AERATION, BIOTECHNOLOGY

1. Introduction

The supply of oxygen to a growing biological species, aeration, in aerobic bioreactors is one of the most critical requirements in biotechnology. It was one of the biggest hurdles that had to be overcome in designing bioreactors (fermenters) capable of turning penicillin from a scientific curiosity to the first major antibiotic (1). Aeration is usually accomplished by transferring oxygen from the air into the fluid surrounding the biological species, from where it is in turn transferred to the biological species itself. The rate at which oxygen is demanded by the biological species in a bioreactor depends very significantly on the species, on its concentration, and on the concentration of the other nutrients in the surrounding fluid (1,2) (see CELL CULTURE TECHNOLOGY). There is no unique set of units used to define this rate requirement, but some typical figures are given in Table 1. The very wide range is noteworthy; during the course of a batch bioreaction, oxygen demand often passes through a marked maximum when the species is most biologically active (1).

The main reason for the importance of aeration lies in the limited solubility of oxygen in water, a value that decreases in the presence of electrolytes and other solutes and as temperature increases. A typical value for the solubility of oxygen

Table 1. **Oxygen Demands of Biological Species**

Biological species	kg $O_2/(m^3 \cdot h)$	References
bacteria/yeasts	1–7	1, 3
plant cells	0.03–0.3	4
seed priming[a]	$1–8 \times 10^{-2}$	5
mammalian cells[b]	$2–10 \times 10^{-3}$	6

[a] Based on a seed density of 100 kg/m^3.
[b] Based on a cell density of 10^{12} cells/m^3.

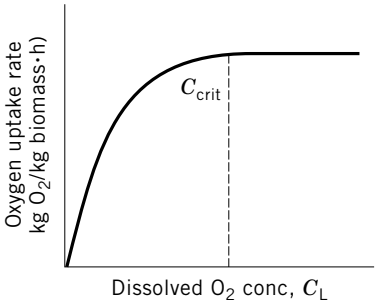

Fig. 1. The relationship between rate of oxygen uptake and dissolved oxygen, concentration where C_{crit} is the critical oxygen concentration.

(the equilibrium saturation concentration) in water in the presence of air at atmospheric pressure at 25°C is ~0.008 kg O_2/m^3 (= 8 ppm = 0.25 mmol/L). Thus, for a yeast or bacterial bioreaction demanding oxygen at the rates given in Table 1, all oxygen is utilized in ~10–40 s (3,7).

In addition to each bioreaction demanding oxygen at a different rate, there is a unique relationship for each between the rate of reaction and the level of dissolved oxygen (1,8). A typical generalized relationship is shown in Figure 1 for a particular species, eg, *Penicillium chrysogenum* or yeast. The shape of the curve is such that a critical oxygen concentration, C_{crit}, can be defined above which the rate of the bioreaction is independent of oxygen concentration, ie, zero order with respect to oxygen. The typical values given in Table 2 indicate that the critical concentration is usually on the order of 1–20% of the oxygen saturation value. Thus for each species, oxygen should be transferred rapidly enough to allow the oxygen demand to be met throughout the volume in which the bioreaction is occurring using a level of dissolved oxygen above the critical value. Failure to do so leads to a reduction in the overall rate and possibly a change of bioreaction to a different and unwanted metabolic pathway. If oxygen concentration falls low enough, an anaerobic reaction may develop. Yeast fermentations are a particularly good example of such possibilities (1,8).

In the cases of pellets (9), flocs (10), and immobilized cells and enzymes (11), aeration becomes more complex (1). Here it is the level of oxygen at the active biological site within the solid particle or aggregate rather than the level of dissolved oxygen in the surrounding fluid that determines the overall rate of reaction. Indeed, in certain cases such as penicillin fermentations, pellets form

Table 2. **Critical Oxygen Concentrations at 30°C**[a]

Organism	C_{crit}, (kg O_2/m^3) × 10^4
Azotobacter vinelandi	6–16
Pseudomonas denitrificans	3
Penicillium chrysogenum	3
Aspergillus oryzae	6.5

[a] Ref. 1.

of such a size that the center of the pellet becomes inactive (12). A similar effect is observed when biofilms form on cooling surfaces. At a certain thickness the oxygen concentration at the base of the film becomes zero, ie, that region becomes anaerobic, and the biological species, eg, *Pseudomonas fluorescens*, at the surface that is being fouled dies and sloughs off (13).

2. Principles of Oxygen Transfer

2.1. The Basic Mass-Transfer Steps. Figure 2 shows the steps through which oxygen must pass in moving from air (or oxygen-enriched air) to the reaction site in a biological species (1,14). The steps consist of transport through the gas film inside the bubble, across the bubble–liquid interface, through the liquid film around the bubble, across the well-mixed bulk liquid (broth), through the liquid film around the biological species, and finally transport within the species (eg, cell, seed, microbial floc) to the bioreaction site. Each step offers a resistance to oxygen transfer. In the last step, the resistance to transport is negligible for freely suspended bacteria or cells that are extremely small, but for immobilized cells and biological flocs and pellets the rate of oxygen diffusion through their structure may be rate limiting. In the more complex situation, the rate of oxygen diffusion to the active sites depends on mass transfer through the external boundary layer followed by diffusion through the solid as governed by Fick's law. The theory is essentially the same as that applied to chemical catalysis (qv.) and is described very well in (1), and other standard texts.

