

A Mathematical Treatment of Rate Data Obtained in Biological Flow Systems under Nonsteady State Conditions

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ABSTRACT

The problem of determining gas exchange rates from flow system data under nonsteady state conditions is analyzed. A correction factor is presented for obtaining constant rates under nonsteady state conditions. A general formula for obtaining any rate under nonsteady state conditions is also given. Turnover time is defined and discussed in terms of the mathematics presented. The origins of nonsteady states and steady states in flow systems are discussed, as are some of the experimental advantages of working under nonsteady state conditions.

The study of gas exchange of plants and plant parts has been largely conducted by postharvest physiologists studying fruit respiration and by biochemists, ecologists, and physiologists studying photosynthesis. There have been and are many similarities in the gasometric methods used by these various researchers. The use of flow systems is one case in point.

In a flow system, tissue is enclosed in a container and air or a modified atmosphere is passed through the container at a known rate. One of the principal advantages of a flow system over the older manometric and volumetric techniques is the ability to closely control the composition of the atmosphere surrounding the tissue. Volume, pressure, flow rate, and usually temperature are constant, and changes in the composition of the gas stream with time are related to the activities of the living tissue. Summaries of the methods for monitoring such changes along with much practical advice and standard operating procedures for some types of flow systems are available (1, 14).

Surprisingly, the mathematics of rate calculations in flow systems has received only passing attention in the biological literature even though the problem is not difficult. The formula usually quoted (1-5, 8, 10-14, 16) for determining the rate R , given the flow rate F and the gas concentration %C, (v/v) corrected for background, is

$$R = \%C \cdot F / 100 \quad (1)$$

This formula is sometimes accompanied by the caveat that it is valid only under steady state conditions, but sometimes this requirement is not made clear.

In this paper we will be concerned with three basic questions: (a) When does equation 1 apply? (b) When equation 1 does not apply what is the appropriate mathematical framework to deal

with the experimental data? (c) What are the potential experimental advantages in using this general solution?

EXPERIMENTAL

The flow system used to obtain the experimental data presented in this paper was assembled in a 30°C room. The cotton explant of the standard abscission bioassay was the subject under investigation. Explants prepared in the standard fashion (15) were placed in chambers through which CO₂- and ETH¹-free air flowed. Explant chambers were wrapped with foil to exclude light. After the insertion of the explants, the chambers were immediately flushed with a rapid stream of the desired atmosphere. The time at which the experimental flow rate was established was taken as the beginning point ($t = 0$) for the rate data. At $t = 0$ the amount of CO₂ and ETH present equalled zero to the precision of our instrumentation. The time between explant cutting and $t = 0$ was minimized. Gas samples were withdrawn from the chambers with hypodermic syringes for chromatographic determination of CO₂ and ETH concentrations. The CO₂ concentrations determined in these experiments were between 0.05 and 0.5%, whereas the ETH determinations were between 2 and 70 nl/l. The maximum error in any measurement is estimated to be 10%; however, the great majority of measurements are accurate to within 3 to 6%.

This flow system was primarily designed to allow measurements of instantaneous rates of CO₂ and ETH production at any point during the abscission process. The need to have the explants free standing, far enough apart for individual treatment, and easily and rapidly accessible for testing or treatment necessitated a relatively large free volume in the chambers. Volumes ranged from 110 to 120 ml in these experiments. The amount of tissue involved was small (twenty explants averaging 1.45 g in total weight) and detection of even a relatively high rate of gas production by this amount of tissue would require a slow flow rate. A maximum flow rate of only 60 ml/hr could be used to detect and measure the lowest critical rates in this system. Large free volume and slow flow rates make this system ideal for the application of nonsteady state techniques (see below).

NONSTEADY STATE MATHEMATICS

Steady state conditions in a flow system are defined as

$$\frac{d\%C}{dt} = 0 \quad (2)$$

and are illustrated for respiration by the horizontal portion of the

¹ Abbreviations: ETH: ethylene; t : time; IRGA: infrared gas analyzer.

