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Aerobic Microbial Growth at Low Oxygen Concentrations

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Sterilizable membrane probes were used to study the relation between oxygen concentration and respiration rate in *Candida utilis* growing on acetate. When the organism was grown in a continuous fermentor at various dissolved oxygen concentrations (0.23×10^{-6} to 32×10^{-6} M), with time allowed for full adaptation to each oxygen concentration, the relationship between oxygen concentration and growth rate simulated Michaelis-Menten behavior, giving an apparent K_m for oxygen of 1.3×10^{-6} M. When respiration rate was measured at various oxygen concentrations without allowing time for adaptation, it was found that the respiration rate was directly proportional to O_2 concentration at low O_2 concentrations, and independent of O_2 concentration at high O_2 concentrations. Transition from one type of behavior to the other was fairly abrupt. The respiration rate in the presence of excess oxygen depended on the O_2 concentration at which the cells were grown, but the rate at low O_2 concentrations did not. There was evidence that, at low oxygen concentrations, oxygen diffusion through the cell substance limits respiration rate, at least in part.

Many investigators have studied the effect of oxygen concentration on the oxygen uptake rate of microorganisms. At oxygen concentrations low enough to give uptake rates well below the maximum, the lowered uptake rate has been ascribed to various causes. Warburg and Kubowitz (10) believed that with yeast cells diffusion of oxygen through the cell substance limited the uptake rate. Longmiur (7) concluded that with larger organisms (yeast, *Bacillus megaterium*) diffusion of oxygen through the cell material was rate-limiting. Winzler (11), also working with yeast, believed the rate-limiting process to be the combination of oxygen with the oxygen-transferring enzyme, presumably cytochrome oxidase. Terui et al. (9) observed differences in apparent K_m for oxygen among various yeasts, but concluded that they were measuring the K_m of cytochrome oxidase.

The effect of oxygen concentration on growth rate may be studied by the use of continuous culture. With oxygen as a limiting nutrient, steady-state growth rate can be studied as a function of steady-state oxygen concentration. This technique was approximated by Terui et al. (9), but they did not allow time for a steady state to be established, and they did not use oxygen concentrations below 10^{-5} M. Button and Garver (1) also used continuous culture to study the effect of oxygen concentration on growth rate, but they did not directly determine the oxygen concentration in the medium. They found an apparent K_m for oxygen for *Candida utilis* (growing on glycerol) of 1.4×10^{-5} M.

The factor that normally limits oxygen uptake by microorganisms is the supply of intermediary metabolites (e.g., adenosine diphosphate and reduced nicotinamide adenine dinucleotide). It is this factor, which can be called the oxygen demand of the cell, that is normally rate-limiting at high oxygen concentrations, where the oxygen-utilization rate is independent of the oxygen concentration. It is also possible that the uptake rate could be limited by the concentration of the oxygen-reducing enzyme. In this case, the maximal rate of oxygen utilization would be the maximal velocity of the enzyme, and a plot of uptake rate against oxygen concentration would have the familiar Michaelis-Menten form. Terui et al. (9) and Longmuir (7) have reported this type of behavior. Their work will be discussed later. In the usual case, oxygen uptake rate at high oxygen concentrations is independent of oxygen concentration, but, if the concentration is allowed to fall, a point is reached where the uptake rate is no longer independent of oxygen concentration, and the uptake rate will begin to decrease. If it is assumed that there is no appreciable resistance to diffusion of oxygen between the bulk of the medium and the oxygen-using enzyme (pre-

sumably cytochrome oxidase), the oxygen concentration at the enzyme is the same as that in the bulk of the medium. In this case, the decrease in uptake rate at low oxygen concentrations would result from lowered reaction velocity due to lack of saturation of the enzyme with oxygen, and would occur when the oxygen concentration fell to a value approaching K_m for the enzyme. The oxygen concentration at which oxygen uptake began to decrease would depend on the amount of enzyme present and on its Michaelis constant for oxygen. For example, if twice as much enzyme were present as was necessary to satisfy the oxygen demand of the cell at high oxygen levels, the oxygen uptake rate would just begin to decrease when the oxygen concentration fell to a value equal to the K_m of the enzyme. If oxygen concentration continued to fall, the uptake rate would decrease in a manner predicted by the Michaelis-Menten relationships. In Fig. 1, the behavior described above is illustrated in curves 1A, 1B, and 1C, which illustrate that the degree of curvature of the low-oxygen end of the curve depends on the ratio of oxygen demand to the maximal capacity of the enzyme. When the enzyme is present in great excess, as in curve 1C, the line is almost straight. The situation illustrated by these curves will be called enzyme-limited oxygen uptake.