The rate-limiting step typically occurs at the air–liquid interface and, for biological species without diffusion limitations, the overall relationship can be simply written at steady state as

oxygen transfer rate from air (OTR) = oxygen uptake rate by biological species (OUR).

Provided this equality is satisfied and the dissolved oxygen concentration in the well-mixed liquid is greater than the critical concentration throughout the bioreactor, then the maximum oxygen demand of the species should be met

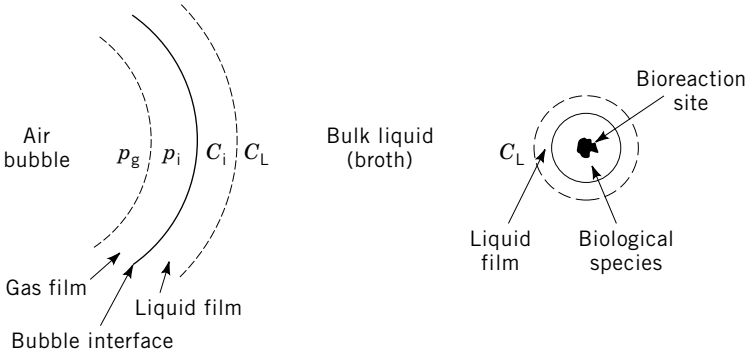

Fig. 2. Steps by which oxygen is transferred from the gas phase to the biological reaction site. Terms are defined in the text.

satisfactorily. Design of the bioreactor must ensure that the above requirements are achieved economically and without damaging the biological species.

When the oxygen demand is low, eg, at the start of a batch fermentation when the amount of biomass is small, the oxygen uptake rate at the bioreaction site is the limiting step and the level of dissolved oxygen is high (near saturation). When the oxygen demand becomes high, however, the rate-limiting step is that associated with transfer across the bubble–liquid interface and this rate of transfer is critically dependent on the fluid flow at the gas–liquid interface as well as the concentration of oxygen in both the gas and the liquid phases. The liquid-phase oxygen concentration is now low, but it should not be allowed to fall below the critical value. It is when this gas–liquid mass-transfer step is rate-limiting that the biggest demands are made on the bioreactor. Then there is the greatest difficulty in getting enough oxygen into the suspending fluid (broth) to satisfy the oxygen demand of the biological species.

2.2. The Basic Mass Transfer Relationship. The basic principles that underlie oxygenation (aeration) are exactly the same as those that determine the rate of transfer of any sparingly soluble gas (oxygen) from the gas stream (air) to the unsaturated liquid (broth). The rate at which this transfer takes place is dependent on four principal parameters (1,14,15). The first is the area of contact between the gas and the liquid. In most bioreactors, this is provided by dispersing air in the suspending fluid (medium or broth) to give a large specific area of contact as implied by Figure 2. However, there are also other methods, especially in wastewater treatment, by which large specific areas of contact are produced. Solid supports are used on which films of the irrigated, biologically active species grow, eg, trickling bed filters and rotating disk contactors (11,14). The other three mass-transfer rate parameters are the driving force available (ie, the difference in concentration of oxygen in the two phases); the two-phase fluid dynamics (including the effect of viscosity); and the chemical composition of the liquid.

The first assumption in all such physical mass transfer processes is that equilibrium exists at the interface between the two phases. This assumption implies that, at the interface, the concentration of the gas in the liquid, C_i, is equal to its solubility at its partial pressure in the gas phase, p_i. Since, for sparingly soluble gases such as oxygen, there is a direct proportionality between the two,

$$p_i = HC_i \tag{1}$$

where H is the Henry's law constant. The second assumption is that as the gas is absorbed, its concentration falls progressively, from a high in the bulk concentration in the gas phase, p_g, to that at the interface, p_i, then again from that on the liquid side of the interface, C_i, to a low in the bulk liquid, C_L (see Fig. 2).

The rate of mass transfer, J, is then assumed to be proportional to the concentration differences existing within each phase, the surface area between the phases, A, and a coefficient (the gas or liquid film mass transfer coefficient, k_g or k_L, respectively) which relates the three. Thus

$$J = k_g A(p_g - p_i) \tag{2}$$

$$= k_L A(C_i - C_L) \tag{3}$$

Equations 2 and 3 also stand as the definitions of k_g and k_L. For sparingly soluble gases, however, $p_i \simeq p_g$ so that from equation 1,

$$C_i = p_i/H = p_g/H = C_g^* \tag{4}$$

and

$$J = k_L A\left(C_g^* - C_L\right) \tag{5}$$

where C_g^* is the solubility of oxygen in the broth that is in equilibrium with the gas phase partial pressure of oxygen. Thus the aeration rate per unit volume of bioreactor, N, is given by

$$N = J/V = k_L\,(A/V)\left(C_g^* - C_L\right) = k_L a\left(C_g^* - C_L\right) \tag{6}$$

where a is the interfacial area per unit volume. At steady state, N must equal the OUR, which should be that demanded by the biological species in the range $C_L > C_{crit}$.