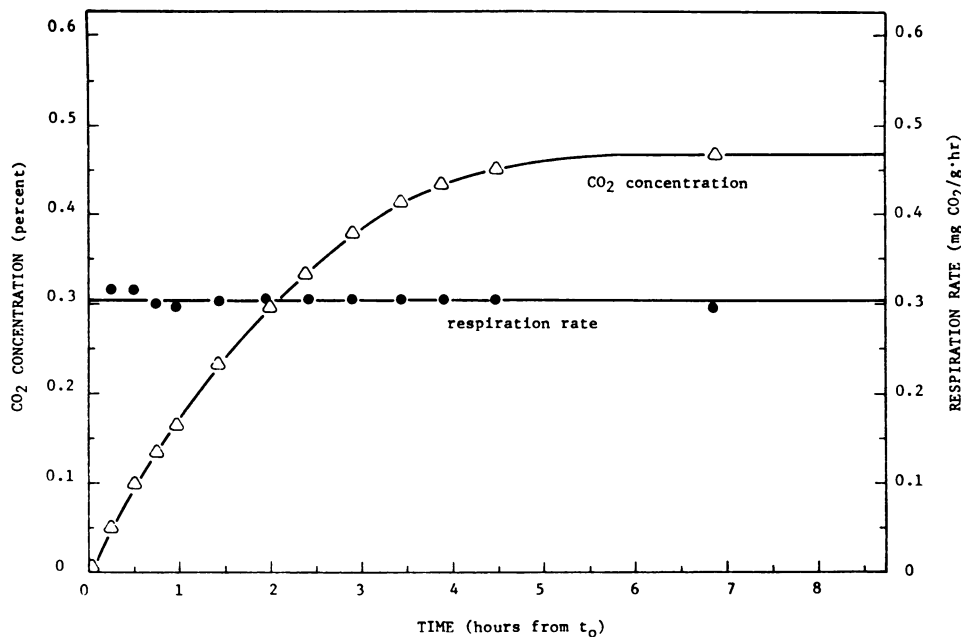


FIG. 1. A constant rate of respiration measured in a flow system. The zero of time corresponds to t_0 . The approach to steady state is clearly seen in the CO_2 concentration plot versus time. The rates of respiration plotted were calculated using equation 5 (see also Table I). Twenty standard cotton explants (1.5 g) were used with $V = 118$ ml and $F = 49.3$ ml/hr. The temperature was 30 C. The experiment was terminated after 21 hr when the rate was still constant to within experimental error.

$\% \text{CO}_2$ versus time curve (Fig. 1). It is only in this portion of the curve that equation 1 is valid.

In this case, in which respiration is constant, one can wait for steady state; however, data such as wound ETH production can, in practice, be obtained in a flow system such as ours only under nonsteady state conditions (Fig. 2). To obtain instantaneous rates of gas exchange under nonsteady state conditions we will consider the differential equation applicable to the type of flow system described above.

$$\frac{dC(t)}{dt} = R(t) - \frac{\%C(t) \cdot F}{100} = R(t) - \frac{C(t) \cdot F}{V} \quad (3)$$

where $C(t)$ is the amount and $\%C(t)$ is the concentration of the gas under consideration in the chamber at time t , $R(t)$ is the net rate of gas exchange at time t , and V is the free volume of the chamber. This equation merely states that the net rate of change of gas in the chamber $[dC(t)/dt]$ is equal to the rate in $[R(t)]$ minus the rate out $[C(t) \cdot F/V]$. If $R(t)$ is constant, this equation is easily solved (6) to yield

$$R = \frac{[\%C(t) - \%C_0 \cdot e^{-F/V \cdot t}] \cdot F}{100} \cdot \left[\frac{1}{(1 - e^{-F/V \cdot t})} \right] \quad (4)$$

where the general boundary conditions are taken as $\%C = \%C_0$ at $t = 0$ (after correction for background). Should the boundary conditions $\%C = 0$ at $t = 0$ be applicable, then equation 4 simplifies to

$$R = \frac{\%C(t) \cdot F}{100} \cdot \left[\frac{1}{(1 - e^{-F/V \cdot t})} \right] \quad (5)$$

The term in brackets may be considered to be a correction factor $[\gamma]$ for nonsteady state conditions. At large t , the correction factor approaches unity and equations 4 and 5 reduce to the steady state equation 1. Also, the time to steady state is decreased with increasing flow rate or decreasing free volume and $\%C$ at steady state is independent of V . Figure 1 and Table I illustrate the appli-

cation of equation 5. We note that it is possible to obtain remarkably accurate values of R at times which are very short compared to the time the system needs to reach approximate steady state conditions.

