = 0$ at time t, D = the O_2 diffusion coefficient in pure water, and $C(0, t)$ = the O_2 concentration (moles/liter) at time t in a plane located at x.

Equation 1 can be solved from the knowledge of $dC/dx\ (0, t)$, the O_2 concentration gradient at the platinum cathode. This can be calculated from the concentration inside the chamber by making use of Fick's second law for diffusion in a volume element dV and the conservation of mass principle.

$$\frac{\partial C}{\partial t}(x, t) = \frac{D\partial^2 C}{\partial x^2}(x, t) + F(x, t), \tag{2}$$

where $F(x, t)$ = number of moles of O_2 produced per second and per liter in volume element dV.

The general solution for equation 2 has been deduced from reference 6.

$$\begin{aligned} C(x, t) = & \int_0^t \int_0^l U(x, t, x', \tau)F(x', \tau)\, dx'\, d\tau \\ & + \int_0^l U(x, t, x', 0)C(x', 0)\, dx' + D \int_0^t \Bigg[C(0, \tau) \frac{\partial U}{\partial n_i}(x, t, 0, \tau) \\ & + C(l, \tau) \frac{\partial U}{\partial n_i}(x, t, l, \tau) \Bigg] d\tau, \end{aligned} \tag{3}$$

where τ, x' are the time and position of *occurrence* of the phenomenon under study, t, x are the time and position of the *measurement* of the phenomenon, l is the length of the electrochemical chamber. $U(x, t, x', \tau)$ is the concentration at x and t due to an instantaneous point source of strength unity located at x' and at τ; for equation 3 to be valid it is a necessary condition that U be a solution to equation 2, that $U = 0$ on all the boundary planes of the chamber, and that $\int_0^l U\, dx = 1$. $(\partial U/\partial n_i)(x, t, x', \tau)$ denotes differentiation of U with respect to x' along the inward-drawn normal at plane x. This corresponds to an instantaneous point doublet of strength unity at plane x' and a time τ, the effect of which is measured at x and time t. If $U = 0$ on plane x', then $D(\partial U/\partial n_i)(\lim x \rightarrow x') = 1$ (x being inward with respect to x'). Therefore, $U(x, t, x', \tau)F(x', \tau)\, dx'\, d\tau$ is the contribution at t and at x of O_2 evolution from a volume element of thickness dx' located at x', of strength $F(x', \tau)$, and duration $d\tau$. $U(x, t, x', 0)C(x', 0)\, dx'$ is the contribution at t and x from the initial concentration at x' in a volume element of thickness dx'. $C(x', \tau)D(\partial U/\partial n_i)(x, t, x', \tau)\, dT$ is the contribution at t and at x from the concentration $C(x', \tau)$ at time τ and for a duration $d\tau$ on the surface lying at plane x'. The sum of these contributions is the total solution we are seeking, $C(x, t)$.

For a chamber of infinite length,

$$U = \left(\frac{1}{2[\pi D(t - \tau)]^{1/2}}\right) e^{-(x'-x)^2/4D(t-\tau)}. \qquad (4)$$

For a chamber of length l, applying the method of images to equation 4,

$$U = \sum_{n=-\infty}^{\infty} \frac{[e^{-[x-(x'+2nl)]^2/4D(t-\tau)} - e^{-[x+(x'-2nl)]^2/4D(t-\tau)}]}{2[\pi D(t - \tau)]^{1/2}}. \qquad (5\,a)$$

By applying the Laplace transformation to equation 5 a, a new series is obtained which is identical to the solution of equation 2 by the Fourier method:

$$U = (2/l) \textstyle\sum_{n=1}^{\infty} \sin\,(n\pi x/l) \sin\,(n\pi x'/l)\; e^{-n^2(t-\tau)/\theta_l}, \qquad (5\,b)$$

where $\theta_l = l^2/\pi^2 D$.

The latter solution (equation 5 b) is often simpler to use and yields a rapidly converging series for $t \geq 3\theta_l$, whereas the former (equation 5 a) converges rapidly for $t \leq 3\theta_l$.

In order to solve equation 1 one needs to know $\partial C/\partial x\ (0, t)$, the O_2 concentration gradient at the cathode. Therefore equation 3 must be differentiated with respect to x, taking $x = 0$; U being the only function of x, it suffices to find $U'(0, t, x', \tau)$ and to substitute its value in equation 3:

$$U'(0, t, x', \tau) = \sum_{n=0}^{\infty} \frac{\mathbf{I}[e^{-(x'+2nl)^2/4D(t-\tau)}(x' + 2nl) + e^{-(x'-2nl)^2/4D(t-\tau)}(x' - 2nl)]}{2\sqrt{\pi}[D(t - \tau)]^{3/2}}. \qquad (6\,a)$$

where $\mathbf{I} = ½, n = 0; \mathbf{I} = 1, n > 0$; or the equivalent solution,

$$U'(0, t, x', \tau) = \frac{2}{l}\sum_{n=1}^{\infty} \frac{n\pi}{l} \sin\left(\frac{n\pi x'}{l}\right) e^{-n^2(t-\tau)/\theta_l}. \qquad (6\,b)$$

We will next proceed to the separate determinations of the contributions to the concentration gradient at the electrode (a) of the O_2 originating from outside the algae and (b) from the sources themselves.