2.3. The Driving Force for Mass Transfer. The rate of mass transfer increases as the driving force, $C_g^* - C_L$, is increased. C_g^* can be enhanced as follows. From Dalton's law of partial pressures

$$p_g = P_g y \tag{7}$$

where y is the mole (volume) fraction of oxygen in the gas phase and P_g is the total pressure, ie, back pressure plus static head. C^*_g (eqs. 4 and 5) is also a function of composition and temperature, decreasing with both. C_L can be low provided it is above C_{crit}. However, in a very large scale bioreactor where circulation times are of the same order as the time for total oxygen depletion (3,7), the average value may need to be kept well above C_{crit} so that local values below it may be avoided. There are very few studies on the variations of C_L in any bioreactor (16,17), partly because of the difficulty of measuring it. C_L is usually measured by an oxygen electrode that gives C_L/C^*_g as a percentage and this value is sensitive to the local velocity over the probe, particularly if the liquid is viscous (18).

In a stirred bioreactor, the liquid is generally considered well-mixed, ie, C_L is spatially constant. The gas phase too may be well mixed (19) so that

$$p_g = \text{constant} = (p_g)_{out} \tag{8}$$

where $(p_g)_{out}$ is the partial pressure of oxygen in the exit gas and

$$C_g^* = (p_g)_{out}/H \tag{9}$$

This situation is most probable with fairly intense agitation and on the small scale. On the other hand, for ease of application, it is often assumed that no oxygen is utilized. This is the so-called no-depletion model. In this case

$$C_g^* = (p_g)_{in}/H \tag{10}$$

Again, it is most reasonable on the small scale, but only with low k_La values. Most of the data leading to k_La values in the literature [especially those in the review in (20)] were obtained by making this assumption.

For large-scale bioreactors (21), especially those of the air lift type (22), the gas phase is best considered as being in plug flow, so that a log mean value of driving force is obtained:

$$\Delta C_{\text{im}} = \frac{\Delta C_{\text{in}} - \Delta C_{\text{out}}}{\ln(\Delta C_{\text{in}}/\Delta C_{\text{out}})} \tag{11}$$

where

$$\Delta C_{\text{in}} = [(p_g)_{\text{in}}/H] - C_L \tag{12}$$

and

$$\Delta C_{\text{out}} = [(p_g)_{\text{out}}/H] - C_L \tag{13}$$

2.4. The Mass-Transfer Coefficient, k_La. Because of the interaction between k_L and a when air is dispersed in the media, the two have not often been measured separately. When they have been measured, k_L has been found to be dependent on the relative velocity between the phases, the level of turbulence in the liquid, and the size of bubbles. However, it is a relatively weak function of all of those. In addition, it appears that impurities, whether in solution or suspension, reduce the ease with which oxygen can pass across the interface (23) and, consequently, reduce the value of k_L.

The Sauter mean bubble size, d_B, is also relatively insensitive to the fluid dynamics, especially in low viscosity broths. On the other hand, it is quite sensitive to the composition of the fluid, especially to the presence of substances that inhibit coalescence. In fact, coalescence and its inhibition plays a much larger role in controlling bubble size than bubble break-up. Theories of break-up suggest that bubble size increases with increasing surface tension, σ. However, in aqueous solutions, alcohols reduce σ whilst electrolytes increase it but both inhibit coalescence and reduce d_B (24). Unfortunately, as a result, a satisfactory equation is not available for calculating d_B. Nevertheless, those interested in CFD predictions of bioreactors generally do not appear to recognise this fact.

When the bubbles are smaller, especially in stirred bioreactors, bubbles very easily circulate with the broth. This increased recirculation when coalescence is inhibited leads to a significantly larger hold-up, ie, increased volume fraction of gas phase, ε_H. d_B, ε_H, and a are all related.

$$a = 6\epsilon_H/d_B \tag{14}$$

Therefore, a values in the presence of coalescence inhibitors are much higher than without them (25), the changes in a outweighing changes in k_L. Thus, it is found that coalescence inhibitors such as electrolytes and low molecular weight alcohols increase k_La by up to an order of magnitude compared to water (26). Coalescence promoters such as antifoams generally lower k_La by

somewhat similar amounts (27,28). In addition, neither d_B nor k_L is a strong function of the fluid dynamics, but the amount of air recirculation is, so k_La and ε_H are both related to the fluid dynamics in similar ways (29). However, exceptions to this interrelationship have been reported (30) and therefore it should be treated with care. For example, if a certain agitator produces a higher hold-up without a commensurate increase in k_La, it represents a reduction in performance because productivity is linked to the volume of broth in the bioreactor.

Increases in broth viscosity significantly reduce k_La and cause bubble size distributions to become bimodal (31). Overall, k_La decreases approximately as the square root of the apparent broth viscosity (32). k_La can also be related to temperature by the relationship (33)

$$k_La = (k_La)_{20}\theta^{(T-20)} \tag{15}$$

where $(k_La)_{20}$ is the value at 20°C, $\theta \simeq 1.022$, and T is the temperature, °C; ie, k_La increases by ~2.5%/ °C.

