Estimation of Dissolved Carbon Dioxide Concentrations in Aerobic Fermentations

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Dissolved carbon dioxide and bicarbonate ions in fermentation broths can (independently) inhibit or promote microbial growth and productivity. In research facilities with a large number of fermenters, dissolved carbon dioxide sensors tend not to be used, and as a result this variable will generally go unmonitored, making the meaningful analysis of data more difficult. For aerobic fermentations, mass transfer of carbon dioxide can be described in an analogous way to oxygen transfer. The mass transfer coefficient for carbon dioxide is 0.89 times that for oxygen. The maximum dissolved carbon dioxide concentration as a function of exit gas composition is compared with the concentration obtained by assuming equilibrium between the broth and exit gas. The difference between these two concentrations is typically 20–40% of the equilibrium concentration. In large fermenters, a degree of plug flow behavior in the gas and the generally lower specific aeration rates will serve to produce a better approach to equilibrium than for research fermenters.

Introduction

Sterilizable sensors for the measurement of the concentration of dissolved carbon dioxide in fermentation broths have been commercially available for several years (Puhar et al., 1980). These sensors, however, cannot be described as an established tool in the industry (Fox, 1984). The sensor is expensive, has only ±10% accuracy when calibrated against standard buffers, has a fairly slow response time, and must be calibrated at two points. Such issues may be expected to hamper in particular the application of these sensors in research facilities, where there are generally a large number of small fermenters. An indication of the dissolved carbon dioxide concentration can in any case be obtained by assuming equilibrium between the gas and liquid phases, and such an approach has been used in the industry for some time (Carleysmith, 1985). For plant-scale fermentors, where the specific aeration rates are generally lower than for research fermenters and there is a significant degree of plug-flow behavior in the gas phase, this may be a good assumption (Yagi and Yoshida, 1977). For research and pilot-scale fermenters, the assumption will be poorer.

The concentration of dissolved carbon dioxide is of particular interest in the case of mycelial fermentations, affecting both the morphology and the productivity of the fermentation (Ho and Smith, 1986). Pirt and Mancini (1975) found that the penicillin synthesis rate of *P. chrysogenum* was decreased by 33% at a carbon dioxide partial pressure of 0.05 atm and by 50% at 0.08 atm compared to the control case. Belmar-Campero (1988) found a 35% decrease in the clavulanic acid production of *S. clavuligerus* for a carbon dioxide partial pressure of 0.08 atm. Inosine production by *B. subtilis* was found to be inhibited above 0.03 atm (Ishizaki et al., 1973). Bicarbonate ions, whose concentration during fermentations depends (at steady state) on the dissolved carbon dioxide concentration and pH, can inhibit or promote microbial growth and reproduction independent of dissolved carbon dioxide (Repaske et al., 1974).

The dissolved carbon dioxide concentration is, therefore, of importance in determining the productivity of a fermentation, in some cases as important as the concentration of dissolved oxygen (Nyiri and Lengyel, 1968). Fermentations are run under conditions that produce dissolved carbon dioxide concentrations that do not seriously affect microbial growth or productivity, without process operators knowing explicitly what these concentrations are. Variations in the (unmonitored) dissolved carbon dioxide concentration will contribute to batch-to-batch variability in fermentation productivity, so rendering

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the meaningful analysis of data from research facilities more difficult.

This article describes how, for aerobic fermentations, the concentration of dissolved carbon dioxide can be calculated routinely on-line from existing fermentation measurements without the need to resort to the use of a dissolved carbon dioxide sensor or assuming gas-liquid equilibrium. The concentration of bicarbonate ions can consequently also be calculated.