Contribution from the Chamber

By substituting the values of U' in the last three integrals of equation 3 with the conditions

$$C(x', 0) = C_0, \forall x',$$

$$C(l, \tau) = C_0, \forall \tau,$$

$$C(0, \tau) = 0, \forall \tau > 0,$$

or by making use of the Fourier method to solve equation 2 with the added condition that $F(x', \tau) = 0, \forall x'$, and $\forall \tau$, since only the contribution from the surface and from the initial concentration is wanted, one obtains

$$\begin{aligned} C'(0, t) &= \frac{1}{2}\sqrt{\frac{\pi\theta_l}{t}}\left(1 + 2\sum_{n=1}^{\infty} e^{-n^2\pi^2\theta_l/t}\right), \\ &= \frac{C_0}{l}\left(1 + 2\sum_{n=1}^{\infty} e^{-n^2 t/\theta_l}\right), \end{aligned}$$

for $t > 5\theta_l$, $C'(0, t) = C_0/l$, and the solution of equation 1 is then

$$I = Z\mathrm{F}AD\, C_0/l = \text{a constant.}$$

This is the electrical current that is measured in the absence of O_2 evolution. It is constant for $t > 5\theta_l$, the concentration in the chamber being in a steady state and the gradient at the electrode being constant. This electrical current can therefore be subtracted from the total current to obtain O_2 production by the algae.

Contribution from the Algae

By substituting the value of U' in the first integral of equation 3 or by using the Fourier method to solve equation 2 with the conditions $C(l, \tau) = C(0, \tau) = C(x, 0) = 0, \forall x$ and $\forall \tau$. One obtains:

$$C'(0, t) = \frac{V}{Ad}\int_0^t \int_0^d \sum_{n=0}^{\infty}$$

$$\frac{\mathbf{I}[e^{-(x'+2nl)^2/4D(t-\tau)}(x' + 2nl) + e^{-(x'-2nl)^2/4D(t-\tau)}(x' - 2nl)]\Psi(x')\cdot dx'\Psi(\tau)\, d\tau)]}{2\sqrt{\pi}[D(t - \tau)]^{3/2}}, \quad (7\,a)$$

or

$$\begin{aligned} C'(0, t) &= \frac{V}{AD}\frac{2}{l}\int_0^t \left(\sum_{n=1}^{\infty} A_n\, e^{-n^2(t-\tau)/\theta_l}\Psi(\tau)\, d\tau\right), \\ A_n &= \frac{n\pi}{2}\Psi_n(x') = \frac{n\pi}{l}\int_0^l \Psi(x')\sin\frac{n\pi x'}{l}\, dx', \end{aligned} \quad (7\,b)$$

where d is the distance from the electrode defining the outer limit of the O_2 sources, $\Psi(x')$ is the ratio between the area of a section through the algae located at x' from the electrode and a standard area S such that $S\cdot d$ = the algal volume (V), $\Psi(\tau)$ is the rate of O_2 evolution per unit of algal volume, Ψ_n is the nth coefficient of the Fourier series for $\Psi(x)$, and $\mathbf{I} = \frac{1}{2}, n = 0; \mathbf{I} = 1, n > 0$.

Equations 7 a and 7 b can be written $C'(0, t) = \int_0^t \beta(t - \tau)\Psi(\tau)\, d\tau(V/[Ad])$,

$$\beta(t - \tau) = \int_0^d \sum_{n=0}^{\infty} \frac{\mathrm{I}[e^{-(x'+2nl)^2/4D(t-\tau)}(x' + 2nl) + e^{-(x'-2nl)^2/4D(t-\tau)}(x' - 2nl)\Psi(x')\, dx']}{2\sqrt{\pi}[D(t - \tau)]^{3/2}}, \quad (8\,a)$$

or

$$\beta(t - \tau) = \frac{2}{l}\sum_{n=1}^{\infty} (A_n\, e^{-n^2(t-\tau)/\theta_l}). \quad (8\,b)$$

These are solutions for a chamber of any type of geometry containing sources of any distribution. We will now seek to determine the influence of the geometry of the chamber and of the algae on the measurements of transient- and stationary-state O_2 evolution.

THE GEOMETRY OF THE CHAMBER AND OF THE CELLS

For the purpose of this study, it is convenient to suppose that O_2 evolution by the algae has the shape of a step function. As will be seen, this facilitates the mathematical treatment and the comparison between the shape of the signal and that of the calculated response.

$$\Psi(\tau) = 0, \tau < 0,$$

$$\Psi(\tau) = 1, \tau \geq 0.$$

We will assume, moreover, that $V/(Ad) = 1$ for the rest of our study. Substituting these values in equations 7 a or 7 b and integrating with respect to τ, one obtains

$$C'(0, t) = \frac{1}{D}\int_0^d \left\{\sum_{n=1}^{\infty} \left[erfc\left(\frac{x' + 2nl}{2\sqrt{tD}}\right) - erfc\left(\frac{x' - 2nl}{2\sqrt{tD}}\right) + erfc\left(\frac{x'}{2\sqrt{tD}}\right)\right]\Psi(x')\, dx'\right\}, \quad (9\,a)$$

$$C'(0, t) = \frac{2\theta_l}{l}\sum_{n=1}^{\infty} A_n\,(1 - e^{-n^2t/\theta_l})/n^2. \quad (9\,b)$$