2.5. The Measurement of k_La. There are two main methods for measuring k_La, the unsteady-state method, and the steady-state method. In the most common unsteady-state method, the level of dissolved oxygen is first reduced to zero, either by bubbling through nitrogen or by adding sodium sulfite (20). Then, the increase in dissolved oxygen concentration as a function of time is followed using an oxygen electrode. One of the assumptions set out earlier about the extent of gas-phase mixing has to be made and the choice can be very significant, giving k_La values when they are high differing by an order of magnitude for the same raw data (34). If two oxygen electrodes, one in the liquid phase and one in the gas, are employed, this difficulty can be eliminated, but the technique is quite difficult to use (35,36). Two other ways of circumventing these gas-phase mixing issues have also been proposed. In one, pure oxygen is used after degassing has been completed so that the driving force is always constant (37). In the other, a step change in backpressure is utilized to produce the transient (38). Both are rather specialized and it is not apparent that they are being used significantly.

In addition, the electrode response time requires consideration (18,20) as does the quality of liquid-phase mixing (22). Most significantly, it should again be emphasized that k_La is very dependent on composition. For example, the k_La of distilled water measured by nitrogen degassing is significantly less than that measured by sulfite deoxygenation. This difference occurs because the sulfite acts as a coalescence inhibitor, greatly enhancing hold-up, and therefore a and k_La.

For stirred bioreactors, the unsteady-state technique can be adapted to give k_La values for real bioreactions (39) including animal cell culture (40). Though the method has the same inherent weaknesses as all the dynamic techniques, its advantage is that the k_La is determined for the system of interest. These experimental values may well be very different from those predicted by literature correlations based on water or idealized liquids. This point is likely to be particularly valid for mycelial fermentations for example. Additionally, the presence of antifoam can cause a dramatic reduction (28).

Steady-state techniques are better and often can be carried out on real bioreactions. In most cases, however, and especially for batch systems, it is necessary to carry out an oxygen balance on the air in order to determine the mass of oxygen utilized and thus the mass flux represented in equation 6. Assumptions must again be made about the gas- and liquid-phase mixing. The significance of the assumptions is less than in the unsteady-state, except on the large scale where the well-mixed liquid assumption breaks down (16,17). No really satisfactory way of overcoming this problem has yet been proposed. Given suitable instrumentation, the steady-state method of measurement for the bioreaction of interest is the recommended technique. However, it is not suitable in systems having very low oxygen demands, where the extent of oxygen utilization is insufficient to give an accurate measure of the drop in oxygen concentration. In that case, the unsteady-state method (39,40) is best, as exemplified by its successful use with seed priming bioreactors (5).

Recently, a new steady-state technique has been introduced and it has become quite widely adopted. It involves the catalytic reduction of hydrogen peroxide either by catalase (41) or manganese dioxide (42). The former catalyst is less robust but is very good with polymer solutions simulating high viscosity fermentation broths where it has been utilised in fermenters up to 80 m^3 (43). However, the catalase is easily denatured especially by electrolytes and in that case, MnO_2 is better (42). The only data required is the constant flow rate of H_2O_2 and the dissolved oxygen concentration.

3. Aeration in Bioreactors

A huge variety of bioreactors has been developed and a thorough review is available (44). It is not feasible to consider them all and large numbers are only curiosities. A useful subdivision has been made into three generic types involving the way in which air is dispersed to give the desired specific surface area. These are bioreactors driven by rotating agitators (stirred tanks), bioreactors driven by gas compression (bubble columns/loop fermenters), and bioreactors driven by circulating liquid (jet loop reactors) (45). The first two are the most important.

3.1. Stirred Tank Bioreactors. Traditionally, stirred tanks have been the most common types of bioreactors for aerobic processes and they remain so even in the face of newer designs. One of the main reasons is their extreme flexibility. Operational designs using controlled air flow rates up to $\sim$1.5 vvm (volume of air/min per unit volume of fermentation fluid) and variable speed motors capable of transmitting powers up to about 5 W/kg with control down to close to zero are suitable for almost any bioreaction. These tanks are also relatively insensitive to fill, ie, to the proportion of liquid added to the bioreactor, and are therefore quite satisfactory for fed batch operations. Control of dissolved oxygen can be carried out by either altering aeration rate and/or agitator power input (via speed control) or for fed batch by adjusting the nutrient feed rate. They are also capable of handling relatively satisfactorily broths that become significantly viscous during the course of a fermentation. In that case, design for good bulk mixing (homogenization) may be the most demanding task that the agitator is required to carry out (46).

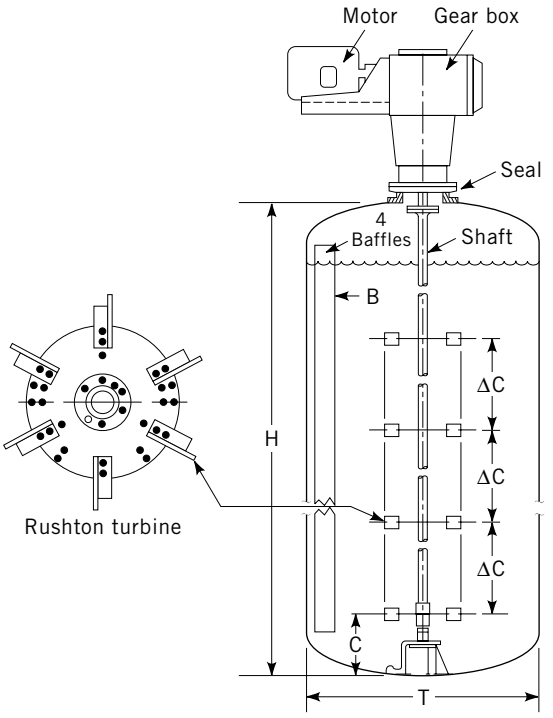

Fig. 3. A large-scale fermenter agitated by Rushton turbines (47) where $B/T = 0.1$, $H/T \simeq 3.3$, $D/T \simeq 0.35$, $C/T = 0.25$, and $\Delta C/T = 0.51$.

