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Reactor design for large scale suspension animal cell culture

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Abstract

The scale of operation of freely suspended animal cell culture has been increasing and in order to meet the demand for recombinant therapeutic products, this increase is likely to continue. The most common reactor types used are stirred tanks. Air lift fermenters are also used, albeit less commonly. No specific guidelines have been published for large scale ($\geq$10 000 L) animal cell culture and reactor designs are often based on those used for microbial systems. However, due to the large difference in energy inputs used for microbial and animal cell systems such designs may be far from optimal. In this review the importance of achieving a balance between mixing, mass transfer and shear effects is emphasised. The implications that meeting this balance has on design of vessels and operation, particularly in terms of strategies to ensure adequate mixing to achieve homogeneity in pH and dissolved gas concentrations are discussed.

Abbreviations: CIP, clean in place; SIP, sterilize in place; FCS, Foetal calf serum; CHO, Chinese hamster ovary; BHK, Baby hamster kidney; PBS, phosphate buffered saline.

Introduction

There is a steadily increasing number of licensed therapeutic proteins derived from mammalian cells and an even larger number in clinical development. Depending on the application and dose level, some of these proteins may be required in very large quantities (possibly hundreds of kg per year). This presents a challenge to produce the proteins in the required quantities cost effectively, leading to an emphasis on increasing the scale and/or volumetric throughput of fermenters and on process optimisation.

Whilst there is emerging competition to the animal cell culture method for producing therapeutic proteins including e.g. gene therapy and cellular therapies (Cooney, 1995) and the use of transgenic animals, the fact that in 1995 half of the $5 billion annual turnover of the biotechnology industry was based on therapeutic production from animal cell culture emphasises the importance of this sector (Cooney, 1995).

Several reviews covering broad aspects of scale up of animal cell culture have been published (e.g. Bliem and Katinger, 1988a, b; Van Brunt, 1988; Horvarth, 1989; Nelson, 1988a, b; Werner, 1994). There are also many papers/reviews which address the issue of scale up criteria for bioreactors in general. However, there has been very little consideration of the important balance between mixing and mass transfer requirements and shear sensitivity of cells in the design of very large scale systems. This is extremely important as it has implications for the maximum feasible scale of operation for animal cell culture fermenters. It is also an important consideration in designing reactors which will function at the high cell densities now being achieved as a result of modern fed batch and perfusion strategies. The essential requirements for selecting a cell culture system are: aseptic operation; mixing without damaging shear sensitive cells; sufficient gas transfer (particularly oxygen and carbon dioxide); ease of scale up; ease of process control and automation; and com-

patibility with upstream and downstream processes (Birch et al., 1985).

In this review, relevant aspects of scale up and reactor design are summarised. The necessary basic principles of mixing, mass transfer and shear as applied to animal cell culture are included. A detailed discussion of current design and use of stirred and air lift fermenters is provided, dealing in particular with important design issues relating to successful scale up of these fermenters for large scale suspension culture of animal cells.

Scale Up

Operating/production volumes for freely suspended batch or fed batch stirred tank animal cell culture reactor of 8–10 m^3 (Pullen et al., 1985; Phillips et al., 1985; Nelson, 1988a, b) and 15 m^3 (Werner, 1994) have been reported. In addition to batch and fed batch systems, large scale continuous (perfused) systems are also operated (e.g. Werner, 1994).

The scale of reactor required to meet a particular demand depends on the reactor system used, the productivity of the cell line and the quantity of product required. Monoclonal antibodies used in diagnostic kits will be at the lower end of the scale, whilst some therapeutic proteins may be required in quantities of kilograms per year (hundreds of kg yr^{-1} in some cases) and, if producers are to benefit from economies of scale, may need to be produced at scales much larger than those currently used i.e. >10 000 L. Successful cost effective scale up is paramount if times to market and costs for new products are to be kept to a minimum.

The main aims of scale up are similar product quality and yield at various scales of operation with bioreactor productivity (mg product L^{-1} day^{-1}) being used to measure success of scale up. In order to maximise productivity at a range of scales of operation, it is essential to: (i) achieve satisfactory and predictable mixing in terms of gas liquid dispersion, suspension of solids and homogenisation of components (homogenisation of components must be sufficient to ensure biochemical and physiological homogeneity (Votruba and Sobotka, 1992); (ii) achieve satisfactory and predictable mass transfer of gases (particularly oxygen and carbon dioxide) and nutrients and (iii) prevent cell damage and minimise adverse alterations to cell physiology. Successful scale up should also be achieved in a minimum time period and should offer cost savings and competitive advantage (Reisman, 1993).

