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A Theoretical Basis for Laboratory Aeration[1]

R. E. Ecker[2] and W. R. Lockhart

Department of Bacteriology, Iowa State University, Ames, Iowa

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Control of aeration has been investigated most frequently in connection with the cultivation of microorganisms of industrial significance, often using culture apparatus of pilot plant or commercial scale. As a result most theoretical studies in this area have been developed with reference to such systems (Cooper, Fernstrom, and Miller, 1944; Hixon and Gaden, 1950; Bartholomew *et al.*, 1950; Karow, Bartholomew, and Sfat, 1953). A complex interaction of variables is involved, and the theoretical basis for experimental results sometimes remains obscure. Workers in general have depended on empirical relationships, defining their respective systems in as great detail as possible.

In many laboratories, controlled aeration in relatively small culture volumes is becoming an established procedure. The theoretical considerations developed here for these less complex systems are intended to establish a basis for prediction of oxygen availability by demonstrating the factors which make significant contributions to oxygen absorption rates.

Materials and Methods

The apparatus used to control gassing rates, shown in figure 1, is a modification of one previously described in detail (Lockhart and Ecker, 1958). Three of the flow meters are equipped with three-way valves and individual pressure reducing valves and gauges, so that each may be used either off the manifold inlet from a compressed air line or independently from any other gas source required. The independent systems are connected to tanks of N_2, O_2, and CO_2. Gas flowing from these three meters may be mixed in a manifold before being led to a single sparger, thus producing any desired mixture of these gases.

The culture vessels, with the exceptions specified in table 1, are 1-L Pyrex bottles of 9.6 cm inside diameter, each containing 500 ml of culture fluid. The surface area of the liquid in vessels of this size is 72 cm². In the

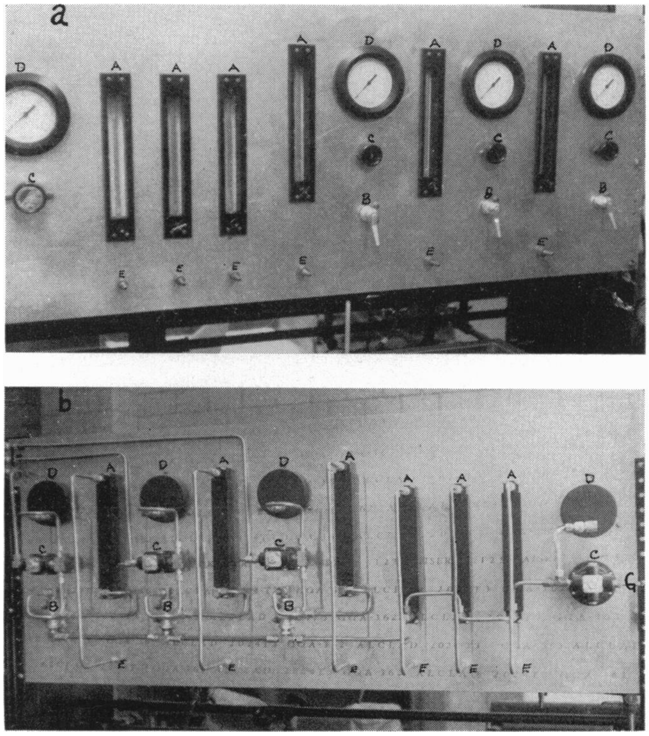

Figure 1. Photographs of aeration control apparatus. *a.* Front view; *b.* rear view. Commercial components from Brooks Rotameter Company, Lansdale, Pennsylvania. *A*, 1355V flow meters; *B*, 108 HD Imperial 3-way positive shut-off valves; *C*, Kendall M30 gas pressure reducing valves; *D*, pressure gauges; *E*, outlets for metered gas; *F*, inlets from individual gas sources; *G*, inlet from compressed air line.

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experiments reported here, the liquid in the culture vessels was a solution of sodium sulfite reagent, and oxygen absorption rates were determined by sulfite oxidation using the modification of Ecker and Lockhart (1959). Aeration experiments were carried out at 37 C.

Spargers were submerged to their maximal possible depth in the culture vessels. Commercial spargers used were Pyrex fritted glass gas dispersion tubes of coarse porosity. The custom-made sparger used in some experiments consists of a nylon cylinder attached by force-fit to a length of stainless steel tubing. The lower portion of the nylon cylinder is machined to a very thin wall into which are drilled 16 holes of known diameter. A diagram of this sparger is shown in figure 2.