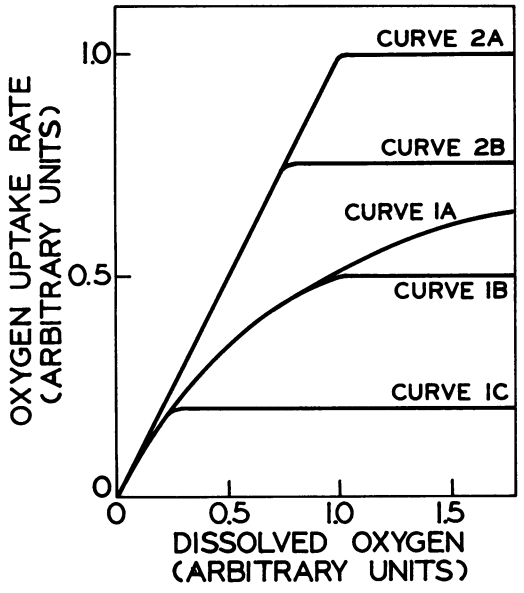

FIG. 1. *Some possible relationships between dissolved oxygen concentration and rate of oxygen utilization. Curves 1A, 1B, and 1C: rate limited at low oxygen concentrations by enzyme concentration and at high oxygen concentrations by oxygen demand. The enzyme concentration is just sufficient to give a rate of utilization of one ordinate unit. K_m for the enzyme is one abscissal unit. Curve 1A, oxygen demand greater than 0.65 ordinate unit; curve 1B, oxygen demand 0.5 ordinate unit; curve 1C, oxygen demand 0.2 ordinate unit. Curves 2A and 2B: uptake rate limited at low oxygen concentrations by the rate of oxygen diffusion. Curve 2A, oxygen demand 1.0 ordinate units; curve 2B, oxygen demand 0.75 ordinate units.*

If it is assumed that the resistance to oxygen diffusion from the bulk of the medium to the enzyme surface is very appreciable, oxygen uptake rates at low oxygen concentrations will be diffusion-limited. This situation is illustrated by curves 2A and 2B of Fig. 1. Here, if the bulk oxygen concentration is allowed to fall, the uptake rate will remain independent of bulk oxygen concentration as long as the concentration drop across the region of resistance to diffusion is considerably less than the bulk oxygen concentration, so that the oxygen concentration at the enzyme surface is appreciable. When the bulk oxygen concentration falls to the point where the oxygen concentration at the enzyme surface is very close to zero, the oxygen uptake rate will fall, maintaining itself at a value such that the concentration drop through the diffusion resistance is roughly equal to the bulk oxygen concentration. The result is a curve consisting of two straight-line portions: the position of the horizontal portion depends on the cell oxygen demand, and the slope of the low oxygen is inversely proportional to the resistance to diffusion.

Recent work from our laboratory (Borkowski and Johnson, *unpublished data*) has indicated that extracellular resistances to diffusion of oxygen are negligible. As will be shown, however, the intracellular diffusion resistance may well be very appreciable.

Behavior of the type idealized in curves 2A and 2B can occur only when a large excess of oxygen-reducing enzyme is present. If such an excess is not present, and if the drop in oxygen concentration inside the cell is small compared with the oxygen concentration in the medium, the behavior indicated in curves 1A, 1B, and 1C may be observed.

In the foregoing, the response of a given cell suspension to changes in dissolved oxygen concentration has been considered. Another type of experiment can also be performed. Let an organism be grown in a continuous fermentor with oxygen as the limiting nutrient. Let it be grown at some constant dilution rate until steady state is reached. The dissolved oxygen concentration is noted, and the dilution rate is changed. The change in dilution rate will cause a change in oxygen concentration and, of course, a change in cell growth rate. The cells will adapt to the new conditions, and a new steady state will be reached.