Therefore when scaling up a process, there are a number of issues that need to be considered, including mixing, mass transfer, shear and pressure effects. These factors will be introduced below, before discussing scale up criteria and design of specific reactor types at large scale.

Mixing

Mixing can be defined and measured by a wide number of means. Mixing times provide useful information in terms of time taken for a degree of homogeneity to be reached. Measurements of mixing time are generally made by measuring the response to injection of a measurable entity (salt, acid or base) in a pulse or step addition. Change in conductivity, pH or concentration with time is then recorded. 95% mixing time is the time taken for the value of e.g. conductivity or concentration to reach 95% of its final value. Analysis of response curves to injections of tracers provides information not only regarding circulation time but also concerning dispersion of the tracer during circulation. A wide number of methods can be used for analysing tracer response curves (e.g. Lu et al., 1994). Also of relevance are fluctuations arising from additions of e.g. basic solutions.

The Bodenstein number (Bo) has been used to compare mixing in different regions in reactors and is a measure of longitudinal mixing. The Bo number is a dimensionless number (Lv/D_1 where L = characteristic length (recirculation length), v = average velocity and D_1 = axial diffusivity). The Bo number essentially provides a measure of dispersion during circulation. When the Bo number approaches zero, the reactor behaviour approaches that of a completely stirred tank; when Bo approaches infinity, the reactor behaves as an ideal plug flow reactor. The Peclet number (Pe) is also used to characterise mixing and is defined as Pe = vD/D_1 (D = tank diameter). Bo and Pe numbers have been used to characterise mixing in a number of reactor types including stirred tanks and air lift reactors.

In general, achieving an even distribution of mass and energy becomes more difficult as scale increases. Mixing is a particularly important issue for animal cells, since they are known to be much more sensitive to shear than microbial cells and this sensitivity can impose limits on operational conditions for animal cell reactors. Until recently however, it was thought that

the relatively low substrate consumption rates (compared with microbial systems) and low rates of change in pH or temperature meant that mixing and circulation times should be much less critical in animal cell culture, particularly when grown freely in suspension. It had been assumed that agitation rates need therefore only be sufficient to keep cells in suspension, with aeration set by altering gas sparging rate (Bliem and Katinger, 1988b). However, it has become increasingly apparent that pH gradients, dispersion of nutrients, waste products and gas dispersion (both oxygen and carbon dioxide) are of particular importance (Wayte et al., 1997; Nienow et al., 1996). Gas dispersion can be quite important for removal of carbon dioxide (produced by metabolism) from solution, and, if dispersion is poor, carbon dioxide concentration, with its adverse effects, can build up. Tramper (1995) calculated the extent of oxygen gradients in animal cell bioreactors (stirred vessels, bubble columns and air lift reactors) at a scale of 10 m^3. Oxygen gradients in the bulk liquid phase and in the stagnant layer surrounding the air bubbles were often quite severe; indeed these gradients were greater than gradients found in stagnant layers surrounding particles (cells and/or microcarrier particles).

There have been many attempts to measure mixing in stirred tanks and air lift reactors, and these are described in detail for each reactor type below. In general, mixing in continuous systems is much better understood than that in batch systems (Wang and Mann, 1992) even though for animal cell culture, batch systems are by far the most common.

Zhang and Thomas (1993) state that hydrodynamics in bioreactors have been studied extensively and that energy dissipation rates, mean velocity, turbulence intensity distributions and energy spectra within bubble columns and stirred tanks have been reported by many workers. However, this does not apply to conditions used for large scale animal cell culture i.e. very low power dissipation rates.

Mass transfer

Mass transfer and distribution of gases, nutrients and toxic products is important in animal cell cultures. In terms of gas transfer, transfer of oxygen and carbon dioxide are important processes particularly when carbon dioxide is used for pH control. Oxygen requirements will be cell line dependent, typically being in the range 0.05–0.5 mmol oxygen L^{-1} hr^{-1} for 10^6cells mL^{-1} for human cells (Fleischaker and Sinskey, 1981) and given as 0.1–2.5 mg.10^8 $cell^{-1}$ hr^{-1} for a range of industrially relevant cell lines by Aunins and Henzler (1993). The gas transfer rate is given by:

$$\text{Gas transfer rate} = k_L a(C^* - C) \qquad (1)$$

where $k_L a$ is the overall mass transfer coefficient, C is the concentration of dissolved gas and C^* is the saturation concentration of the dissolved gas.