THEORY

To be useful, aeration theory should demonstrate relationships between oxygen availability (or oxygen absorption rates) and the controllable experimental parameters. In small, laboratory apparatus it is convenient to consider the volume flow rate of gas (the gassing rate) as the primary adjustable variable, although it will be shown that other factors can also be controlled to affect oxygen availability. In this discussion, however, all factors except the gassing rate will be considered constant in any given expression.

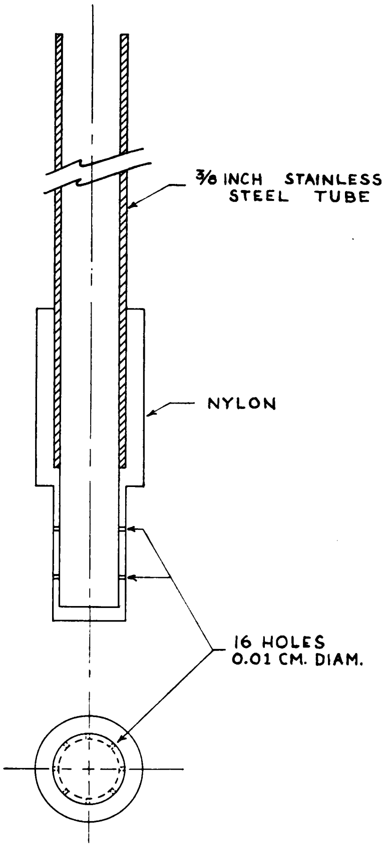

Figure 2. Diagram of custom-built nylon sparger described in text.

We shall be concerned here only with straight-walled vessels of the sizes ordinarily used for laboratory cultivation, into which gas from any source producing a known gassing rate is introduced through a single sparger or bubble tube. Since the liquid depth in such vessels is not great, changes in the volume (and, thus, surface area) of bubbles and the changes in partial pressure through loss of oxygen from bubbles during their ascent through the liquid are not considered to be significant.

Consider a liquid system, such as sulfite solution, in which oxygen demand is always greater than the rate at which it can be supplied. If we assume, when this liquid is in contact with an oxygen-containing gas, that the passage of oxygen across the interface is the rate-limiting step, then the absolute rate of oxygen absorption may be considered dependent only on the gas-liquid interfacial area and the rate constant for passage of oxygen across the interface. This constant must in turn depend on the proportion of oxygen in the gas mixture. Therefore

$$\delta = k_1 A f \quad \text{mM/hr} \qquad (1)$$

where δ is the absolute oxygen absorption rate, k_1 is the oxygen transfer constant in mM/cm²/hr, A is the interfacial area in cm², and f is the fraction of oxygen in the gas mixture; that is, the ratio of the partial pressure of oxygen to the total gas pressure.

In the system under consideration the area, A, is the sum of two quantities, a constant value, A_0, which is the surface area of the liquid in the vessel, and a value A_g, contributed by the gas being introduced through the sparger:

$$A = A_0 + A_g \quad \text{cm}^2 \qquad (2)$$

At any instant there is a given amount of interfacial area contributed by the bubbles rising in the liquid. Under set conditions this value (A_g) is constant, for although the bubbles are rising they are being replaced at the sparger at the same rate as they disappear at the surface. Thus the rate of formation of area due to gassing can be shown as:

$$\begin{aligned} \text{rate of formation} &= \frac{(\text{volume/unit time})(\text{area/bubble})}{\text{volume/bubble}} \\ &= \frac{R(4\pi r^2)}{4/3\pi r^3} \quad \text{L/cm-hr} \\ &= \frac{3{,}000R}{r} \quad \text{cm}^2\text{/hr} \qquad (3) \end{aligned}$$

where R is gassing rate in L/hr and r is the average bubble radius in cm. Then the total interfacial area due to gassing at any instant will be the rate of formation multiplied by the time of ascent (t) in hours.