Until recently most industrial scale, and even bench scale, bioreactors of this type were agitated by a set of Rushton turbines having about one-third the diameter of the bioreactor (47) (Fig. 3). In this system, the air enters into the lower agitator and is dispersed from the back of the impeller blades by gas-filled or ventilated cavities (48). The presence of these cavities causes the power drawn by the agitator, ie, the power required to drive it through the broth, to fall and this has important consequences for the performance of the bioreactor with respect to aeration (36). The parameter $k_L a$ has been related to the power per unit volume, P/V, in W/m^3 (or $P/\rho V$ in W/kg) and to the superficial air velocity, v_s, in m/s (20), where v_s is the air flow rate per cross-sectional area of bioreactor. This relationship in water is

$$k_L a = 2.6 \times 10^{-2} \left(\frac{P}{V}\right)^{0.4} v_s^{0.5} \tag{16}$$

and for electrolyte solutions:

$$k_L a = 2.0 \times 10^{-3} \left(\frac{P}{V}\right)^{0.7} v_s^{0.2} \tag{17}$$

Each equation is independent of impeller type. As pointed out earlier, the absolute $k_L a$ values vary considerably from liquid to liquid. However, similar

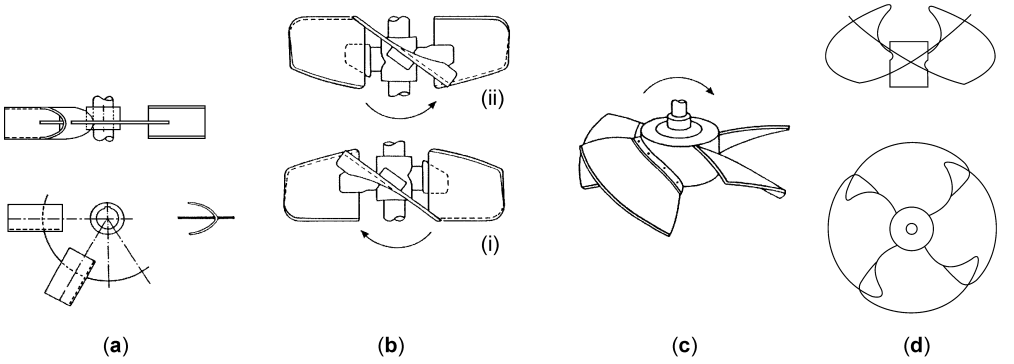

Fig. 4. Examples of agitators (53) progressively replacing the Rushton turbine: (**a**) radial Scaba 6SRGT, (**b**) (i) down-pumping Lightnin' A315[which has also been shown to work effectively in the up-pumping mode (ii) (52)], (**c**) down-pumping Prochem Maxflo T, and (**d**) up-pumping Hayward Tyler (formally APV) B2.

relationships have been found for other fluids, including fermentation broths, and also for hold-up, ε_H. Therefore, loss of power reduces the ability of the Rushton turbines to transfer oxygen from the air to the broth.

There are two other features of Rushton turbines that require consideration (49). First, as these turbines disperse the gas–liquid mixture, they drive it radially outward at each agitator leading to rather poor top-to-bottom mixing. Second, if the air flow rate is too high, they can no longer disperse the air and the impellers are said to become flooded (50). New impellers have been introduced that are better able to handle the large quantities of air needed in large scale fermentations and that do not lose power so significantly. The most common have been the so-called, hollow-blade radial flow impellers, especially the Chemineer CD6 and the Scaba 6SRGT [Fig. 4(**a**)] (49). However, as with Rushton turbines, these other radial impellers in mutiple configurations as found in industrial scale fermenters, also provide relatively poor top-to-bottom bulk blending of nutrients (including air) (49). Therefore there has been another trend which has been aimed at improving this characteristic by using multiple down-pumping, wide blade hydrofoil impellers such as the Lightnin' A315 [Fig. 4(**b**)] and the Prochem Maxflo T [Fig. 4(**c**)] (49). These types do improve vertical mixing but on aeration, they lose power and have very unstable torque, power and flow characteristics (49). A quite counter-intuitive procedure is the utilization of multiple up-pumping, wide blade hydrofoils such as the Hayward Tyler (formerly APV) B2 [Fig. 4(**d**)] (51) or the Lightnin' A315 (52). These give stable and high aerated power characteristics (49), a very high air handling ability without flooding and good vertical blending, even in viscous broths at specific power inputs up to $\sim$2 W/kg (52). The Ekato Intermig (not shown) was proving popular in Europe but it has been shown to give intense vibrational problems because of the large gas-filled cavities that it develops, especially in viscous broths (53) and the manufacturers no longer recommend it for aeration.

These new impellers are progressively taking over from Rushton turbines. However, the reported improvements achieved by them are attributable to better bulk blending compared to Rushton turbines rather than to improved rates of

aeration, ie, k_La (46). Indeed, though higher k_La values may be achieved, it is only as a result of increases in either P/V or v_s in equations 16 or 17.