The new steady-state oxygen concentration is then noted. In this way, a plot can be made of growth rate of adapted cells as a function of oxygen concentration. The response of the organisms to changes in oxygen concentration would be expected to be different from that observed in a short-term experiment where adaptation could not occur.

In the present work, both types of experiments have been performed on *C. utilis* cells grown at 30 C in a mineral medium with acetate as the sole carbon source. Cells were grown in steady state at various dissolved oxygen levels, and the resulting cell suspensions were tested for short-term response to changes in dissolved oxygen concentration.

MATERIALS AND METHODS

Organism and growth conditions. The organism used was *C. utilis* No. 3, Wisconsin collection. It was grown at 30 C on the following medium (concentrations in grams per liter): acetic acid, 0.5; ammonium acetate, 0.64; KH_2PO_4, 0.05; $MgSO_4 \cdot 7H_2O$, 0.02; Triton X-100 (a nonionic detergent), 0.1. To the medium were added 60 μM Fe^{+++} and 20 μM Zn^{++}, Cu^{++}, and Mn^{++}. The *p*H of the autoclaved medium was 4.70.

Fermentor. The continuous fermentor used had a volume of 167 ml. It was operated entirely full of medium. The medium fed to the fermentor was saturated with air at 30 C. The air dissolved in the medium was the only oxygen supply for the organisms. The dilution rate was controlled by controlling the rate of withdrawal from the fermentor with a dilution-rate controller that will be described elsewhere.

The controller, at 10-sec intervals, withdrew from the fermentor a measured volume (0.04 to 0.17 ml) such that the desired dilution rate was maintained.

Oxygen probes. The dissolved oxygen level in the fermentor was monitored with a sterilizable oxygen probe of the type previously described (4). The probes used were constructed for rapid response and low zero current. Rapid response was obtained by using a 1-mil Teflon membrane, and low zero current was obtained by having a large area of Pb anode as close as possible (less than 1 mm) to the silver cathode. Probes were calibrated in the fermentor at low oxygen levels by adding to the almost oxygen-free fermentor a known volume of medium of known oxygen content. Probes were generally used with a load resistor of 20,000 ohms, which gave a sensitivity of 0.6×10^{-6} to 1.0×10^{-6} M O_2 per mv, depending on whether 6- or 7-mm glass tubing was used for the probe. In all cases, the amount of oxygen used by the probe was negligible compared with the amount of oxygen used by the organisms.

Experimental procedure. The continuous fermentor could be operated for from 5 to 7 days before growth on the walls of the fermentor became appreciable. During operation of the fermentor, the dilution rate was set at a known constant value, and the dissolved oxygen was monitored with a millivolt recorder. Steady state, as indicated by constancy of dissolved oxygen in the fermentor, was reached in from 10 to 20 hr after a change in the dilution rate. The dissolved oxygen level, depending on dilution rate, varied, approximately, from 10^{-5} to 10^{-6} M. Since the oxygen concentration in the feed was 2.28×10^{-4} M, at least 95% of the oxygen was used. The constancy of the level of unused oxygen, therefore, was a good indicator of a steady state. After a steady state was reached, continuous feeding was stopped, and fresh medium was rapidly added to the fermentor in an amount giving, in the fermentor, a dissolved oxygen level of the order of 10^{-5} M. The rate at which the organisms used this oxygen was determined by recording the output of the oxygen probe. From the slope of the curve, the rate of oxygen utilization at various oxygen concentrations was calculated. Care was taken that the rate of oxygen utilization was not so rapid that appreciable error was caused by the response time of the probe. This possibility was checked by diluting the cell suspension with oxygen-free medium and repeating the exhaustion curve.

RESULTS

Steady-state growth rate as a function of oxygen concentration. The steady-state oxygen concentrations observed at various dilution rates were recorded in four continuous culture experiments, made over a period of 2 months. All of the data obtained are summarized in Fig. 2, where the reciprocal of the growth rate is plotted against the reciprocal of the oxygen concentration. From the data, the maximal growth rate (μ_{max}) was about 0.44 hr^{-1}, and the apparent Michaelis constant (K_m) for oxygen was about 1.34×10^{-6} M. It is not believed that this apparent K_m is the Michaelis constant of an enzyme. When the dilution rate was changed, considerable adaptation time elapsed before a stable dissolved oxygen level was established. It does not appear likely that the level of oxygen-using enzyme present in the cells was independent of the oxygen concentration at which they were equilibrated. Herbert (2) reports that levels of some cytochrome components vary greatly with dissolved oxygen level. Also, it is probable that the rate of diffusion of oxygen into the cell was one of the factors that limited the rate of oxygen uptake. The statement that the apparent K_m was 1.34×10^{-6} M and the maximal growth rate was 0.44 hr^{-1} can be regarded only as a convenient method of summarizing the data of Fig. 2.