At equilibrium the latter equation yields (for an infinitely long time)

$$C'(0, \infty) = 2\theta_l/l \textstyle\sum_{n=1}^{\infty} A_n/n^2.$$

Geometry of the Electrochemical Chamber

We will now study how the geometry of the electrochemical chamber and particularly its length with respect to the thickness of the algal layer affects the measurement of transient- and stationary-state O_2 evolution. In order to simplify the calculations we will assume the O_2 sources to be evenly distributed up to distance d from the cathode.

$$\Psi(x') = 1,\ x' \leq d,$$
$$\Psi(x') = 0,\ x' > d.$$

It will be shown that the conclusions are applicable to any possible type of source distribution and that a low enough ratio of d/l is a good approximation to an infinitely long chamber. Some properties of such a chamber will be given.

From equations 9 *a* and 9 *b* the following solution for $C'(0, t)$ can be drawn:

$$C'(0,t) = 2\sqrt{\frac{t}{\pi D}} + \left\{- i\,erfc\left(\frac{d}{2\sqrt{tD}}\right) + \sum_{n=1}^{\infty}\left[2i\,erfc\left(\frac{nl}{\sqrt{tD}}\right) - i\,erfc\left(\frac{2nl+d}{2\sqrt{tD}}\right) - i\,erfc\left(\frac{2nl-d}{2\sqrt{tD}}\right)\right]\right\} 2\sqrt{\frac{t}{D}}, \qquad (11\ a)$$

where $i\,erfc\ x = \int_0^x erfcx\ dx$,

$$C'(0,t) = (2\theta_l/l)\sum_{n=1}^{\infty}\left[1 - \cos\left(n\pi d/l\right)\right]\left(\frac{1 - e^{-n^2 t/\theta_l}}{n^2}\right). \qquad (11\ b)$$

The solution for $C'(0, \infty)$ is obtained from equation 10:

$$C'(0, \infty) = \frac{d}{D}\left(1 - \frac{d}{2l}\right) = \left(\frac{2\theta_d}{d}\right)\pi^2\left(1 - \frac{d}{2l}\right), \qquad (12)$$

where $\theta_d = d^2/(\pi^2 D) = \theta_l\, d^2/l^2$. For $D = 1.98 \cdot 10^{-5}\ \text{cm}^2 \cdot \text{sec}^{-1}$ (in 0.17 M NaCl at 18°C, according to reference 7) and $d = 4\ \mu$, the value of θ_d is $8.2 \cdot 10^{-4}$ sec.

Some important features of the above equations will next be commented upon.

The Stationary State. Equation 12 shows that for stationary-state O_2 evolution the electrical current varies both with d and l. In the present section d will be supposed to have a fixed value, and l will be varied. The efficiency of a chamber of any length is given by $(1 - d/2l)$ which is, in other words, an expression of the percentage of the evolved O_2 that is measured. This value of l varies from $l = d$ to $l = \infty$, the greatest change occuring between $l = d$ and $l = 4d$.

Transients. Fig. 2 computed from equation 11 shows the response of the method to a step function of O_2 evolution. In this figure the sources are uniformly distributed up to distance d (held constant) from the electrode. The initial kinetics for values ranging from $l = d$ to $l = \infty$ are superimposed up to θ_d. Any two curves are superimposed up to θ_l, θ_l being defined with respect to the smaller of the two l's. One-half of the maximum current is attained between $0.5\theta_d$ for $l = d$, to

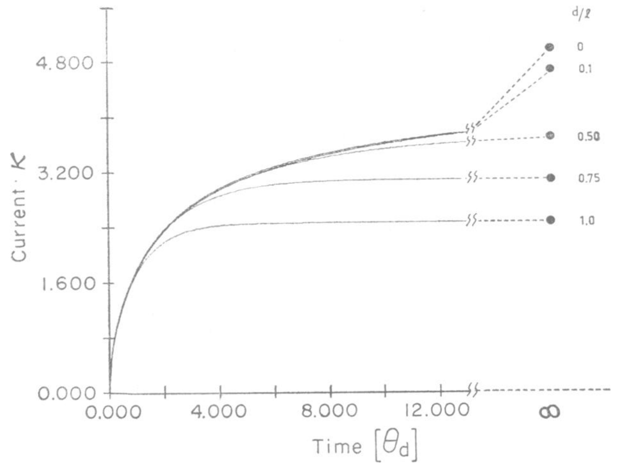


FIGURE 2 The kinetics of the O_2 influx at the cathode due to a step function impulse from uniformly distributed sources. The different curves correspond to various d/l ratios. The ordinate is dimensionless and proportional to the current. $K = \pi^2/2ZFV\psi(\tau)$, where $\psi(\tau)$ is held constant. The time (abscissa) is expressed in θ_d units. Computed from equation 11.

$2\theta_d$ for $l = \infty$. If one compares the time taken to reach 91 % of the maximum current a much greater variation is observed: its value goes from $2.2\theta_d$ for $l = d$ to $110\ \theta_d$ for $l = \infty$.