Theory

Oxygen mass transfer

Most of the resistance to oxygen transfer in aerobic fermentations lies in the liquid film near the gas-liquid interface (for example, Steel and Maxon, 1966). Only the liquid-film mass transfer coefficient, $K_L^{O_2}$, is needed to describe the oxygen transfer across this interface, as the gas-film mass transfer coefficient is quite high in agitated vessels, leading to negligible gas-phase resistance (Fair et al., 1973).

$$d(OTR) = \frac{K_L^{O_2} \cdot a}{H^{O_2}} \cdot (p_g^{O_2} - p_L^{O_2}) \cdot dV \tag{1}$$

where OTR is the oxygen transfer rate, $p_L^{O_2}$ and $p_g^{O_2}$ are the oxygen partial pressures in the liquid and gas phases, respectively, V is the aerated broth volume, a is the interfacial area per unit aerated broth volume and H^{O_2} is the Henry's law constant for oxygen. The output from an oxygen sensor is proportional to the liquid-phase oxygen partial pressure. The broth volume V is taken by convention to be the aerated volume. If a load cell measurement rather than level measurement is available, it may be more convenient to define the interfacial area on the basis of the unaerated broth volume.

For research fermenters, the environment in the fermenter is relatively homogeneous. Steel and Maxon (1966) found no variation in dissolved oxygen concentration with position, in a 15-m^3 novobiocin fermentation, suggesting that the assumption of a well mixed liquid may be good even for quite large fermenters. Gas circulation rates are commonly an order of magnitude higher than the supply rates in small, agitated, aerated vessels (Westerterp et al., 1963). Hence, the gas phase can be considered completely backmixed, and the gas-phase oxygen partial pressure of interest is the exit gas partial pressure.

Both a and $K_L^{O_2}$ are functions of specific power input and hence of position (Lopes de Figueiredo and Calderbank, 1979). This functionality need not be determined as the integral of their product can simply be defined to be the fermenter volumetric oxygen mass transfer coefficient:

$$OTR = \frac{K_L^{O_2} a}{H^{O_2}} \cdot (p_g^{O_2}(\text{out}) - p_L^{O_2}) \cdot V \tag{2}$$

where $K_L^{O_2}a$ is the fermenter volumetric oxygen mass transfer coefficient and $p_g^{O_2}(\text{out})$ is the oxygen partial pressure in the exit gas.

The oxygen transfer rate can be calculated from gas analyses. Under the pseudo-steady-state conditions normally applicable during fermentations, it is equal to the oxygen uptake rate by the organisms:

$$OTR = \frac{G}{100} \cdot \left(\%O_2^{in} - \%O_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} \right) \approx OUR \tag{3}$$

where OUR is the oxygen uptake rate, G is the inlet air flow rate, and the compositions of the inlet and exit gas streams are mole percentages. In this work, gas analyses (and hence the aeration rate, G) are assumed to be on a dry basis, such as produced by mass spectrometers, which are increasingly widely used in fermentation facilities.

Carbon dioxide mass transfer

As the cell membrane is relatively impermeable to ionic species (Jones and Greenfield, 1982), the carbon dioxide produced by microbial metabolism enters the broth as dissolved carbon dioxide. It can then be involved in any of the reversible processes below:

$$CO_2(g) \underset{k_L^{CO_2}a}{\overset{k_L^{CO_2}a}{\rightleftharpoons}} CO_2(aq) \overset{k_1}{\underset{k_2}{\rightleftharpoons}} H_2CO_3 \overset{K_{ac}}{\rightleftharpoons} H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

where

k_1, k_2 = rate constants for the indicated reactions, s^{-1}

K_{ac} = carbonic acid dissociation constant, $mol \cdot m^{-3}$

Fermentation processes are operated in the pH range 4–8. At such pH values, the concentration of carbonate ions is negligible, complexing of carbon dioxide with amine groups of protein molecules can be ignored, and reaction of dissolved carbon dioxide with hydroxyl ions is negligible (Sherwood et al., 1976). Of the chemical species in the liquid phase, only dissolved carbon dioxide, $CO_2(aq)$, contributes to the transfer of carbon dioxide across the gas-liquid interface. The concentration of carbonic acid is three orders of magnitude less than that of carbon dioxide. The concentration of bicarbonate ions increases with increasing pH, being equal to that of dissolved carbon dioxide at a pH of about 6.3. This bicarbonate concentration, however, has no impact on carbon dioxide mass transfer for pseudo-steady-state conditions (that is, static or slowly varying pH and carbon dioxide evolution rate) which are applicable during most fermentations. Unsteady-state conditions can be accommodated where necessary (Royce et al., 1989). The concentration of dissolved carbon dioxide, $[CO_2]$, will settle at a value that provides a concentration gradient such that the carbon dioxide transfer rate, CTR, will be equal to the carbon dioxide evolution rate, CER, by the organism.