Recently, an equation based on data obtained by the steady-state hydrogen peroxide technique has been obtained (54). The fluids used were water and viscous shear thinning sodium methyl carboxy−cellulose solutions. The impellers used were a Rushton turbine, a Lightnin' A315 , a hollow-blade impeller and a pair of Intermigs. The effect of viscosity was allowed for by the method of Metzner and Otto (53). The equation is

$$k_La = 4.7 \times 10^{-4}(P/V)^{0.34}(v_s)^{0.27}(\mu_a)^{-0.75} \quad (18)$$

where μ_a is in kg/m s. This equation fitted the data to $\pm 20\%$ that is typical for such work (20). However, it is important to recognise that there are many other equations available and it is impossible to determine which is the best.

3.2. Bubble Column and Loop Bioreactors. Air driven bioreactors are said to offer these advantages (44): No opening for a shaft is required and therefore they are less likely to become contaminated; and they are very simple to operate on the very small scale and more economic on the very large scale where huge agitators and motors would otherwise be required (55). Initially, it was also considered that they were less likely to damage bioreactions involving fragile material such as plant (4) or mammalian cells. However, a recent paper on the former, which acknowledges that such systems are often rather viscous, recommends up-pumping hydrofoils (56). Also, for the latter, stresses associated with bursting bubbles rather than rotating agitators have been shown to be the main determinants of cell damage (57).

Examples of air driven bioreactors are given in Figure 5. The bubble column is clearly the simplest of these bioreactors to construct. However, because of its rather ill-defined liquid circulation, air-lift reactors having either internal (draught tube) or external (loop) circulation of broth have been introduced. The major disadvantages of all three types are the poor capability of handling very viscous fermentations, especially those having a yield stress; the inflexibility, especially of the airlift types, which only work well using a fill closely matched to the size of the bioreactor and its internals (this match affects both circulation rates, mixing and mass transfer, and bubble disengagement); and lack of independent control of dO_2 and mixing, since both are closely linked to the aeration rate. Reference 58 presents a very interesting literature review and analysis of bubble columns. In contrast to stirred tank bioreactors, the bubble size may in certain cases be very dependent on the way the air is introduced, ie, on the type of sparger employed. For systems having hindered coalescence, very fine (0.25−1 mm diameter) bubbles can be formed by using porous disks, provided the superficial gas velocity is less than $\sim 10^{-2}$ m/s. These conditions lead to very high k_La values. However, unless coalescence is very repressed, the bubble size grows within $\sim$0.5−1 m to give k_La values similar to those found in coarse bubble systems. There may also be disengagement problems if coalescence does not occur.

Coarse bubble systems are typically found with orifice, perforated disks, or pipe spargers. Under most realistic conditions, bubbles of $\sim$4−6 mm are

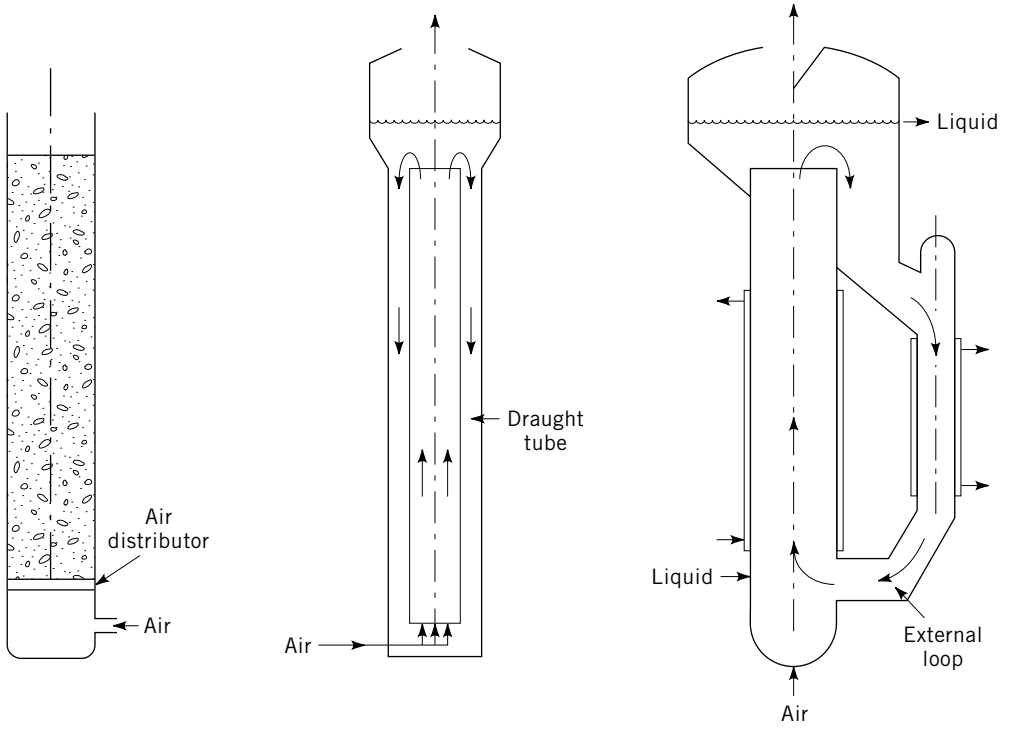

Fig. 5. Examples of air driven bioreactors: (**a**) bubble column, (**b**) draught tube, and (**c**) external loop.

formed. For a wide range of sizes,

$$k_L a = 0.32 v_s^{0.7} \tag{19}$$

Figure 6 enables a comparison to be made of $k_L a$ values in stirred bioreactors and bubble columns (58). It can be seen that bubble columns are at least as energy-efficient as stirred bioreactors in coalescing systems and considerably more so when coalescence is repressed at low specific power inputs (gas velocities).