Also interesting is the comparison between the rise curve and the relaxation curve to a given signal. If the signal is long enough for $C'(0, t)$ to reach an equilibrium, it is obvious that for any l both curves are identical and that both the rise time and the relaxation time increase identically as l goes up.

If the square signal is short compared to the equilibrium time, then the rise time is longer than the relaxation time and their difference grows with l. This can be explained qualitatively as follows. Some of the O_2 molecules produced during the square wave accumulate in the chamber and constitute an extra reservoir whose rate of emptying is inversely proportional to its size. This reservoir is closer to the empty-state equilibrium than to the full-state equilibrium. As in all diffusion phenomena, the rate of change of $C'(0, t)$ is rapid when far away from the equilibrium and slow when close to it. This is another way of saying that the rise time is faster than the relaxation time in the situation described.

The Infinitely Long Chamber. With the information summarized on Fig. 2, a choice can be attempted between the various possible d/l ratios. Such a choice depends of course on the type of measurement required. However two important criteria may be retained: the sensitivity of the method and its time resolution. For any given algal diameter, the sensitivity varies at most by a factor of 2 between a chamber of $l = d$ and a chamber of $l = \infty$. This cannot be an important element of choice.

The other parameter, the time resolution, is somewhat more complex to deal with.

It is remarkable, however, that for any given type of source distribution, chambers of different lengths yield the same current value up to θ_d. This means that the ability of the method to follow fast O_2 transients is not affected by the value of d/l. It is true that the equilibrium current is reached after a longer time as l increases. In that respect, for a signal of duration greater than θ_d the longer chambers will cause amplitude distortion of the current. This would result in inaccurate amplitude ratios between short O_2 bursts and the stationary state of O_2 evolution. This is the only important distortion that is introduced by the longer chamber. But again it is never greater than by a factor of 2.

Noteworthy also, is that the rate of change of the stationary-state current as l increases is maximum between $l = d$ to $l = 4d$. As l increases further, the stationary-state current approaches asymptotically its maximum value, obtained when $l = \infty$. At the same time the kinetics are superimposable for longer and longer durations.

Hence, two extreme chamber configurations appear worthy of interest: $l = d$, and $l = \infty$. The first configuration is difficult to realize in practice since a small error in the estimation of d/l would result in a large error in the current; moreover one would have to take into account the thickness of the dialysis membrane or, if the latter could be removed, the thickness of the water layer above the cells. It should be mentioned also that the background electrode current would be large as predicted by the equation $I = ZFAD\ C_0/l$.

The second configuration does not suffer from these drawbacks. As noted above, the infinitely long chamber situation is rapidly approached as l increases. The difference in the electrical currents measured in chambers of lengths $l \neq \infty$ and $l = \infty$ (for a fixed d) varies from 0% up to θ_d, to $(d/2l)$ % at the stationary state for a symmetrical distribution of the sources. As an example, for $d/l = 1/50$ the stationary-state current is 99% that of an infinitely long chamber. For small organisms a "long" chamber is easily realized experimentally: an error in l has no serious effect on the measurement and the thickness of the dialysis membrane need not be taken into account. This configuration is physically well defined and mathematically approximated by the $l = \infty$ solution.

It can be deduced from equation 12 that the latter solution is valid for any type of source distribution. The maximum (stationary-state) observable difference for a volume thickness dx' located at distance $x = 0$ or $x = d$ from the electrode is (d/l) %. For an infinitely long chamber the effect is nil. For a chamber of $d/l = 1/50$, the current produced by a slice located at $x = 0$ is 100% and by a slice of the same thickness at $x = d$ is 98%. This effect is negligible.

The solution of $l = \infty$ from equation 9 *a* is

$$C'(0, t) = \frac{1}{D} \int_0^d erfc\left(\frac{x'}{2\sqrt{tD}}\right) \Psi(x')\, dx', \qquad (13)$$

and will be used frequently hereafter for any distribution type.

Space Distribution of the Sources

Before proceeding to study a more realistic model of source distribution we will extend the validity of equation 12 to any symmetrical distribution of the sources about plane $d/2$.

From equation 9 *b* it can be shown that the stationary-state current from two layers of thickness Δd placed symmetrically with respect to $d/2$ is given by

$$C'(0, \infty) = \frac{d}{D}\left(1 - \frac{d}{2l}\right)\frac{2\Delta p}{d}.$$

Therefore the same number of sources uniformly distributed or merely plane symmetrically distributed will yield the same stationary-state current. Equation 12 applies to any plane symmetrical shape of algae and noticeably to spherical cells.

The Spherical Distribution. Let us suppose a uniform distribution of the sources within spherical chloroplasts surrounded by a spherical envelope formed by an inactive membrane of thickness ϵ. The algae are assumed to rest directly on the platinum electrode.