The principal resistance to transfer of carbon dioxide across the gas-liquid interface again lies in the liquid film, with the gas film resistance being negligible. It can be shown that the ratio of concentration gradients in the liquid and gas films is around 50 in agitated gas-liquid contactors for no internal circulation in the gas bubbles (Calderbank, 1959). In practice, there is considerable internal circulation, and this ratio is in reality much higher (Calderbank, 1959). The ratio of partial pressure gradients in the liquid and gas films (which are the measurements available during fermentations) is thus at least $50.0(H/RT)$, where H, R and T are the Henry's law constant

for the component being transferred, the ideal gas constant, and the temperature. The respective Henry's law constants for oxygen and carbon dioxide at a typical fermentation temperature of 30°C are 86,000 and 3,400 $Pa \cdot m^3 \cdot mol^{-1}$ at 30°C (Schumpe and Quicker, 1982). Hence, the ratios of the partial pressure gradients in the liquid and gas films at 30°C are at least 1,700 for oxygen and 70 for carbon dioxide, making the gas film resistance negligible in both cases. Carbon dioxide transfer can therefore be described in an analogous way to oxygen transfer:

$$CTR = \frac{G}{100} \cdot \left(\%CO_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} - \%CO_2^{in} \right) \tag{4}$$

$$= K_L^{CO_2} a \cdot \left([CO_2] - \frac{p_g^{CO_2}(out)}{H^{CO_2}} \right) \cdot V \tag{5}$$

$$\approx CER$$

where $K_L^{CO_2} a$ is the fermenter volumetric carbon dioxide mass transfer coefficient.

The fermenter volumetric mass transfer coefficients for oxygen and carbon dioxide involve the same interfacial area, the same solvent properties, and the same agitation variables. Schneider and Frischknecht (1977) found the volumetric coefficients to be equal; however, in view of the complexity of their fast transient approach and their ignoring the extent of gas backmixing and sensor response times, their claimed accuracy seems optimistic. Yagi and Yoshida (1977) recognized the dependence of the film coefficient on the liquid diffusivity and suggested that the ratio of the volumetric coefficients should depend on the square root of the diffusivity ratio, yielding a value of 0.92, which corresponds to the large bubble correlation of Calderbank (1959). In practice, the high concentration of electrolytes in fermentation media makes it quite difficult to generate large bubbles (Calderbank, 1959), so the small bubble correlation (Calderbank, 1959) is more relevant, though the outcome is not very different. Using diffusivity data for water at 25°C (Incropera and de Witt, 1990), this yields:

$$\frac{K_L^{CO_2} a}{K_L^{O_2} a} = \left(\frac{D_L^{CO_2}}{D_L^{O_2}} \right)^{2/3} = \left(\frac{2.0 \times 10^{-9} m^2 \cdot s^{-1}}{2.4 \times 10^{-9} m^2 \cdot s^{-1}} \right)^{2/3} = 0.89 \tag{6}$$

where $D_L^{O_2}$ and $D_L^{CO_2}$ are the liquid-phase diffusivities of oxygen and carbon dioxide. This result agrees better with the experimental data of Fox (1984), who obtained a ratio of 0.80. While the liquid diffusivities are a function of broth composition (Ho and Ju, 1988) and temperature, their ratio is constant (Wilke and Chang, 1955).

For there to be no mass transfer enhancement of the desorption of CO_2 by reaction in the liquid film, the following inequality must be satisfied:

$$\frac{k_1 \cdot D_L^{CO_2}}{2 \cdot (K_L^{CO_2})^2} \ll 1 \tag{7}$$

Appendix 1 shows the derivation of this inequality and calculates the value of the expression to be of order 10^{-3}, indicating that there is negligible mass transfer enhancement.