For microbial systems, there are a wealth of empirical correlations relating $k_L a$ or k_L to power input: these are of the following general form:

$$k_L a = c\left(\frac{P}{V}\right)^m U_s^n \qquad (2)$$

where P/V is power input per unit volume of dispersion, U_s is the superficial gas velocity and c, m and n are empirical constants.

Bliem and Katinger (1988b), Reuss (1993), Asai et al. (1992) and Chisti and Moo Young (1987) provide good overviews of correlations for $k_L a$ for a range of geometries of air lift and stirred tank reactors with different physical properties of solution. However, most of the correlations available have been derived for oxygen $k_L a$ in microbial fermentations at high agitation speeds and are not generally appropriate for animal cell culture systems.

The overall mass transfer coefficient is a function of the physical properties of the liquid phase and it has been shown that $k_L a$ can vary by up to 50% depending on medium composition (Dorresteijn et al., 1994). There are many methods for measuring $k_L a$ including on line measurements and these are described in depth elsewhere in the literature (e.g. Dorresteijn et al., 1994; Reuss, 1993). New methods are continually being developed to predict and control oxygen and carbon dioxide mass transfer in bioreactors. For example, Smith et al. (1990) have developed such a strategy and demonstrated that it was capable of controlling dissolved gas concentrations in studies involving *Catharanthus roseus* plant cells.

The means of injecting gas into fermenters may affect the mass transfer rate, as it will influence the interfacial area available for mass transfer. For small scale cultures, oxygen transfer through the liquid surface is generally adequate. Various researchers (e.g. Hu et al., 1986) have shown that surface aerators can be used to improve oxygen transfer without causing cell damage as a result of shear effects at the liquid surface, however this is only of use for small

scale reactors; as scale increases additional means of gas injection must be used to meet mass transfer requirements. The usual methods used to introduce gas include: gas permeable membranes and direct sparging with air or oxygen (or enriched air). Other methods suggested at small scale, but impracticable at large scale include (i) pure oxygen sparging within a vibrating wire gauze oxygenator (Katinger, 1979) and (ii) in situ generation of oxygen using, e.g., hydrogen peroxide and the enzyme peroxidase. Oxygen transfer can be increased by adding oxygen complexing agents to the medium e.g. modified haemoglobin or fluorocarbon oils. However, this provides an additional expense, can cause problems in downstream processing (Katinger et al., 1979) and is not known to be used industrially.

Moreira (1995) provides a comparison between oxygen transfer by different aeration methods i.e. surface, sparger and membrane aeration. Correlations for power as a function of Reynolds number, bubble size for each sparger design and k_La are reported for each method. Surface aeration was found to be applicable only for small volumes with the highest k_La found for sparged aeration. The effect of the addition of commonly used polymers such as Pluronic F-68 on mass transfer was also considered; the addition of Pluronic was found to reduce k_La. Chisti (1993) describes a 300 L fermenter (with one marine impeller) scaled up from 20 L for growth of hybridoma cells. Two sparger designs were considered: porous metal spargers (180–200 $\times 10^{-6}$ m) and larger (>0.001 m) multi hole spargers. The larger spargers were found to be preferable mainly due to foaming problems caused by the porous metal spargers.

The design of some gas injection methods has focused around the perceived need to achieve complete bubble dissolution before the bubbles reach the surface; therefore reducing any cell damage as bubbles collapse at the liquid surface. Other methods are designed to provide gas liquid transfer without producing air bubbles e.g. gas permeable membranes and vibrating silicone tubes (Monahan and Holtzapple, 1993). However, there are some disadvantages of membrane diffusion, including difficulties in maintenance e.g. CIP (clean in place) and SIP (sterilise in place), particularly at larger scale (Moreira, 1995). One inherent disadvantage of such systems is that the reduced interfacial area means that dissolution of CO_2 from the solution to the gas phase will be limited with a consequent build up of dissolved CO_2, which, as discussed below, can be a significant process problem.

Interfacial area (a) will also obviously affect k_La. Zhang et al. (1992) measured oxygen transfer rates in aerated animal cell bioreactors as a function of bubble diameter. Three ranges of bubbles were studied 5 mm, 1 mm and 100 μm in diameter. Oxygen transfer rates increased as the size of bubbles decreased. The effects of additives including new born calf serum and Pluronic F-68 were found to depend on the size of the bubbles. Although it is known that bubble coalescence is important, as it affects bubble size and hence interfacial area and k_La, the effect of fluid properties including surface properties is still not well established (Reuss, 1993).