Data from exhaustion curves. After a steady state had been established at a constant dilution rate and a constant dissolved oxygen level, the rate of utilization by the organisms of rapidly added oxygen was determined as described under "Experimental procedure." A typical record is shown in Fig. 3. From measurements of the slopes of such records, curves such as those of

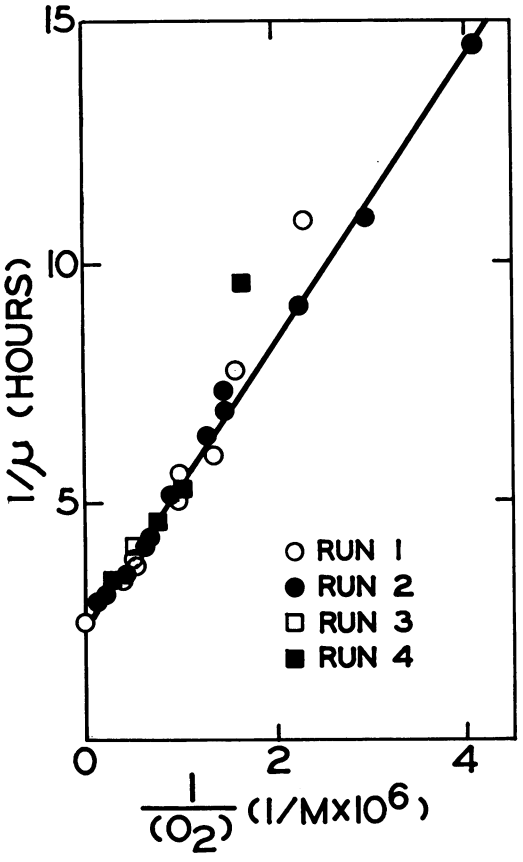

FIG. 2. *Reciprocal plot of steady-state growth rate as a function of oxygen concentration. The line corresponds to a Michaelis constant of 1.34 × 10⁻⁶ M, and a maximal growth rate of 0.44 hr⁻¹. The figure includes all the data obtained during the investigation, and includes the results from four fermentation experiments.*

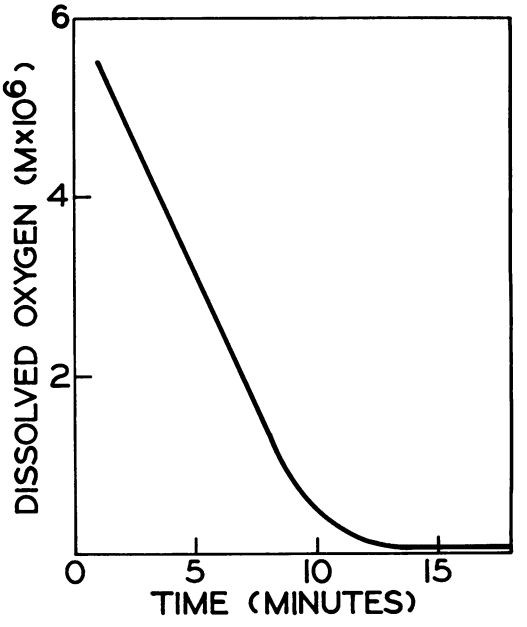

FIG. 3. *Typical exhaustion curve. Rate of use of dissolved oxygen by the contents of a continuous fermentor, grown to steady state at a dilution rate of 0.105 hr⁻¹, at a dissolved oxygen concentration of 0.62 × 10⁻⁶ M. The concentration of oxygen (the limiting nutrient) in the feed medium was 2.28 × 10⁻⁴ M.*