Discussion

Dissolved carbon dioxide excess

The concentration of carbon dioxide dissolved in the fermentation broth, $[CO_2]$, will in general be higher than the concentration predicted by assuming carbon dioxide in the broth to be in equilibrium with the exit gas from the fermenter, $[CO_2]_{eq}$, as a partial pressure gradient for desorption is required. The extent of this difference is limited by two constraints. First, the respiratory quotient (the ratio of the CER to the OUR) is near unity for most fermentations. Hence, the CTR and OTR are nearly equal. Also, the liquid-phase oxygen partial pressure must be greater than zero to avoid oxygen starvation. Noting also that $\%CO_2^{in}$, being that in air, is very small (typically 0.035%), Eqs. 8 describe the carbon dioxide equilibrium concentration, the maximum concentration possible, $[CO_2]_{max}$, and the ratio of these concentrations (called here the carbon dioxide excess, ξ_{max}), in terms of the exit gas composition.

$$[CO_2]_{eq} = \frac{p_g^{CO_2}(out)}{H^{CO_2}} = \frac{\%CO_2^{out}}{100} \cdot \frac{(P - p_w)}{H^{CO_2}}$$

$$[CO_2]_{max} = \frac{\%CO_2^{out}}{100} \cdot \frac{(P - p_w)}{H^{CO_2}} + \frac{1}{0.89} \cdot \frac{\%O_2^{out}}{100} \cdot \frac{(P - p_w)}{H^{O_2}} \tag{8}$$

$$\xi_{max} = \frac{[CO_2]_{max}}{[CO_2]_{eq}} = 1 + \frac{1}{0.89} \cdot \frac{H^{CO_2}}{H^{O_2}} \cdot \frac{\%O_2^{out}}{\%CO_2^{out}}$$

where P is the operating pressure, and p_w the partial pressure of water in the exit gas.

Values for the Henry's law constants for water are available in the literature (for example, Schumpe and Quicker, 1982). Their dependence on temperature is indicated below.

$$H^{O_2} = \exp\left\{ 12.74 - \frac{133.4}{(T - 206.7)} \right\}$$

$$H^{CO_2} = \exp\left\{ 11.25 - \frac{395.9}{(T - 175.9)} \right\}$$

At 30°C, H^{O_2} and H^{CO_2} are 86,000 and 3,400 $Pa \cdot m^3 \cdot mol^{-1}$, respectively. Figure 1 plots Eqs. 8 for operation at atmospheric pressure and 30°C, assuming the inlet air to contain 21% oxygen.

From Figure 1 it is clear that the difference between the maximum and equilibrium carbon dioxide concentrations is a fairly constant quantity. The extent of this difference cannot be very large because of the need to work at oxygen mass transfer coefficients that will maintain the concentration of the sparingly soluble oxygen above a level that affects respiration. This difference becomes a smaller proportion of the total dissolved carbon dioxide concentration with increasing $\%CO_2^{out}$, and hence ξ_{max} is seen to decrease. While this means that the equilibrium assumption becomes relatively more accurate, the organism will in most cases be more sensitive to a given error at the higher dissolved carbon dioxide concentration.

Figure 1 indicates that the carbon dioxide excess may be important and can be evaluated more precisely from Eq. 9.

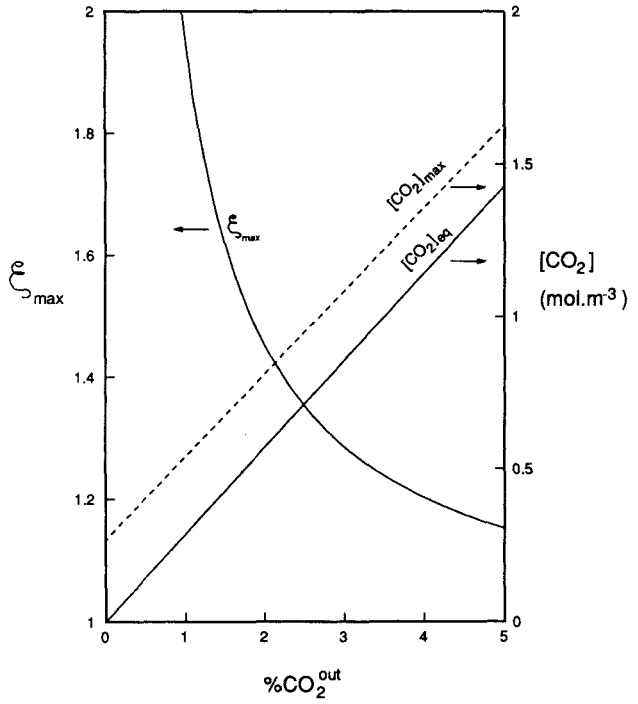

Figure 1. Maximum and equilibrium concentrations of dissolved carbon dioxide and their ratio as a function of the exit gas composition at 30°C and 1 atm for an $RQ = 1$.