$$A_g = \frac{3{,}000Rt}{r} \quad \text{cm}^2 \qquad (4)$$

Combining equations (1), (2), and (4),

$$\delta = k_1 f\left(A_0 + \frac{3{,}000Rt}{r}\right) \text{ mM/hr} \tag{5}$$

Under most conditions the effects of bubble radius and time of ascent cannot be separated experimentally. Therefore let

$$\frac{3{,}000t}{r} = \alpha \tag{6}$$

Thus far we have considered only bubbles formed at the sparger orifice and rising directly to the surface, but gassing will also contribute power to the system. Agitation, the effect of this power input, will serve to cleave bubbles, thereby increasing interfacial area, and to hold the bubbles below the surface longer than expected. In other words, turbulence will tend to increase the value of α. So α becomes a function of power, P:

$$\alpha = \alpha_1 + \alpha(P) \tag{7}$$

where α_1 is the value of $(3{,}000\ t/r)$ under nonturbulent conditions. In nonturbulent systems t is a direct function of the depth below the surface at which the bubbles are released. Therefore, α_1 equals βd, where β is a constant which must be determined empirically and d is the depth in centimeters. So

$$\alpha = \beta d + \alpha(P) \tag{8}$$

Determination of the precise nature of the factors contributing to turbulence would be prohibitively difficult. However, it is possible to determine the power input during gassing and by assuming a relationship with turbulence it may be possible to determine the effect of agitation on oxygen absorption. Work must be done to build up pressure in the gassing system. Or, since we are speaking of a constant flow of energy, a certain amount of power must be invested to maintain the pressure in the system. This energy is given up when the pressure returns to normal as gas leaves the sparger. Although much of the energy released at this time is dissipated as heat, some of it contributes to the turbulence of the liquid. If we assume that the relationship between total power input and power expended as turbulence is not altered with changes in gassing rate, we can measure the power input and relate it to agitation.

If pressure in the system is measured by means of a mercury U-tube manometer placed between the metering device and the sparger, the pressure is indicated by the height of the column of mercury. Call this height h, in centimeters. The pressure (p) may then be expressed as

$$\begin{aligned} p &= (9.8)\ (0.0136)\ h \\ &= 0.13\ h \quad \text{newtons/cm}^2 \end{aligned} \tag{9}$$

where 9.8 is the acceleration due to gravity in m/sec^2 and 0.0136 is the density of mercury in kg/cm^3.

The work done in compressing the gas in the system is equal to the change in pressure multiplied by the volume of gas compressed. We do not know the volume of gas in the system but we know the volume compressed per unit time, for this is the gassing rate. If the pressure above the liquid in the culture vessel is the same as that on the open arm of the manometer, the change in pressure is given by equation (9).

Thus the power input can be expressed as:[3]

$$\begin{aligned} P &= 0.13hR \quad \text{newton-meters/hr} \\ &= \left(\frac{0.13h}{3{,}600}\right) R \quad \text{watts} \end{aligned} \tag{10}$$

Although we now know the power input, we have no means of describing, from theoretical considerations, the relationship between power and oxygen absorption rate. It has been assumed that most of the energy put into the system will be lost as heat, but the actual contribution to turbulence will have to be inferred from experimental observations.

The equations so far developed express absolute rates of oxygen absorption, but oxygen absorption rate (OAR) is generally expressed on a unit volume basis with dimensions such as mM/L/hr. For convenience, the final expression therefore becomes

$$\text{OAR} = \frac{k_1 f}{V}\ \{A_0 + R[\beta d + \alpha(P)]\} \quad \text{mM/L/hr} \tag{11}$$

where P is the value shown in equation (10) and V is the culture volume in liters.

EXPERIMENTAL RESULTS AND DISCUSSION

It is noted that the above considerations apply in their entirety only to the calibration of aeration apparatus. The relationships presented may give the maximal rates of oxygen absorption (and, presumably, of oxygen availability to cultures) under calibration conditions, but omit the actual rate of intracellular oxygen availability to microorganisms in any given cultural environment, (Finn, 1954; Schultz and Gaden, 1956; Phillips and Johnson, 1959). If the proposed theoretical treatment is valid with this qualification, however, it should be possible to justify the assumptions made and to evaluate the proposed constants from experimental data.