In this case,

$$\Psi(x') = \frac{6d}{d - 2\epsilon}\left[\frac{x' - \epsilon}{d - 2\epsilon} - \left(\frac{x' - \epsilon}{d - 2\epsilon}\right)^2\right] \quad \text{where} \quad \epsilon < x' < d - \epsilon,$$

$$A_n = \frac{12dl}{(d - 2\epsilon)^2\pi n} \sin\,(n\pi d/2l) \tag{14 a}$$

$$\cdot\left[\frac{2l}{\pi n(d - 2\epsilon)} \sin\,(n\pi(d - 2\epsilon)/2l) - \cos\,(n\pi(d - 2\epsilon)/2l)\right],$$

$$\beta(t - \tau) = \frac{12d^2}{\sqrt{\pi}(d - 2\epsilon)^3}\left[(erfc(z) - erfd(w))\,\frac{\sqrt{\pi}}{2}\,(e^{-w^2} - e^{-z^2})/y\right], \tag{14 b}$$

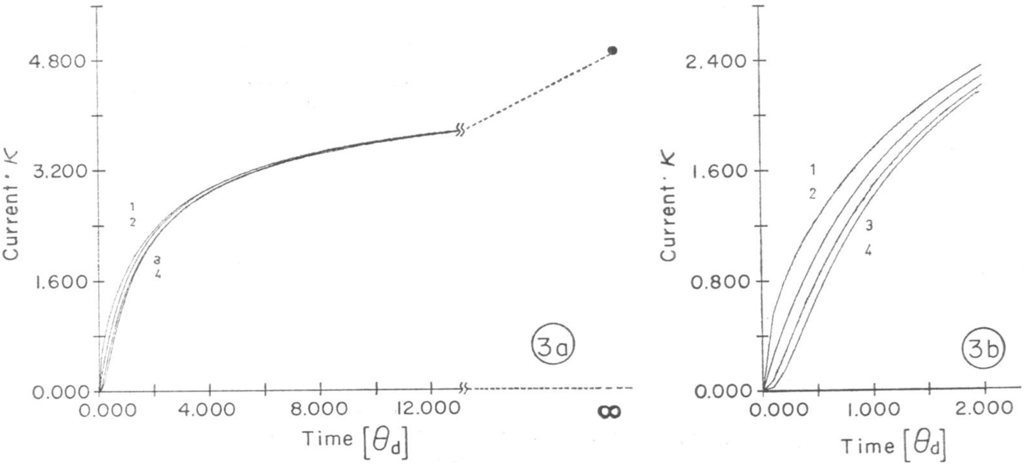
where $w = ([d - \epsilon]/d)y$, $y = (\pi/2)\sqrt{\theta d/(t - \tau)}$, and $z = (\epsilon/d)y$.

By substituting these values in equations 8 *a* and 8 *b*,

$$C'(0, t) = \pi^2\left(\frac{2\theta_d}{d}\right)\left(\frac{d}{y(d - 2\epsilon)}\right)^3\left[y\left(3 - \frac{4\epsilon}{d}\right)i^2erfc(z) - ierfc(z)\right.$$
$$\left. + y\left(1 - \frac{4\epsilon}{d}\right)i^2erfc(w) + ierfc(w)\right], \tag{15 a}$$

$$C'(0, t) = \frac{2\theta_l}{l}\sum_{n=1}^{\infty} A_n(1 - e^{-n^2t/\theta_l})/n^2. \tag{15 b}$$

Fig. 3 *a* and *b* shows the identity of the measured kinetics (for $t > 0.2\theta_d$) corresponding to a square wave of O_2 evolved by uniformly distributed sources or by



FIGURES 3 *a* and *b* The kinetics of the O_2 influx at the cathode due to a step function impulse from sources of various distribution in an infinitely long chamber. Curve 1: uniform distribution of sources up to distance *d* from the cathode. Curves 2–4: spherical distribution of algae of diameter *d* with an envelope of thickness ϵ. Curve 2: $\epsilon = 0$; curve 3: $\epsilon = 0.125\ d$; curve 4: $\epsilon = 0.25\ d$. Same units as in Fig. 1. Computed from equations 11 (curve 1) and 15 (curves 2–4).

sources within spherical cells. There is, however, a $0.25\theta_d$ time shift between the two curves. Note also that in the latter case when membrane thickness varies from $\epsilon = 0$ to $\epsilon = d/4$ the time shift varies from 0 to $0.25\ \theta_d$ (for $d = 4\ \mu$ and $D = 1.98 \cdot 10^{-5}$ cm^2 sec^{-1}, $0.25\theta_d = 2 \cdot 10^{-4}$ sec).

For times smaller than $0.25\theta_d$ the time shift between uniform and spherical distribution tends smoothly to zero. But within the spherical distribution the time shift between membrane thickness $\epsilon = 0$ and $\epsilon = d/4$ remains constant for a longer time.

The Thickness of the Cell Layer. From equations 11 and 12 the effect of the thickness *d* of a layer of uniformly distributed sources is as follows. When *l* is fixed, $C'(0, t)$ is independent of *d* up to θ_d : for measurements made on algae of increasing *d*, the initial kinetics are superimposed up to θ_d, θ_d being defined with respect to the smallest *d* of the series. This is shown on Fig. 4. If the number of the sources is assumed to increase linearly with *d*, the stationary-state current increases with *d* (equation 12 and Fig. 4).