$$\xi = \frac{[CO_2]}{[CO_2]_{eq}} = 1 + \frac{CTR \cdot H^{CO_2}}{(K_L^{CO_2}a) \cdot V \cdot p_g^{CO_2}(\text{out})}$$

$$= 1 + \frac{Q \cdot (1-\epsilon) \cdot H^{CO_2}}{60RT_0 \cdot K_L^{CO_2}a} \cdot \left(\frac{P_0}{P - p_w}\right) \cdot \left(\frac{\%CO_2^{out} \cdot \frac{\%N_2^{in}}{\%N_2^{out}} - \%CO_2^{in}}{\%CO_2^{out}}\right)$$

$$\approx 1 + \frac{Q \cdot H^{CO_2}}{60RT_0 \cdot (0.89 \cdot K_L^{O_2}a)} \cdot \left(\frac{P_0}{P}\right) \quad (9)$$

where Q is the specific aeration rate at standard temperature and pressure, T_0 and P_0, are the standard temperature (298 K) and pressure (1.01×10^5 Pa), R is the ideal gas constant (8.314 J·mol^{-1}·K^{-1}), ϵ is the gas void fraction, and ξ is the dissolved carbon dioxide excess over the equilibrium assumption. The specific aeration rate Q is normally based on the unaerated volume, so the void fraction ϵ enters the expression. The void fraction is typically less than 10%, while p_w is normally around 5% of the operating pressure, so these variables can be considered to cancel out with little error. The term involving gas analyses falls away as it is close to unity.

For a given operating pressure, the value of the carbon dioxide excess ξ by Eq. 9 is therefore determined mainly by the values of Q and $K_L^{O_2}a$. It should be noted that these variables are not wholly independent. While Westerterp et al. (1963) suggest that they are independent, most authors have found a weak power law dependence of $K_L^{O_2}a$ on the superficial gas flow rate, and hence on Q. They are treated here as approximately independent ($K_L^{O_2}a$ depending mainly on the agitation rate) to clarify the discussion. The ranges of these variables is quite limited in practice, so the range of ξ for most practical cases can be plotted as a function of these two variables. Figure 2 plots Eq. 9 for operation at the atmospheric pressure and a temperature of 30°C.

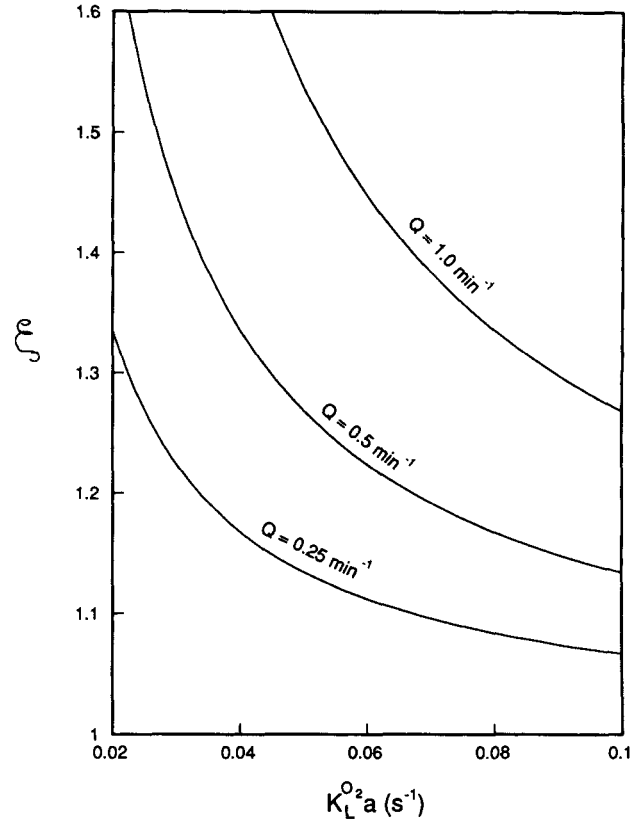

Figure 2. Variation of the carbon dioxide excess with specific aeration rate and volumetric mass transfer coefficient at 30°C and 1 atm.