In the formulation of the basic relationship expressed in equation (1), two assumptions were made: (a) The rate-limiting step in oxygen absorption is the passage of

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[3] If the gas flow measuring device has been calibrated at a pressure other than that found in the culture vessel, the measured value of the gassing rate must be modified before it can be used in equation (10).

$$R_c = (P_0/P_a)R$$

where R_c = corrected gassing rate, P_0 = calibration pressure, and P_a = pressure above the liquid in the vessel.

oxygen across the interface. There is some difference of opinion among workers in the field as to the validity of this assumption for sulfite systems. Finn (1954) discusses this question extensively. Because it is not the purpose of this paper to defend the merits of the sulfite method and there has been no clear-cut evidence to the contrary, this assumption is accepted subject to whatever limitations may actually exist. (b) There is a linear relationship between the fraction of oxygen in the gas mixture and the oxygen absorption rate. The validity of this assumption is demonstrated in figure 3. These calibrations were conducted using the individual metering capabilities of the aeration control apparatus, so that the relative proportions of nitrogen and oxygen could be varied predictably. Both the slopes and intercepts of these lines are related in the ratio of 2.0:3.5:5.0, which is the ratio of the fractions of oxygen in the gas mixtures used.

The calibration curves in figure 3 may be used to evaluate several of the constants found in the basic theoretical expressions. Extrapolation of the lines to zero gassing rate provides values, $(\mathrm{OAR})_0$, for oxygen absorbed through the surface of the liquid in the vessel. From equation (11), this value is equal to $k_1 f A_0/V$. A number of extrapolations, using the lower curve in figure 3 and similar calibration curves at $f = 0.2$, have provided an average value for $(\mathrm{OAR})_0$ of 4.5 mM/L/hr. Since the other terms are known, k_1 may be calculated.

$$k_1 = \frac{V(\mathrm{OAR})_0}{f A_0} \tag{12}$$

$$= \frac{(0.5)(4.5)}{(0.2)(72)}$$

$$= 0.16 \text{ mM/cm}^2\text{/hr}$$

This is the oxygen transfer constant, which should be the same for any sulfite system.

Although figure 3 describes the basic relationship, the curves shown have limited applicability to the theory developed earlier. This calibration was carried out using commercial spargers, which cannot be used extensively for predictions of OAR. Although they are graded according to porosity, there is a rather wide distribution of pore sizes within a given sparger. If gas is forced through the sparger at a low rate, only a few of the larger pores will be used, producing bubbles of maximal size. When gas is fed at a higher rate, more of the pores are used and those added are of smaller diameter than the original few. This causes heterogeneity in bubble size and decreases the average bubble diameter, so that β does not remain constant. Figure 3 suggests this effect at the highest gassing rate shown; other experiments have demonstrated its occurrence at still higher gassing rates. Predictions of OAR in systems using commercial spargers must therefore be limited to lower gassing rates, where β can be approximated as a constant.

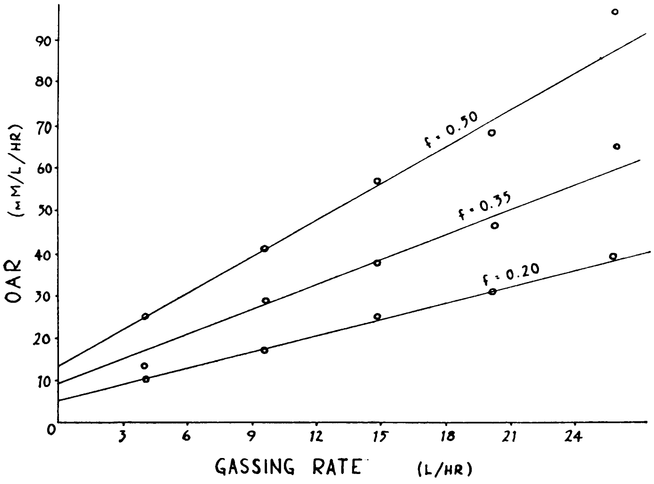

Figure 3. Calibration curves using a fritted glass sparger and three different partial pressures of oxygen in the gas mixture.

Determinations of pressure in the system at various gassing rates indicate that the power input is negligible and that it does not change appreciably as gassing rate increases. The linear portions of the curves in figure 3 confirm that there is no appreciable effect from turbulence at these lower gassing rates. Although is is not possible to use commercial spargers interchangeably, a straight line can be fitted to individual calibration data and β can be given an approximate value which is characteristic for any given sparger. Thus β is a somewhat more sophisticated approach to the "sparger factor" proposed in an earlier paper (Ecker and Lockhart, 1959).