It is also interesting to use Figure 6 to make a comparison of different aeration devices on the basis of energy efficiency. From equation 6 and assuming a constant driving force,

$$N \propto \mathrm{OTR} \propto k_L a \tag{20}$$

and from Figure 6

$$k_L a \propto (P/V)^{0.6} \tag{21}$$

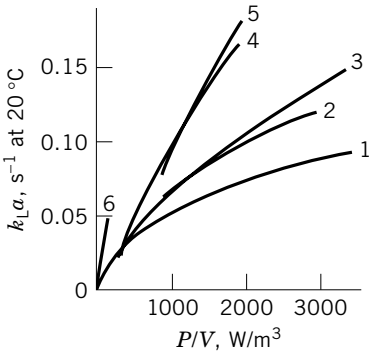

Fig. 6. A comparison of k_La values (58). Represented are 1, stirred bioreactor using water, $v_s = 0.02$ m/s, k_La (eq. 16); 2, stirred bioreactor using water, $v_s = 0.04$ m/s, k_La (eq. 16); 3, bubble column using water, k_La (eq. 19); 4, stirred bioreactor using salt water, $v_s = 0.02$ m/s, k_La (eq. 17); 5, stirred bioreactor using salt water, $v_s = 0.04$ m/s, k_La (eq. 17); and 6, bubble column using salt water (noncoalescing).

Therefore,

$$\frac{\text{OTR}}{P/V} \propto \left(\frac{P}{V}\right)^{-0.4} \tag{22}$$

or

$$\frac{(\text{OTR})V}{P} \propto \left(\frac{P}{V}\right)^{-0.4} \tag{23}$$

so that

$$\left(\frac{\text{kg O}_2}{\text{kW·h}}\right) \propto \left(\frac{P}{V}\right)^{-0.4} \tag{24}$$

From equation 24, it can be seen that the higher the power input per unit volume, the lower the oxygen-transfer efficiency. Therefore, devices should be compared at equal transfer rates. All devices become less energy efficient as rates of transfer increase (3).

External and internal loop airlifts and bubble column reactors containing a range of coalescing and non-Newtonian fluids, have been studied (59,60). It was shown that there are distinct differences in the characteristics of external and internal loop reactors (61). Overall, in this type of equipment

$$k_La \propto \mu_a^{-0.9} \tag{25}$$

showing a greater fall as viscosity increases than in stirred tank bioreactors ($k_La \propto \mu_a^{-0.5} - \mu_a^{-0.7}$) and supporting the contention that such devices are unsuitable for viscous broths. The complete lack of oxygen transfer found in the

downcomer in one high viscosity study (59) is also a significant factor and could easily lead to values of $C_L < C_{crit}$ being found in that region.

4. Applications to Different Biological Species

4.1. Mycelial Fermentations. Mycelial fermentations typically become viscous and shear thinning and difficult to mix (53). For such systems, k_La and mixing are inextricably interlinked. Agitators that give better bulk blending per unit of energy can also give higher k_La values by involving more of the fermenter volume in the mass-transfer process (46). In practice, this requirement suggests low power number, large impeller-to-tank diameter ratio impellers such as those shown in Figure 4 should be used. Agitation levels link with mycelial structure and if pelleted growth can be encouraged, for example, using *P. chrysogenum* (12), viscosity does not significantly increase and high levels of k_La can be maintained at satisfactory specific power inputs. Difficulties may arise, however, if the pellets are too large, as a result of diffusion resistance within them (1), leading to oxygen starvation of the potentially active sites at the center of the pellet (9).

Because of the high viscosity, adequate bulk blending, oxygen transfer, and cooling often require high specific power inputs. Recent work has clearly shown that such levels of agitation can cause significant mycelial breakage. In the case of *P. chrysogenum*, such breakdown leads to loss of productivity (62). However, with *Aspergillus oryzae*, though breakdown occurs, productivity is unaffected (63). Models of this breakage have been developed and validated and the reader is referred to the original papers (62,63) for further information as it is outside the scope of this article. Clearly, however, if breakage does not affect productivity, short periods of intense agitation might be used to break hyphae in order to lower viscosity (63). Such an action should then lead to enhanced k_La and allow lower agitation intensity to be used subsequently.

4.2. Xanthan Gum Fermentations. Xanthan gum (and other polysaccharide) fermentations become very viscous toward the end whether batch (64) or fed-batch (65). Therefore, satisfying the oxygen demand at this stage is very demanding of agitation power. Because the broth also possesses a yield stress, bulk blending to maintain dissolved oxygen above the critical concentration throughout the fermenter has a major impact on the productivity and the quality of the gum (66). Stirred bioreactors are therefore preferable using large impeller/tank diameter ratios and more closely placed impellers (66).