Figure 2 shows that the error in the equilibrium assumption becomes relatively smaller, as the volumetric mass transfer coefficient rises or as the specific aeration rate falls. The typical error in the equilibrium assumption will be 20–40%. At high aeration and low agitation, the error can be more than 40%. Such conditions will tend to coincide with a low value of the $\%CO_2^{out}$ and hence of $[CO_2]$ (Figure 1), and so this error may be of relatively little importance. Under opposing conditions of low aeration and high agitation, the error in the equilibrium assumption will be only 10–20%. Such conditions will in general be associated with a high value of the $\%CO_2^{out}$ and hence of $[CO_2]$. At high dissolved carbon dioxide concentrations, organisms are relatively more sensitive to variations in the dissolved carbon dioxide concentration, hence even a small error in the equilibrium assumption may be of importance.

The accuracy required is clearly determined by the sensitivity of the organism to the dissolved carbon dioxide concentrations involved. The assumption of equilibrium may be inadequate where the organism is sensitive to the dissolved carbon dioxide concentration, particularly if the aeration rate is high or the volumetric mass transfer coefficient is low. A low mass transfer coefficient could be caused not only by a highly viscous broth,

which would have the effect of lowering the film mass transfer coefficient $K_L^{O_2}$, but also by low agitation rates.

The carbon dioxide concentration can be calculated on-line during the fermentation using Eqs. 2 to 6. The concentration of bicarbonate ions in the bulk broth under steady-state conditions may be obtained from this:

$$[HCO_3^-] = \frac{k_1 \cdot K_{ac}}{k_2 \cdot 10^{-pH}} \cdot [CO_2] = \frac{K_{ac}^*}{10^{-pH}} \cdot [CO_2] \qquad (10)$$

where K_{ac}^* is the pseudo-dissociation constant for carbon dioxide, which has a value of 4.7×10^{-4} mol·m^{-3} at 30°C in water (Arrua et al., 1990). While pH electrodes are sensitive to hydrogen ion activity, they are calibrated against hydrogen ion concentrations in buffers, and hence $[H^+]$ can be obtained from the pH measurement directly. Arrua et al. (1990) provide data for cases where the ionic strength affects the equilibrium.

The above discussion explains why the equilibrium assumption will be better for large fermenters than for small ones. Part of the reason was pointed out by Yagi and Yoshida (1977), who noted that with increasing scale, the specific aeration rate Q must be reduced to maintain the gas superficial velocity (and hence, the gas void fraction, ϵ) within practical limits. Figure 2 shows a decrease in the specific aeration rate at constant $K_L^{O_2}a$ to improve the relative accuracy of the equilibrium assumption. Such reductions, however, are limited by the increasing $\%CO_2^{out}$ which ultimately causes severe inhibition in most aerobic organisms. As a result, the minimum specific aeration rates used are around 0.2 min^{-1} even for large fermentors (Siggig, 1982).

A more important reason for the good approach to carbon dioxide equilibrium in large fermenters concerns the extent of gas backmixing. The high solubility of carbon dioxide means that the concentration gradient required for meeting the CTR translates into a relatively small partial pressure gradient. If there is a substantial degree of plug-flow character in the gas phase, as may be expected on larger fermenters, large partial pressure gradients will exist at the bottom of the fermenter, as the inlet air has a very low carbon dioxide content. The dissolved carbon dioxide concentration cannot vary greatly in the axial direction, as it is an order of magnitude greater than the dissolved oxygen concentration. Hence, the CTR for the fermenter will be met rapidly at the bottom of the fermenter, and the gas will be in carbon dioxide equilibrium with the broth as it reaches the broth surface.