The curves in figure 3 are of the form $y = ax + b$, where y is the dependent variable, OAR; x is the independent variable, R; a is the slope of the line; and b is the intercept on the y-axis. We have already seen that the intercept is equal to $k_1 f A_0/V$. From equations (5) (6), and (8), the slope is equal to $k_1 f \beta d/V$. The value of d in this calibration was 6 cm. Since the actual value of the slope can be determined graphically and the remainder of the terms are known, β can be computed. The slope, a, at $(f = 0.2)$ is 1.3 mM/L^2. Therefore

$$\beta = \frac{aV}{k_1 f d} \tag{13}$$

$$= \frac{(1.3)(0.5)}{(0.2)(0.16)(6)} = 3.4 \text{ hr-cm/L}$$

Using this particular sparger, the conditions described earlier and gassing rates up to 24 L/hr, OAR is equal to $1.3\,R + 4.5$ mM/L/hr. This is a specific application of the general equation for rate of oxygen absorption in nonturbulent systems:

$$\mathrm{OAR} = \frac{k_1 f}{V}(\beta d R + A_0) \quad \text{mM/L/hr} \tag{14}$$

Within a certain range, the conditions may be changed and this equation still can be used to make predictions of OAR. It is necessary only to adjust the appropriate parameters. The value of β is known for each sparger, k_1 is a true constant and all the other variables are subject to experimental control. But there are limits beyond which the relationships in equation (14) may be expected to break down. For example, since power is distributed on a unit volume basis, in very small culture volumes the effect of turbulence, even at low gassing rates can no longer be considered negligible. Using a single sparger as this system requires errors also would be introduced at very large volumes due to inadequate stirring. The conditions were defined to include only culture vessels of such depth as to allow a negligible loss of oxygen from bubbles during their ascent. As a rule of thumb in this laboratory, we have considered negligible a loss of 10 per cent or less. The average loss can be computed from the *absolute* rate of oxygen absorption and the volume flow rate of oxygen (20 per cent of the gassing rate when using air), remembering that 1 mM occupies 22.4 cm^3 at standard conditions.

We have been considering only those systems in which the effect of turbulence is not significant. Since it is often impossible to obtain adequate aeration without having a turbulent system, it is desirable to extend these relationships to include such cases. To determine the feasibility of such expansion, it was necessary to achieve experimental control of all the variables, a condition which was not possible with commercial spargers. The nylon sparger shown in figure 2 has 16 holes of uniform diameter (0.01 cm). This is near the minimal number and size of sparger orifices allowing a reasonable gassing rate at the maximal pressure possible with this apparatus (10 psig).

It is readily observed that greater pressures are required to maintain higher gassing rates through such a sparger. That is, the pressure is a function of the gassing rate. Since the pressure and the height (h) of mercury in a U-tube manometer are related through a constant, we can say that h is a function of R.

To determine the nature of this function it is necessary only to place a manometer in the system between flow meter and sparger and record the height of the mercury column at various gassing rates. A plot of such data for the nylon sparger immersed in water at a depth of 6 cm is shown in figure 4. Since the function appears to be of the form

$$h - h_0 = k_2 R^n, \tag{15}$$

one can plot $\log(h - h_0)$ *versus* $\log R$ and determine n from the slope of the resulting line and $\log k_2$ from the intercept on the $\log(h - h_0)$ axis[4] (figure 5).

The value of n is 2.2 in this case. Other work has indicated that this is a true constant for any sparger with orifices of uniform diameter. The value of k_2 need not be determined at this time since it will be combined with other constants before these expressions are used. Therefore the relationship between $(h - h_0)$ and R is

$$h - h_0 = k_2 R^{2.2} \text{ cm} \tag{16}$$

Placing this value into equation (10) and combining constants,

$$\begin{aligned} P &= \frac{0.13 k_2 R^{2.2}}{3{,}600} R \\ &= k_3 R^{3.2} \text{ watts} \end{aligned} \tag{17}$$

The relationship between power input and OAR may be determined from a calibration for OAR in a turbu-

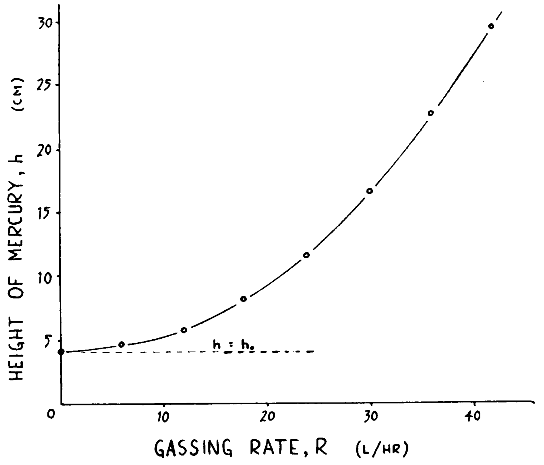

Figure 4. Plot of manometer reading as a function of gassing rate using the nylon sparger.