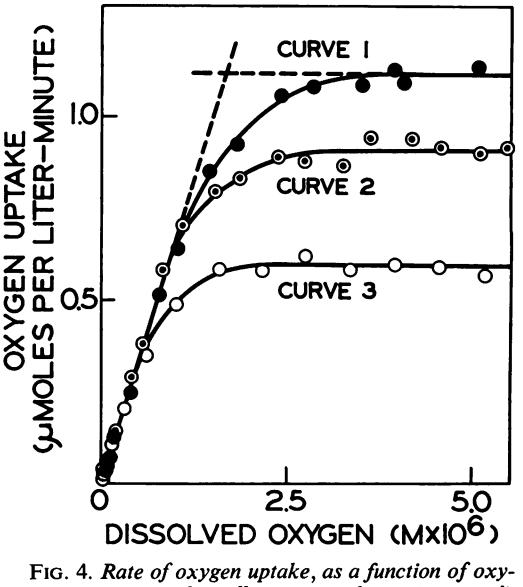

FIG. 4. *Rate of oxygen uptake, as a function of oxygen concentration, for cells grown under various conditions. Curve 1: cells grown at 2.84 × 10⁻⁶ M O₂; μ = 0.293 hr⁻¹. Curve 2: cells grown at 0.97 × 10⁻⁶ M O₂; μ = 0.190 hr⁻¹. Curve 3: cells grown at 0.62 × 10⁻⁶ M O₂; μ = 0.105 hr⁻¹.*

Fig. 4 were constructed. Although care was taken to reduce the error in the potentiometric record as far as possible, slope determinations from short segments of the record gave curves with considerable scatter of points, as may be seen from Fig. 4. In Fig. 4, curve 3 corresponds to the record reproduced in Fig. 3. All three curves in Fig. 4 were made from the same fermentation run. A number of conclusions may be drawn from the figure. First, the curves have a straight-line portion, passing through the origin, which indicates that the oxygen-using enzyme is far from saturated. That is, the curves resemble Fig. 1, curves 1C or 2A, rather than curve 1B. Second, the transition region of the curves, that is, the part between the two straight-line portions, might be due either to the spread in cell size alone, or to

this spread plus approaching saturation of the enzyme. That is, it might be concluded that curves 1 and 3 of Fig. 4 are analogous to Curves 1B and 1C of Fig. 1. Third, the initial slopes of all three curves are the same. This means that if the curves are analogous to Curves 1B and 1C of Fig. 1, the amount of oxygen-using enzyme in the cells was constant, and did not change when the oxygen concentration in the medium was changed. If, however, the curves of Fig. 4 are considered as analogous to curve 2A of Fig. 1, the constancy of initial slope indicates only that the diffusion barrier within the cell did not change with growth conditions.

To compare the shapes of the curves of Fig. 4, both ordinates and abscissas of curves 1 and 2 were multiplied by a number (0.6/1.12 for curve 1, and 0.6/0.91 for Curve 2) such that the maximal uptake rate for all curves was the same. The resulting curve is shown in Fig. 5. It can be seen that, within experimental error, the curves coincide. They are not as different in shape, for example, as curves 1B and 1C of Fig. 1. This constancy of shape indicates again that the curves are similar to Curves 2A and 2B of Fig. 1, and not to Curves 1B and 1C. During the course of this investigation, a number of other sets of curves, similar to those of Fig. 4, were obtained. The shapes of the curves obtained were always similar to those of Fig. 4.

Figure 5 is perfectly consistent with the assumption that diffusion is the chief limiting factor in oxygen uptake. The driving force (i.e., the concentration difference) necessary to cause diffusion of oxygen through the cell at the observed rate may be approximately calculated if values are assumed for the diffusivity of oxygen through the cell substance and for the distance the oxygen must diffuse through cell substance before being used.