4.3. High Oxygen Demanding Fermentations. High oxygen demanding fermentations often require a higher level of oxygen over relatively short periods of time. The use of enriched air, or pure oxygen, as a separate feed stream and/or back pressure for a short period to enhance C_g^* as well as maximizing power and aeration rate to give the highest k_La may be worth considering. In such circumstances, the flexibility of the stirred bioreactor is a distinct advantage.

4.4. Animal Cell Culture. Airlift (67) and bubble column bioreactors have been considered necessary for handling fragile animal cells. However, more recent work has shown that bursting bubbles are much more damaging

to these cells than agitation unless protective agents such as Pluronic F68 are included in the media (68,69). On the other hand, sparged aeration in the presence of Pluronic in standard stirred bioreactors have been found not to damage hybridoma cells even at rather high (0.25 kW/m^3) agitator power inputs on a small scale (68,69). Such power inputs even at low aeration rates (say 0.05 vvm) would meet the needs of most industrial animal cell culture processes at the cell densities presently achievable and the use of enriched air and back pressure would further extend the range of cell densities that could be handled. Furthermore, it has recently been shown that the typical very low power inputs initially used industrially ($<\sim$0.01 W/kg) lead to major homogenization problems with respect to pH (70). A trend to higher specific power inputs at the industrial scale in order to overcome these problems is discernable.

4.5. Plant Cell Culture. Airlift bioreactors have been favored for plant cell systems since these cultures were first studied (4). However, they can give rise to problems resulting from flotation of the cells to form a "meringue" on the top and they are often rather viscous (56). It is interesting to note that some reports indicate that stirred bioreactors do not damage such cells (4), and a recent paper (56), after an extensive review of the literature, has recommended the use of up-pumping, wide-blade hydrofoil impellers in agitated bioreactors.

4.6. Seed Priming Bioreactors. Seed priming is a relatively new technique enabling seeds suspended in an osmotica to imbibe moisture and thus be brought to the point of germination (5). However, germination does not occur. Subsequently, on sowing, germination is very rapid and synchronous. While the process is taking place (up to $\sim$14 days), the seeds require oxygen. Both bubble column (71) and stirred bioreactors (5) have been used successfully, although the former requires high air rates to keep seeds in suspension (72). In addition, some seeds such as onions, appear to have a critical oxygen concentration greater than saturation with respect to air that may be due to diffusion limitations within the seed. In that case, enriched air must be used and, in order for the process to be economic, stirred bioreactors are appropriate (71).

4.7. Single-Cell Protein. Systems involving single-cell proteins are often very large throughput, continuous processing operations, such as the Pruteen process developed by ICI. These are ideal for airlift bioreactors of which the pressure cycle fermenter is a special case (55).

4.8. Biological Aerobic Wastewater Treatment. Biological aerobic wastewater treatment is a rather specialized biotechnical application (73). The activated sludge process consists of an aerated bioreactor to which the basic principles of oxygen transfer discussed here apply. Either aerated agitators or air spargers (diffusers) are used. Where the effluent has especially high oxygen demands, however, pure oxygen or oxygen-enriched air is employed. Two examples are the UNOX process (74), which involves agitation and the ICI deep-shaft process (equivalent to a loop fermenter) that is very effective where space is a premium (75). For maintaining the aeration of large quantities of relatively pure water having a low oxygen demand, where space is not a limitation, such as in reservoirs, simple plunging jets having low k_La values but very high energy efficiency are suitable (27). There is also a range of specialized devices in which the area for oxygen transfer is achieved by the use of extended solid surfaces on which a biofilm, irrigated by the water being treated, grows, eg, trickle beds and

rotating disk contactors (73). Also, three-phase systems (fluidized beds) have been developed (11). These are bubble columns in which the air flow keeps inert solids such as sand in suspension giving a large surface area on which the biofilm can grow (11).

5. Nomenclature

Symbol	Definition	SI units
a	interfacial area of air per unit volume of liquid	m^2/m^3
A	interfacial area available for mass transfer	m^2
C_{crit}	critical dissolved oxygen concentration	kg/m^3
C_i	concentration of oxygen in the liquid phase at the interface	kg/m^3
C_{L_4}	concentration of oxygen in the bulk liquid	kg/m^3
C_g^*	saturation concentration of oxygen in the liquid for an oxygen partial pressure p_g	kg/m^3
d_B	Sauter mean bubble size	m
H	Henry's law constant	$kPa \cdot m^3/kg$
J	oxygenation rate	kg/s
k_g	gas film mass-transfer coefficient	$kg/(s \cdot m^2 \cdot kPa)$
k_L	liquid film mass-transfer coefficient	m/s
N	oxygen mass flux	$kg/(m^3 \cdot s)$
P	power imparted to the liquid	W
P_g	total pressure of the gas phase	kPa
p_g	partial pressure of oxygen in the bulk of the gas phase	kPa
p_i	partial pressure of oxygen in the gas phase at the interface	kPa
T	temperature	°C
v_s	superficial gas velocity	m/s
y	volume (mole) fraction of oxygen in gas phase	dimensionless
ϵ_H	hold-up	dimensionless
σ	surface tension	N/m
θ	temperature coefficient = 1.022	dimensionless
μ_a	apparent dynamic viscosity	$kg/(m \cdot s)$
ρ	broth density	kg/m^3
	Subscripts	
in	air entering the bioreactor	
out	gas leaving the bioreactor	
lm	ln mean	

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