If $R(t)$ is not constant then there is no general analytic solution to equation 3, but numerical solutions may be easily obtained by noting that $d\%C/dt$ is simply the slope of a concentration versus time plot (Figs. 1 and 2). This slope is obtained numerically by quadratic approximation. A small region of the concentration curve defined by $\%C_1$, $\%C_2$, $\%C_3$, t_1 , t_2 , and t_3 is approximated by $\%C(t) = at^2 + bt + c$, where $t_1 < t_2 < t_3$ and $t_1 < t < t_3$, the constants a and b are solved for exactly, and the derivative $d\%C(t)/dt = 2at + b$ is calculated and used in equation 3. The general solution for variable R then becomes

$$R(t_2) = \frac{V/100[\%C(t_1) \cdot (t_2 - t_3)^2 + \%C(t_2) \cdot (t_1(t_1 - 2t_2) - t_3(t_3 - 2t_2)) - \%C(t_3) \cdot (t_1 - t_2)^2]}{t_1^2(t_2 - t_3) - t_2^2(t_1 - t_3) + t_3^2(t_1 - t_2)} + \frac{\%C(t_2) \cdot F}{100} \quad (6)$$

where the rate $R(t_2)$ is always calculated at the time point lying between t_1 and t_3 and the points are close enough together in time for the quadratic approximation to be valid. When possible, the points should be at equal time intervals Δ , and then equation 6 reduces to

$$R(t_2) = \frac{V}{200} \left[\frac{\%C(t_3) - \%C(t_1)}{\Delta} \right] + \frac{\%C(t_2) \cdot F}{100} \quad (7)$$

This formula could be especially useful in conjunction with continuous monitoring devices, when it is possible to obtain concentrations for any time. Figure 2 illustrates the application of equation 6 for wound ETH data. It should be noted that the rate peaks before the concentration peaks. This is a general characteristic of all open flow systems.

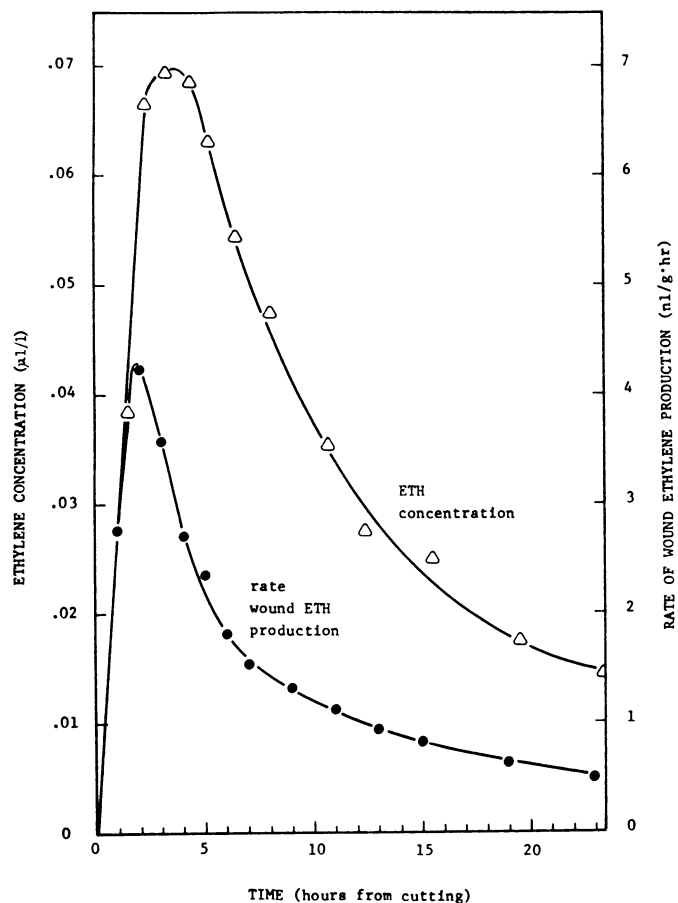


FIG. 2. An example of the application of the general formula: a varying rate of ETH production measured in a flow system. The zero of time corresponds to the cutting time of the explants. Steady state is never obtained since the rate of ETH production approaches zero (data are available for 60 hr but not shown). The rates of ETH production here have been calculated from equation 6. Twenty standard cotton explants (1.4 g) were used with $V = 120$ ml and $F = 56.3$ ml/hr. The temperature was 30 C.

TURNOVER TIME

If we define the turnover time T as the time needed for a volume of gas equal to the volume of the chamber to pass through the system, then $T = V/F$. It is of interest to know what percentage of the gas originally in the chamber remains after one turnover [$\%G(t)$]. The general form of the relevant differential equation is that of equation 3 with $R(t) = 0$. The solution is

$$\%G(t) = 100 e^{-F/V \cdot t} \quad (8)$$

After one turnover we obtain $\%G = 100e^{-1} = 36.79\%$. After two turnovers $\%G = 13.53$, and so on. Steady state conditions are approximated after three turnovers, when $\%G = 5.00$ and γ , the nonsteady state correction factor for the constant R cases equals 1.05.