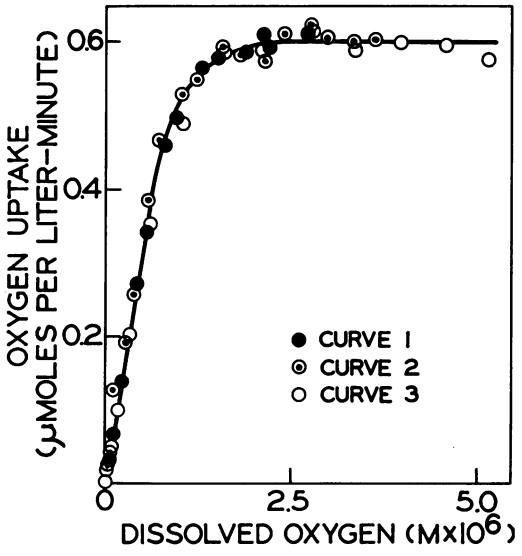

FIG. 5. *Curves of Fig. 4 normalized to equal oxygen uptake rates. Ordinates and abscissas of curve 1 multiplied by 0.535, and ordinates and abscissas of curve 2 multiplied by 0.66.*

It is a reasonable simplification to assume that the bulk of the oxygen utilization in a microbial cell is localized in a shell located some distance from the cell surface. In bacteria, most of the oxygen is apparently utilized in the plasma membrane. In yeasts, the mitochondria tend to cluster just inside the plasma membrane. If it is assumed that, in a spherical microorganism of radius r, the oxygen uptake occurs at an average distance nr from the center of the cell, the drop in oxygen concentration between the cell surface and the point at which the oxygen is used may be calculated from the following formula, which is derived in the appendix.

$$M_s - M_e = \frac{3.12r^2\mu(1/n - 1)}{D_c Y} \qquad (a)$$

where M_s = oxygen concentration at cell surface, moles per liter; M_e = oxygen concentration at enzyme, moles per liter; r = cell radius, centimeters; μ = cell growth rate constant, sec^{-1}; n = distance from cell center to enzyme, as fraction of r; D_c = diffusivity of oxygen through the cell, $cm^2\ sec^{-1}$; y = cell yield constant based on oxygen (grams of cells formed per gram of oxygen used).

It is reasonable to assume that the diffusivity of oxygen through the cell is about 40% of its diffusivity through the medium. Krogh (6) found that the diffusivity of oxygen through muscle tissue was 41% of the diffusivity through water, and Kreuzer (5) found that diffusivity of oxygen through a 30% serum protein solution was from 40 to 45% of its diffusivity through water. If it is assumed that the location of the oxygen-using enzyme is such that half of the cell volume is outside the enzyme shell, and half inside, n, the radius of the enzyme shell in terms of cell radius, becomes $0.5^{1/3}$, or 0.7937. The drop in oxygen concentration within the cell for various values of n and for various values of diffusivity of oxygen is shown in Table 1. The table is calculated for a cell with a growth rate (μ) of 0.3 hr^{-1}. This is approximately the rate at which the cells of Fig. 4, curve 1 were growing. In Fig. 4, the (extrapolated) low-oxygen portion of the curves intersects the maximal uptake line for curve 1 at about 1.7×10^{-6} M. Hence, the drop in oxygen concentration within the cell at maximal oxygen uptake rate

could not exceed this figure. Table 1, however, shows that the drop inside the cell was probably a very substantial proportion of 1.7×10^{-6} M.

It is interesting to compare, for the exhaustion curves from which Fig. 4 was derived, and for other curves in the same fermentation run, the rate at which oxygen was taken up during steady-state growth of the organism with the maximal rate (as determined by the exhaustion curve) at which the cells could utilize oxygen at high oxygen concentrations. It is apparent from Fig. 4 that cells grown at high oxygen concentrations could use oxygen at a more rapid rate (in the presence of excess oxygen) than those grown at low oxygen tensions. In Table 2, data from a number of different steady states are summarized. It is evident that cells adapted to growth at a low oxygen concentration cannot (in the presence of excess O_2) use oxygen as rapidly as cells adapted to a higher oxygen concentration, giving more rapid growth. It is also apparent that cells grown at a low oxygen concentration are able (immediately, without adaptation) to use oxygen more rapidly than the rate at which it was used during growth.

TABLE 1. *Oxygen concentration drop within cells*[a]

n	Per cent of cell vol traversed by O_2	O_2 concn drop within cell, $M_s - M_e$ (M × 10^6)		
		D_c/D_w[b] = 0.60	D_c/D_w = 0.40	D_c/D_w = 0.30
0.9	27	0.23	0.34	0.46
0.8	49	0.52	0.77	1.03
0.7	66	0.88	1.33	1.77

[a] The drop is calculated for spherical cells of 2.5 μ radius, growing at a rate of 0.3 hr^{-1} with a yield constant (for oxygen) of 0.483.