Conclusions

The absolute difference between the actual and equilibrium carbon dioxide concentrations is limited for aerobic fermentations by practical considerations. Figure 1 can provide an *a priori* assessment of the maximum value of this difference. If significant, the precise value of the excess carbon dioxide concentration over the equilibrium assumption can be evaluated from the curves presented in Figure 2. The data presented are for operation at 30°C, but as the dependence on temperature is rather weak, they can provide a first approximation at other temperatures.

For systems where the aeration rate is high and the mass transfer coefficient is low, the excess over the equilibrium assumption may be more than 40%. For opposing conditions of small aeration rates and large mass transfer coefficients, the concentration of dissolved carbon dioxide will be only 10–20% greater than the equilibrium assumption. Under such circumstances, the accuracy of the equilibrium assumption may be better than that of a dissolved carbon dioxide probe. In large fermentors, the approach to carbon dioxide equilibrium between the broth and exit gas will be better than that in research fermentors for the reasons discussed.

The equations presented can be used to monitor routinely the concentrations of dissolved carbon dioxide and bicarbonate ions in fermentation broths. This ability means that the concentrations of dissolved oxygen and carbon dioxide can be independently controlled. Stricter control of these variables may facilitate more meaningful comparisons to be made between different strains and operating conditions during fermentations in research facilities, and improve productivities in production systems.

Notation

a = interfacial surface area per unit volume of aerated broth, m^2·m^{-3}
CER = carbon dioxide evolution rate, mol·s^{-1}
CTR = carbon dioxide transfer rate, mol·s^{-1}
$[CO_2]$ = concentration of dissolved carbon dioxide, CO_2(aq), mol·m^{-3}
$[CO_2]_{eq}$ = concentration of dissolved carbon dioxide assuming equilibrium between the exit gas and fermentation broth, Eq. 8, mol·m^{-3}
$[CO_2]_{max}$ = maximum possible concentration of dissolved carbon dioxide assuming $RQ = 1$, mol·m^{-3}
$D_L^{CO_2}$ = liquid diffusivity of carbon dioxide, m^2·s^{-1}
$D_L^{O_2}$ = liquid diffusivity of oxygen, m^2·s^{-1}
G = inlet air flowrate (dry basis), mol·s^{-1}
H^{CO_2} = Henry's law constant for carbon dioxide, Pa·m^3·mol^{-1}
H^{O_2} = Henry's law constant for oxygen, Pa·m^3·mol^{-1}
$\%i^{in}$ = mole percentage of component i in inlet air to fermenter (dry basis)
$\%i^{out}$ = mole percentage of component i in exit gas from fermenter (dry basis)
k_1, k_2 = rate constants governing carbon dioxide hydration, s^{-1}
K_{ac} = carbonic acid dissociation constant, mol·m^{-3}
K_{ac}^* = pseudo-dissociation constant for carbon dioxide defined in Eq. 10, mol·s^{-1}
$K_L^{CO_2}$ = liquid film mass transfer coefficient for carbon dioxide, m·s^{-1}
$K_L^{CO_2}a$ = fermenter volumetric carbon dioxide mass transfer coefficient, s^{-1}
$K_L^{O_2}$ = liquid film mass transfer coefficient for oxygen, m·s^{-1}
$K_L^{O_2}a$ = fermenter volumetric oxygen mass transfer coefficient, s^{-1}
OTR = oxygen transfer rate, mol·s^{-1}
OUR = oxygen uptake rate, mol·s^{-1}
P = total pressure in fermenter, Pa
P_0 = standard pressure, 1.01×10^5 Pa
$p_g^{O_2}$ = partial pressure of oxygen in the gas phase, Pa
$p_g^{CO_2}$(out) = partial pressure of carbon dioxide in the exit gas, Pa
$p_g^{O_2}$(out) = partial pressure of oxygen in the exit gas, Pa
p_w = partial pressure of water in the exit gas, Pa
Q = specific aeration rate, volumetric aeration rate at standard temperature and pressure per unit unaerated broth volume, min^{-1}
R = ideal gas constant, 8.314 J·mol^{-1}·K^{-1}
RQ = respiratory quotient, the ratio of CER to the OUR
T = temperature of broth, K
T_0 = standard temperature, 298 K
V = aerated broth volume, m^3
V_{film} = liquid film volume, m^3