If, the number of O_2 sources remaining constant, the thickness of the algal layer varies from *d* to $(d + \Delta d)$, then $\Psi(x') = d/(d + \Delta d)$. The effect at the stationary state is a current variation of $\Delta d/(2l - \text{d})$. When $l = \infty$, there is no current change, whatever the shape of the cells. The current at the beginning of the time curve decreases by $\Delta d/d$. This is shown on Fig. 5.

For spherical sources with a shell of fixed ϵ/d ratio, a Δd diameter variation causes a time shift and a decrease of the initial current by $2\ \Delta d/d$, by $\Delta d/d$ for $t = 2.5\theta_d$ and

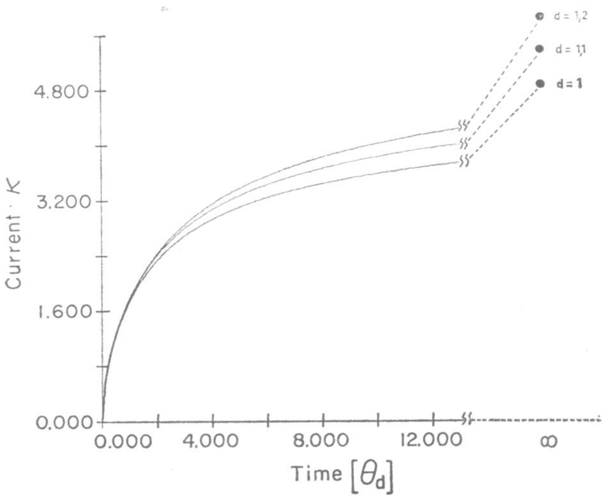


FIGURE 4 The kinetics of the O_2 influx at the cathode due to a step function impulse in an infinitely long chamber. The sources are uniformly distributed up to distance d from the cathode. The curves correspond to a variation of the relative value of d, the source concentration being kept constant. Same units as in Fig. 1. Computed from equation 11. The θ_d scale is defined here for the case $d = 1$.

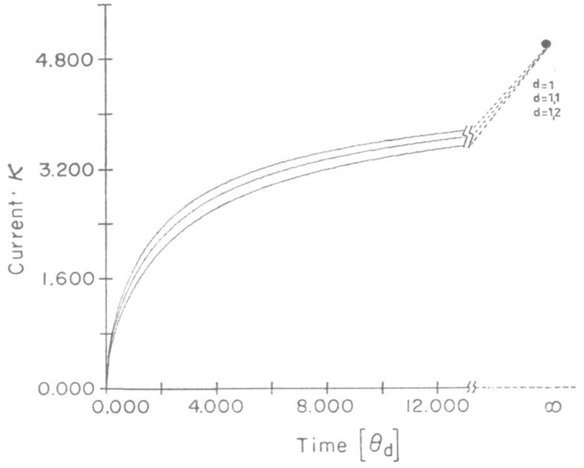


FIGURE 5 The kinetics of the O_2 influx to the cathode due to a step function impulse in an infinitely long chamber. Uniform distribution of the sources up to distance d from the cathode. The curves correspond to a variation in the relative value of d. The source concentration is inversely proportional to d. Same units as in Fig. 1. Computed from equation 11. The θ_d scale is defined here for the case $d = 1$.

by $\Delta d/(2l - d)$ for $t > t_{0.91}$. For an infinitely long chamber the current change goes from $2 \Delta d/d$ at the beginning of the kinetics to zero at the stationary state.

More generally for algae of any shape a change in the diameter of the cells will distort the kinetics as follows: from equation 11 *a* each volume element dV of thickness dx' located at x' contributes to the total current by the amount

$$erfc\left(\frac{x'}{2\sqrt{tD}}\right)\Psi(x')\,dx'\,\frac{1}{D}.$$

When d is changed to $(d + \Delta\, d)$, point x' is displaced at

$$(d + \Delta\, d/d)x'.$$

The shape of the measured kinetics is therefore the same. But the time scale differs by factor $[(d + \Delta\, d)/d)]^2$, therefore by $(2\Delta\, d/d)\,\%$.

This is true for a square wave of O_2 evolution. However for kinetics of any shape the distortion is more complex. This is made clear when this curve is decomposed into its square wave elements: every square wave is distorted by a change in its time scale, every time scale having a different origin.

CORRECTION EQUATION

The measured kinetics of O_2 evolution is distorted by diffusion as described above. Is it possible to reconstruct the original kinetics from the recorded measurements?

In principle, this can be done as follows. (*a*) Suppose O_2 evolution stops at instant $(t - \Delta t)$. (*b*) Take the difference between the current read at instant t and that predicted by condition *a*. (*c*) Attribute this difference to O_2 evolution of strength $\Psi(\tau)$ at instant t.

From equation 8 *a*.

$$C'(0, t) = \left[\int_0^{t-\Delta t} \beta(t - \tau)\Psi(\tau)\,d\tau + \int_{t-\Delta t}^{t} \beta(t - \tau)\Psi(\tau)\,d\tau\right]\frac{V}{Ad}, \quad (16)$$

Where $\Psi(\tau)$ is known for $\tau < t - \Delta t$, and $\Psi(\tau)$ is unknown for $\tau > t - \Delta t$.