[b] D_c/D_w = ratio of oxygen diffusivity in the cell to its diffusivity in water, which is taken as 2.71×10^{-5} cm^2/sec (30 C).

TABLE 2. *Effect of growth conditions on maximum oxygen uptake rate of Candida utilis cells*

Conditions during steady-state growth			O_2 uptake with excess O_2[b]
μ	O_2 concn	O_2 uptake rate[a]	
hr^{-1}	$M \times 10^6$	$M \times 10^6/min$	$M \times 10^6/min$
0.0514	0.234	0.195	0.312
0.105	0.62	0.399	0.594
0.190	0.97	0.721	0.912
0.207	1.29	0.788	0.942
0.294	2.84	1.11	1.12

[a] Uptake rate during growth = O_2 concentration in feed (2.28×10^{-4} M) times μ (in min^{-1}).

[b] From exhaustion curve.

DISCUSSION

The effect of oxygen tension on oxygen uptake of yeast has been investigated by three previous investigators, all of whom, using exhaustion curves, reported K_m values for oxygen. Winzler (11) reported, for *Saccharomyces cerevisiae*, a K_m value of 1.85 mm of O_2 tension, constant between 23.4 and 34.3 C. At 30 C, this value corresponds to 2.84×10^{-6} M. Longmuir (7) reported a K_m value of 6.45×10^{-7} M at 19 C. Terui et al. (9) reported values that depend on the oxygen concentration at which the cells were grown, and that range from 0.73×10^{-6} to 3.4×10^{-6} M.

Winzler reported a maximal Q_{O_2} (microliters of O_2 per milligram of dry yeast per hour) of about 75 to 30 C. Terui et al. found for *S. cerevisiae* a maximal Q_{O_2} of 68. In the present work (with *C. utilis*), the maximal Q_{O_2} values for the suspensions of Fig. 4 were from 227 to 428. The method of calculation is given in the appendix. The low Q_{O_2} values for *S. cerevisiae* are to be expected, because this yeast does not possess an oxidative system capable of supplying energy at a rate necessary to meet cell demands (8). In the exhaustion curve experiments of Winzler, or Longmuir, and of Terui et al., glucose was present, and the yeast was able, at low oxygen concentrations, to obtain its needed energy anaerobically. *S. cerevisiae* will, even in the presence of excess oxygen, convert two-thirds of the glucose used to ethyl alcohol and CO_2 (8). In our experiments, acetate was the only carbon source present.

The low rates of oxygen utilization in Winzler's experiment made it unlikely that oxygen diffusion could limit oxygen uptake. Moreover, by his ingenious use of carbon monoxide, he showed conclusively that diffusion was not limiting. In *S. cerevisiae*, the level of oxygen-using enzyme is apparently so low that its concentration limits oxygen uptake rate. From our experiments, in which diffusion certainly partially limited oxygen uptake, it is difficult to estimate the minimal value of K_m that would be consistent with out results. However, it appears to be higher than the K_m reported by Winzler. Longmuir unfortunately does not report Q_{O_2} values. He reports K_m values for various organisms, but concludes that diffusion of oxygen is, with larger organisms, "a most important factor." If his conclusion is true, he should have obtained curves like those of Fig. 4, rather than curves like curve 1A, Fig. 1, which he reported obtaining. Longmuir reported that K_m, as calculated from his curves, coincided with the oxygen concentration at half-maximal uptake rate. This could not be true if diffusion was a

factor in rate limitation, and could not be true, even if diffusion were not a significant factor, unless the amount of enzyme present was so small that it was operating at maximal velocity at high oxygen concentrations. Neither in Winzler's experiments nor in ours was the behavior reported by Longmuir observed.

Terui et al. (9) reported obtaining curves of the type of curve 1A, Fig. 1, under a wide variety of conditions, and concluded that they were measuring the K_m of the oxygen-reducing enzyme. Their findings appear to require that, under all the conditions they employed, concentration of the oxygen-reducing enzyme always limited oxygen uptake rate. They also reported that K_m for the oxygen-reducing enzyme varied by a factor of more than 4, depending on the oxygen concentration under which the cells were grown. These results are difficult to interpret.