Greek letters

ϵ = gas void volume as a fraction of the aerated broth volume

ξ = dissolved carbon dioxide excess, the ratio of the dissolved CO_2 concentration to the equilibrium concentration, Eqs. 8

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Appendix: Mass Transfer Enhancement by Liquid-Film Reaction

The liquid-phase reactions involving carbon dioxide have already been discussed. At pseudo-steady state (static or slowly changing *CER* and pH), chemical equilibrium can be assumed in the bulk broth. In the liquid film, however, $[CO_2]$ drops from its value in the bulk broth to an interfacial value dependent on the gas-phase composition. At the interface, there will therefore be a gradient driving the flux of carbon from H_2CO_3 to CO_2(aq). If this flux is significant in comparison to the *CTR*, it will have the effect of enhancing mass transfer.

If it is initially assumed that the film effect is negligible, then the concentration of H_2CO_3 will be the same at the interface as it is in the bulk broth. This will have the effect of maximizing the concentration gradient driving the film effect. If the resulting expression for the film effect indicates that it is negligible, the initial assumption will have been justified.

The problem is solved using boundary layer theory in linear coordinates, as bubble diameters in fermenters are much larger than the thickness of the liquid film. If the interface is at $x=0$ and the edge of the liquid film at $x=\delta$, then:

$$[CO_2]^{x=0}=\frac{p_g^{CO_2}(\text{out})}{H^{CO_2}}$$

$$[CO_2]^{x=\delta}=\frac{p_g^{CO_2}(\text{out})}{H^{CO_2}}+\frac{1}{K_L^{CO_2}}\cdot\frac{CTR}{a\cdot V}$$

$$[H_2CO_3]^{x=0}=[H_2CO_3]^{x=\delta}=\frac{k_1}{k_2}\cdot[CO_2]^{x=\delta}$$

As the reactions are first order:

$$\begin{aligned}\text{film effect } (\text{mol}\cdot\text{s}^{-1})&=\frac{1}{2}\text{ at } x=0+x=\delta\\&=\frac{1}{2}\text{ at } x=0\\&=\frac{1}{2}\left(k_2\cdot[H_2CO_3]^{x=0}-k_1\cdot[CO_2]^{x=0}\right)\cdot V_{\text{film}}\\&=\frac{k_1}{2\cdot K_L^{CO_2}}\cdot\frac{CTR}{a\cdot V}\cdot V_{\text{film}}\end{aligned}$$

where V_{film} is the volume of the liquid film. In terms of boundary layer theory, the volume of the liquid film (V_{film}) is:

$$V_{\text{film}}=\delta\cdot a\cdot V=\frac{D_L^{CO_2}}{K_L^{CO_2}}\cdot a\cdot V$$

Hence,

$$\frac{\text{film effect}}{\text{mass transfer}}=\frac{\left(\frac{k_1}{2\cdot K_L^{CO_2}}\cdot\frac{CTR}{a\cdot V}\cdot\frac{D_L^{CO_2}}{K_L^{CO_2}}\cdot a\cdot V\right)}{CTR}=\frac{k_1\cdot D_L^{CO_2}}{2\cdot(K_L^{CO_2})^2}$$

At 25°C, k_1 has a value of 0.028 s^{-1} (Pinsent et al., 1956), and D_{CO_2} has a value in water of 2.0×10^{-9} $m^2 \cdot s^{-1}$ (Incropera and de Witt, 1990). Godbole et al. (1984) give values for the film mass transfer coefficient of around 2×10^{-4} $m \cdot s^{-1}$. Hence,

$$\frac{\text{film effect}}{\text{mass transfer}} = 0.0007$$

Therefore, the film effect is negligible, and assumption *a* above is justified. It has been assumed that there is no catalysis of carbon dioxide hydration by the enzyme carbonic anhydrase, as this is an intracellular enzyme and is present in only a few micro-organisms.

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