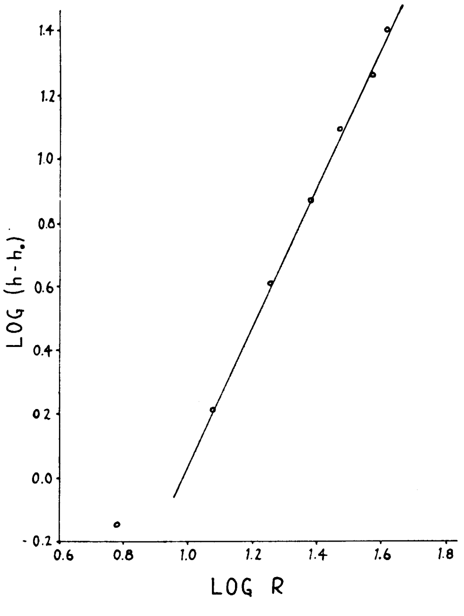

Figure 5. Log-log plot of the data from figure 4

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[4] The value h_0 is the height reached by the mercury column before the gas begins to flow and relates to the limiting value of the pressure as the gassing rate approaches zero. This constant pressure does not contribute to power input and must be subtracted as shown. Thus the quantity $(h - h_0)$ in equation (15) is equivalent to the quantity h in equations (9) and (10).

lent system. The points in figure 6 show data from such a calibration. Note first that the change in spargers did not affect the value of $(\mathrm{OAR})_0$ (*cf.* figure 3). From the slope of the linear portion of this curve and knowing that d is 6 cm for this calibration, β can be computed for this sparger. This value is found to be 0.44 hr-cm/L. In the systems considered previously this was the only constant determined from experimental data. However, this calibration includes control of the parameters which affect turbulence. It should therefore be possible to evaluate the constants necessary for establishing the relationship between power input and OAR. This relationship is responsible for the nonlinearity of the calibration curve in figure 6 at gassing rates above 12 L/hr.

The simplest assumption would be that $\alpha(P)$ is a linear function of P. That is, $\alpha(P) = k_4 P$, where P is the expression shown in equation (17) and k_4 is a constant. To test this assumption we can place this value of $\alpha(P)$ into equation (11) and check the resulting expression against experimental data. Thus

$$\mathrm{OAR} = \frac{k_1 f}{V}\left[A_0 + R(\beta d + k_3 k_4 R^{3.2})\right] \tag{18}$$

Rearranging and combining constants,

$$\mathrm{OAR} = \frac{k_1 f}{V}\left(k_5 R^{4.2} + \beta d R + A_0\right) \tag{19}$$

Which gives us a general equation of the form

$$\mathrm{OAR} = xR^{4.2} + yR + z \tag{20}$$

in which y and z are, respectively, the slope and intercept of the linear portion of the curve in figure 6 (*cf.* equation (14) and figure 3).

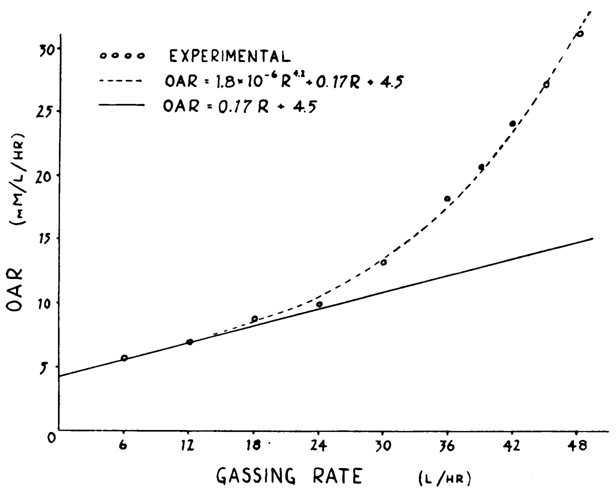

Figure 6. Calibration curves using the nylon sparger with compressed air ($f = 0.2$) as the gas source. Points represent experimental determinations of OAR at the indicated gassing rates. *Solid line*, calculated curve for nonturbulent conditions; *broken line*, calculated curve for turbulent conditions.