Let us suppose that $\Psi(\tau)$ is a linear function of time such that $\Psi(\tau)$ varies from $\Psi(t - \Delta t)$ (known) to $\Psi(t)$ (unknown) when τ goes from $t - \Delta t$ to t,

$$\Psi(\tau) = \Psi(t - \Delta t) + \frac{\Psi(t) - \Psi(t - \Delta t)}{\Delta t}(\tau - t + \Delta t).$$

Substituting this value of $\Psi(\tau)$ in equation 16,

$$C'(0, t) = \left\{\int_0^{t-\Delta t} \beta(t - \tau)\Psi(\tau)\,d\tau + \left[i\beta(\Delta t) - i^2\frac{\beta(\Delta t)}{\Delta t}\right]\Psi(t) + \left[i^2\frac{\beta(\Delta t)}{\Delta t}\right]\Psi(t - \Delta t)\right\}\frac{V}{Ad}, \quad (17)$$

from which the unknown $\Psi(t)$ can be calculated, and where $i\beta(t) = \int_0^t \beta(\Delta t - \tau)\,d\tau$, and $i^2\beta(t) = \int_0^t i\beta(\tau)\,d\tau$.

For a source distribution of any type, since only A_n is affected by the distribution pattern

$$i\beta(\Delta t) = \frac{2\theta_l}{l}\sum_{n=1}^{\infty}(1 - e^{-n^2\Delta t/\theta_l})A_n/n^2,$$

$$i^2\beta(\Delta t) = \frac{2\theta_l}{l}\sum_{n=1}^{\infty}\left(\frac{1 - e^{-n^2\Delta t/\theta_l}}{n^4}\right) - \left(\frac{\Delta t e^{-n^2\Delta t}}{n^2}\right)A_n.$$

For spherical algae of diameter d with a shell of thickness ϵ in an infinitely long chamber,

$$i\beta(\Delta t) = \left(\frac{2\theta_d}{d}\right)\pi^2\left(\frac{d}{y(d - 2\epsilon)}\right)^3\left[y\left(3 - \frac{4\epsilon}{d}\right)i^2erfc(z)\right.$$
$$\left. - ierfc(z) + y\left(1 - \frac{4\epsilon}{d}\right)i^2erfc(w) + ierfc(w)\right],$$

$$i^2\beta(\Delta t) = \left(\frac{2\theta_d}{d}\right)3\theta_d\frac{\pi^4}{(d - 2\epsilon)^2y^4}\left[\left(1 - \frac{2\epsilon}{d}\right)i^4erfc(w)\right.$$
$$\left. + \frac{2}{y}i^5erfc(w) + \left(1 - \frac{2\epsilon}{d}\right)i^4erfc(z) - \frac{2}{y}i^5erfc(z)\right],$$

where $y = (\pi/2)\sqrt{\theta d/\Delta t}$, $w = ([d - \epsilon]/d)y$, and $z = (\epsilon/d)y$.

Application of the Correction Equation

For spherical algae with a shell, a square wave of O_2 evolution is detected only after a lag time. This is explained by the shape of function $\beta(t - \tau)$ whose maximum is at $(t - \tau) = t_p \neq 0$. $\beta(t - \tau)\,d\tau$ is the contribution to the current measured at t of an instantaneous O_2 evolution having occured at τ. Thus, current measured at t can yield little information on any event more recent than $\tau = t - t_p$. Therefore $\Psi(t)$ of equation 17 has no real meaning, being merely a linear extrapolation of $\Psi(t - t_p)$. We will therefore assume that only $\Psi(t - t_p)$ can be deduced accurately and attempt to find the value of $\Psi(\tau)$ for $\tau > t - t_p$ from subsequent measurements. This is done by using measurements taken at $(t + nt_u)$ from which are deduced values of $\Psi(t - t_p + nt_u)$ with $\Delta t = t_u + t_p$.

$$\Psi(t - t_p) = \frac{(\Delta t - t_p)\Psi(t) + t_p\Psi(t - \Delta t)}{\Delta t},$$

where $\Psi(t)$ has the same value as in equation 17, Δt is the time interval between the instant corresponding to a known $\Psi(\tau)$ and the next measurement, and t_u is the smallest useful time interval between two measurements.

TIME RESOLUTION OF THE METHOD

An absolute accuracy at all times in the current determination would permit, through the use of the correction equation, the recovery of the exact kinetics of O_2 evolution. This condition cannot be realized because of noise in the measuring devices which can be assumed to be time invariant. There occurs also a time-dependent weakening of the measured signal due to a loss of information inherent in the diffusion process itself.

The diffusional loss of information at the stationary state can be calculated from the entropy increase concomittant with the isothermic volume expansion of evolved O_2 (8). However this method is not easily applied to changing phenomena. Our diffusion model provides an answer more directly applicable to kinetics.

Any inaccuracy in the amplitude of a phenomenon is accompanied by an uncertainty in the time of occurrence of that phenomenon. We will study separately the effect of an error in current and in the geometry estimate of the sources.

Erroneous Current Determination ($l = \infty$)

The loss of information over any given time interval t_r is a function of the ratio of the number of oxygen molecules evolved to the number of oxygen molecules measured during that time interval. That ratio is inversely proportional to t_r because of the finite source-to-electrode diffusion time. The number of counted molecules decreases with t_r leading to a smaller signal-to-noise ratio.