It is believed that the measurements of oxygen concentration in the present study are more reliable than those obtained before the availability of membrane probes. Well-designed membrane probes have a very low residual current, retain their calibration for weeks, and can be steam-sterilized. The curves obtained at low oxygen concentrations in the present study are consistent with the assumption that oxygen uptake is limited chiefly by diffusion. Calculations show that with reasonable assumptions for diffusivity of oxygen within the cell, diffusion, at the oxygen uptake rates observed in this study, constitutes certainly an appreciable limitation, and possibly the chief limitation, to oxygen transfer at low oxygen concentrations. The data are consistent with a low intracellular diffusion barrier only if it is assumed that the cells adapted to various oxygen concentrations contained equal concentrations of oxygen-using enzyme.

APPENDIX. OXYGEN DIFFUSION WITHIN THE CELL

Notation

M_s = oxygen concentration at cell surface, moles per liter
M_e = oxygen concentration at enzyme, moles per liter
r = cell radius, cm
μ = cell growth rate constant, sec^{-1}
n = distance from cell center to enzyme, as fraction of r
D_e = diffusivity of oxygen through the cell, $cm^2\ sec^{-1}$
y = cell yield constant based on oxygen (grams of cells formed per gram of oxygen used)
C_s = oxygen concentration at cell surface, g/cm^3
C_e = oxygen concentration at enzyme, g/cm^3
f = distance (cm) from the cell center of some point within the cell such that $r > f > nr$.
C = oxygen concentration at f, g/cm^3
Q = integration constant
k = a constant evaluated below
T = rate of transfer of oxygen at cell surface, g per cell per second

Derivation

Consider the diffusion of oxygen within the cell, at a distance f from the cell center, where $r > f > nr$, in other words, in the region between the cell surface and the surface at which the oxygen is assumed to be used. The oxygen concentration gradient (dC/df) in this region is positive (C is greater at a greater value of f) and, since the area in which the diffusion takes place is proportional to f^2,

$$\frac{dC}{df} = \frac{k}{f^2}$$

integrating,

$$C = -\frac{k}{f} + Q$$

When $f = r$, $C = C_s$, therefore

$$Q = C_s + \frac{k}{r}$$

Hence,

$$C = C_s + \frac{k}{r} - \frac{k}{f}$$

$$C_s - C = \frac{k(r - f)}{rf}$$

When $f = nr$, $C = C_e$, and

$$C_s - C_e = \frac{k}{r}\left(\frac{1}{n} - 1\right).$$

The constant k may be evaluated as follows: when $f = r$ (at cell surface)

$$\frac{dC}{df} = \frac{k}{r^2}$$

The cell surface area is $4\pi r^2$.
Consider the oxygen transfer across this area:

$$T = 4\pi r^2 \cdot \frac{k}{r^2} \cdot D_e = 4\pi k D_e$$

The transfer is also equal to the cell weight times its specific oxygen utilization rate; that is, the oxygen transferred per second by 1 g of cells is μ/y. The growth rate μ is, in continuous culture at steady state, equal to the dilution rate. The cell yield y (grams of cells produced per gram of oxygen used) may be directly measured, or it may, for strictly aerobic growth, be calculated, from material balance considerations, from the cell yield on the carbon source (3). In our experiments, the yield y was 0.483, as calculated from the yield on acetic acid.

Incidentally, when μ is expressed in hr^{-1} rather

than sec^{-1}, $\mu/y \times 700 = Q_{O_2}$, which is microliters of O_2 per milligram of dry cells per hour.

The oxygen transfer rate per cell is μ/y times the dry weight of one cell. If the dry content of the cells is assumed to be 0.3 g per cm^3, then

$$T = \frac{0.4\pi r^3 \mu}{y}$$

Therefore

$$\frac{0.4\pi r^3 \mu}{y} = 4\pi k D_c$$

$$k = \frac{0.1 r^3 \mu}{D_c Y}$$

and

$$C_s - C_c = \frac{0.1 r^2 \mu \left(\frac{1}{n} - 1\right)}{D_c Y}$$

or, if oxygen concentrations are expressed as molarities,

$$M_s - M_c = \frac{3.12 r^2 \mu \left(\frac{1}{n} - 1\right)}{D_c Y}$$

ACKNOWLEDGMENT

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