Equation (20) may be rewritten as

$$\log(\mathrm{OAR} - yR - z) = 4.2 \log R + \log x \tag{21}$$

When the data in figure 6 were replotted with $\log(\mathrm{OAR} - yR - z)$ as one axis and $\log R$ as the other, it was possible to fit the points to a straight line with a slope of 4.2. The intercept of this line with the $\log(\mathrm{OAR} - yR - z)$ axis gives the value of $\log x$. Taking the antilog of this quantity yielded a value of 1.8×10^{-6} for x. Therefore, under the conditions defined,

$$\mathrm{OAR} = 1.8 \times 10^{-6} R^{4.2} + 0.17\, R + 4.5 \quad \mathrm{mM/L/hr} \tag{22}$$

The broken line in figure 6 is a plot of the above function, which seems to fit the experimental points quite well.

By factoring equation (22), using the known values of all the terms except k_5, we may restore the form of equation (19):

$$\mathrm{OAR} = \frac{(0.16)(0.2)}{(0.5)}\left[2.8 \times 10^{-5} R^{4.2} + (0.44)(6.0)R + 72\right] \tag{23}$$

and the value of k_5 is found to be 2.8×10^{-5}.

From this more general expression relating OAR and gassing rate for the nylon sparger, it should now be possible to predict oxygen absorption rates in turbulent systems. Table 1 shows the result of such experiments. The values found experimentally for OAR agree reasonaby well with those predicted from equation (23), confirming the values for the constants β and k_5. Manipulation with f, V, d, R, and A_0 is the same in this case as in the nonturbulent systems considered earlier, but the effect of culture volume on turbulence is now taken into account and is no longer a limiting factor in the accuracy of predictions. However, there remain limits beyond which these relationships no longer apply. The primary limitation will be significant loss of oxygen from the bubbles during their ascent, in which case the interface can no longer be considered the limiting barrier in oxygen supply.

Higher values of OAR were obtained in the non-

TABLE 1

*Comparison of predicted and actual rates of oxygen absorption during aeration with the nylon sparger**

Expt	Values of Variables†					OAR (mM/L/hr)	
	V	d	A_0	f	R	Predicted	Actual
I	0.2	7	27	0.2	30	27	25.6
II	0.4	8	44	0.2	24	12	11.6
III	0.8	10	72	0.4	36	26	24.1
IV	3.0	15	180	0.2	42	6	5.2

* Actual values for oxygen absorption rate determined by sulfite oxidation in straight-walled vessels of various sizes.

† V = liquid volume (L), d = sparger depth (cm), A_0 = liquid surface area (cm²), f = decimal fraction of oxygen in gas mixture, R = gassing rate (L/hr).

turbulent systems shown than in the turbulent systems used, since the nylon sparger was constructed to permit control of certain experimental parameters rather than to achieve higher rates of oxygen absorption. We desired to learn what factors affect OAR and how an understanding of the interaction of these factors might be used to make predictions of OAR in specific culture apparatus. The basic relationship illustrated by equation (11) and applied to nonturbulent and turbulent systems in equations (14) and (19) permits such predictions. Determination of the oxygen transfer constant k_1 (0.16 mM/cm^2/hr for sulfite systems) is possible from equation (12); determination of the sparger constants is illustrated for β in equation (13) and for k_5 in equations (20 to 23). All other factors used in these expressions are subject to experimental control.

We have not attempted to modify these expressions for application outside the conditions initially defined; it appears that such attempts would be of doubtful value. These relationships are useful in the study of laboratory aeration, but large-scale fermentation apparatus presents problems which properly are the concern of the engineer. Many factors which could be simplified or ignored altogether under the narrowly confined conditions specified here assume new importance when "scale up" to the pilot plant is contemplated. By manipulating variables in the manner suggested, it should be possible to achieve rather precise control of aeration in the laboratory and to determine optimal conditions for most microbiological processes. OAR, as determined here, is easily converted to K_La, the measure preferred in engineering circles (Finn, 1954). The latter parameter can then provide a starting point for carrying the fermentation beyond the laboratory stage.

SUMMARY

For culture systems involving laboratory-scale volumes and aeration through a single sparger, theoretical expressions are developed relating rate of oxygen absorption with various experimentally adjustable parameters, such as gassing rate, culture volume, and partial pressure of oxygen. Apparatus used in testing these hypotheses is described and the expressions are verified experimentally. Use of these expressions for predictions of oxygen absorption rates in both turbulent and nonturbulent systems is discussed.

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