It is possible to define a certain t_r over which any signal can be measured with any degree of accuracy. The t_r chosen here corresponds to a signal-to-noise ratio of one.

Suppose a noise of fixed value ΔI inherent in the measuring devices: it can be attributed to a noise in function Ψ. A square variation of amplitude $\Delta\Psi$ and duration t_r leading to current variation ΔI is the equivalent of that noise. So defined, t_r is the time over which the O_2 square wave $\Delta\Psi$ is known with a total inaccuracy; t_r is also the lower limit of time resolution of $\Delta\Psi$.

From equation 15, with $\epsilon \longrightarrow 0$,

$$6Z\,FAD(2\theta_d t_r/d^2)(1 - 8t_r^{1/2}/3\pi^{3/2}\theta_d^{1/2})\Delta\Psi = \Delta I.$$

Neglecting the second term in the second parenthesis,

$$t_r = d^2\Delta I/\Delta\Psi(12Z\,FAD\,\theta_d) = (\Delta I/\Delta\Psi)\cdot\text{constant}.$$

The value of the constant is more conveniently expressed as a function of θ_d and of I, the current at the stationary state of the O_2 square wave, Ψ.

$$t_r = \Psi\pi^2\theta_d\Delta I/6I\Delta\Psi.$$

If $\Psi/\Delta\Psi = 1$, t_r corresponds to the time interval over which Ψ is completely undetermined (100% error). For $\Delta I/I = 1/100$, $t_r \simeq 1.3\cdot 10^{-5}$ sec, if $\theta_d = 8\cdot 10^{-4}$ sec.

From the above the amount of error in the time constant of a known function of O_2 evolution can be calculated. The noise of Ψ, $\Delta\Psi$, can be equated to the difference between two identical curves Ψ_1 and Ψ_2. The same applies to current I corresponding to Ψ and its noise ΔI. For any given measurement the error in amplitude is $\Delta\Psi \cdot \Delta I/I$.

In the case of an exponential curve $\Psi = (1 - e^{-kt})$, the error in the constant k can be calculated from the above relationship.

$$\Delta\Psi/\Delta k \simeq d\Psi/dk = te^{-kt},$$

whence

$$\Delta k = \Delta\Psi/te^{-kt},$$

or

$$\Delta k = (e^{kt} - 1)\Delta I/It.$$

If the recording of I as a function of time is an exponential (the reaction is slow with respect to diffusion), Δk is minimum at $t = 1/k$. For a measurement taken at that time

$$\Delta k = (e - 1)k\Delta I/I \simeq 2k\Delta I/I.$$

If the reaction is fast compared to diffusion, Δk is minimum at a time slightly different from $t = 1/k$, but the above relationship is still valid. The effect of diffusion is to reduce I.

False Geometry Estimate

The use of the correction equation with a false algal dimension or shape has no pronounced effect on t_r since the relative error in Ψ varies slowly with time.

A square Ψ from spherical algae whose diameter is known with Δd accuracy will be in error by $2\Delta d/d\%$ at the beginning of the kinetics and by 0% at the stationary state. If Ψ is an exponential function, its error is fairly constant $(2\Delta d/d\%)$ in time. The error in its rate constant is about $\Delta\ d/d\%$.

CONCLUSION

A question often asked by experimentalists is whether under nonstationary-state conditions the Haxo and Blinks method permits the direct measurement of rates of O_2 evolution. The answer is no. This is made clear by equation 7 b: $\Psi(\tau)$ cannot be evaluated from the current value alone since both $\Psi(\tau)$ and the summation

$$\sum_{n=1}^{\infty} A_n e^{-n^2}(t - \tau)/\theta_l$$

are time-independent variables. However, after a long time, when the stationary state has been attained equation 7 *b* becomes

$$C'(0, \infty)2V\theta_l/A\ dl = \sum_{n=1}^{\infty} (An/n^2)\Psi(\tau)$$

and the proportionality factor between $\Psi(\tau)$ and $C'(0, t)$ becomes time independent.

Application of correction equation 17 to the raw data leads to $\Psi(\tau)$ and to the obtainment of the rate of oxygen evolution even at short times. This correction equation should be applied until the stationary state is attained.

By holding $\Psi(\tau)$ constant from t_0 it has been possible to study how several parameters affect $C'(0, t)$.

One of the most interesting results of this study is that for small organisms it is probably best to use a long electrochemical chamber. Besides being easily feasible, this condition is a close approximation to the infinitely long chamber. This is particularly useful in the application of the correction equation.

Another important feature for the measurement of rapid kinetics is the spatial distribution of the sources. For times longer than $0.2\theta_d$ there is a $0.2\theta_d$ time shift between the curves yielded by uniform and spherically distributed sources. There is also a $0.2\theta_d$ time shift between the curves from spherical algae with a shell thickness varying from 0 to $d/4$. An error of 10 % in the diameter of the cells leads to an error in time smaller than 2×10^{-4} sec.

The limitation introduced by diffusion on the resolution of fast kinetics is thus smaller than might be thought, the rise time of the method for an infinitely long chamber being of the order of 10^{-4} sec.

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