DISCUSSION

While more sophisticated techniques for obtaining numerical first derivatives exist (7) and could easily be used, they are not necessary here. In addition, the quadratic approximation allows us to write an explicit formula for the rate, given three points, while more sophisticated approximations would not provide such a simple algebraic expression. Rates calculated using equations

4 to 7 are instantaneous in the precise mathematical definition of the word. They are by no means average rates.

The mathematics presented here are generally valid for all open flow systems for which instantaneous mixing is a good approximation. It is important to note that our mathematical treatment deals with gas concentrations in the chamber. If there were any significant length of tubing connecting the chamber to the site of sampling (as is common when IRGA instruments are used) the approximation of instantaneous mixing would not be valid and our mathematical treatment would not necessarily apply. The sampling, as well as the mathematics, must reflect the instantaneous situation in the chamber.

Errors implicit in nonsteady state rate calculations are principally errors in gas and time measurements. The effects of these errors are most easily discussed in terms of the constant R equation 5. It is clear from this equation that errors in R are directly proportional to errors in $\%C$. It is a simple matter to minimize these errors by using equations 4 or 5 only when $\%C$ is known to the desired accuracy. Errors in time are potentially more troublesome, since the nonsteady state correction factor depends exponentially on time. If $t = 0$ is defined as the point at which the flow starts, a small uncertainty in time is possible. This could give rise to large errors in γ for times near $t = 0$ (although we certainly do not observe them in our system). In any case, this error can be virtually eliminated by defining $t = 0$ with a gas measurement $\%C_0$ and using equation 4. The general solution essentially takes care of this potential problem automatically by requiring three measurements. Other types of errors inherent in flow systems have been discussed previously (3, 4, 9). Depending on the accuracy of the available measuring equipment, we believe that nonsteady state techniques will allow accurate measurements of rates

Table I. A Comparison of Two Methods of Calculating Rates

The CO_2 concentration data of Figure 1 are presented in tabular form. The correction factors for the constant R formulae (equations 4 and 5) are shown as are the results of using equation 1 on this data. The rates calculated using equation 5 are compared to those calculated using the general formula (equation 6). The average rates obtained from both equations are in excellent agreement.

$\% \text{CO}_2$	Time	$\% \text{CO}_2 \cdot K^1$	γ^2	R^3	R^4
	hr			mg $\text{CO}_2/\text{g tissue} \cdot \text{hr}$	
0	0				
0.048	0.22	0.028	11.387	0.320	0.327
0.100	0.47	0.058	5.609	0.328	0.305
0.137	0.73	0.080	3.804	0.305	0.275
0.167	0.95	0.098	3.052	0.298	0.296
0.239	1.42	0.140	2.235	0.312	0.325
0.298	1.97	0.174	1.783	0.311	0.308
0.335	2.40	0.196	1.580	0.310	0.324
0.384	2.90	0.225	1.424	0.320	0.343
0.421	3.43	0.246	1.313	0.323	0.323
0.440	3.88	0.257	1.246	0.321	0.308
0.456	4.47	0.267	1.183	0.316	0.300
0.475	6.88	0.278	1.060	0.294	
				0.313 avg	0.312 avg

¹ Rates calculated from equation 1, $K = F(\text{ml/hr}) \cdot (\text{mg/ml } \text{CO}_2 \text{ at } 30 \text{ C})/100 \cdot \text{tissue weight (g)} = 0.585 \text{ mg } \text{CO}_2/\text{g} \cdot \text{hr}$ for these data.

² γ is the nonsteady state correction factor for the constant R case.

³ Rates calculated from equation 5 (product of columns 3 and 4); standard deviation = 0.010, 3.2% error.

⁴ Rates calculated from equation 6; standard deviation = 0.019, 6.1% error.

from two to twenty times faster than the usual steady state procedures.

Nonsteady state techniques are useful whenever a large free volume or a slow flow rate, or both, is either unavoidable or desirable, or when speed in obtaining data is critical. Whenever a rate to be measured in a flow system is rapidly changing relative to the time needed to obtain steady state, nonsteady state techniques are essential. In other cases where work under steady state is practical, the elimination of the wait for steady state with use of nonsteady state rate calculations could result in a significant saving of time.

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