There are a range of correlations available for predicting bubble size at the sparger. As the gas flow rate increases four regimes of bubble formation can be seen: separate bubble formation, chain bubbling, jet regime and the turbulent regime. The jetting regime is only approached at Reynolds number (Re) >2000, where Re is for gas flow through the orifice. It is unlikely that this regime is ever approached for gas flow rates and spargers used for animal cell cultures. Therefore the separate bubble formation and chain bubbling regimes are most relevant. For separate bubble formation:

$$d_{vs} = 1.7 \left\{ \frac{\sigma d_o}{\Delta\rho\, g} \right\}^{1/3} \quad (3)$$

where d_{vs} = Sauter mean diameter, σ = surface tension, d_o is orifice diameter, $\Delta\rho$ is density difference and g is gravitational acceleration.

For the chain bubbling regime:

$$d_{vs} = 1.17 v_o^{0.4}\, d_o^{0.8}\, g^{-0.2} \quad (4)$$

where v_o is the gas velocity at the sparger. Equations have been proposed for bubble size for turbulent flow (generally stirred sparged vessels) far from the sparger, both for non coalescing and coalescing solutions (e.g. Parthasarathy et al., 1991; Machon et al., 1997). However, at the low gas flow rates and low agitation rates used in animal cell culture, the bubble size far from the sparger is likely to be equal to the bubble size at the sparger. Bubble sizes will generally be in the region of 4–6 mm.

The partial pressure of metabolically produced carbon dioxide (pCO_2) and hence mass transfer of carbon dioxide from solution to the gas bubbles is important in animal cell culture. Rises in pCO_2 can cause rises in H_2CO_3 and hence a drop in pH. pCO_2 must therefore be carefully monitored and controlled and the effect

of scale up on this parameter considered. This is particularly important since carbon dioxide is often used for pH control and control of the ratio of CO_2/HCO^{3-} is critical over a narrow range. High levels of dissolved carbon dioxide can have an adverse effect on cells. Aunins and Henzler (1993) provide a useful account of oxygen and carbon dioxide gas transfer in cell cultures.

Shear

There have been a great number of studies of the shear sensitive nature of cells, because of the obvious implications for reactor design and operation. There are a number of reviews published in this area, dealing with both freely suspended cultures and anchorage dependent cells, e.g. Papoutsakis (1991). There have been many suggestions of the reasons and locations of cell damage resulting from agitation and aeration in bioreactors. For example Leist (1990) listed the following possible causes of cell damage: direct collisions of cells with stirrers or baffles; pressure differences between front and back side of impellers; turbulence mediated by differences in vector length of the vector field of non laminar flow and micro eddies with mean diameter smaller than the cellular diameter. However, the general conclusion from the wealth of research literature in this area is that there are two main potential causes of cell damage.

Firstly, it has been proposed by several authors that cell damage is likely when the Kolmogorov eddy size is similar to or smaller than the cell size itself (Papoutsakis and Kunas, 1989; Kunas and Papoutsakis, 1990). However, doubt has been thrown on this by several researchers. Kioukia et al. (1992) carried out a range of batch cultures in baffled and unbaffled bioreactors (head space aeration and air sparged) and found that the most important region for cell damage was the bubble disengagement region; the more frequently cells passed through this region the greater the cell damage. Kioukia et al. (1992) claimed that use of Kolmogorov's theory of turbulence to explain damage could be misleading.

Secondly it has been proposed that cell bubble interactions are responsible for cell damage; there being 3 main regions where damage may occur:

i) bubble generation region (including region where impeller induces coalescence and break up),
ii) bubble rising region,
iii) bubble disengagement region.

Recent research indicates that bubble rising generally has little effect on cell death/damage (e.g. Michaels et al., 1996) and that the major influences are bubble generation and bubble bursting, with bubble bursting being the most important event (Kioukia et al., 1992; Cherry, 1993; Jobses et al., 1991).

Some researchers have proposed that both of the reasons above are important and that the actual cause of damage will be dependent on conditions. For example Kunas and Papoutakis (1990) proposed that for a particular hybridoma cell line, at high speeds ($>$700 rpm) damage was a result of micro scale turbulent eddies, whilst at lower speeds gas entrainment and break up were the major factor. In recent years there has been fundamental work to actually quantify the effect of bubble rupture on cells (e.g. Trinh et al., 1994). Other researchers have gone further and proposed models for cell inactivation in the presence of bubbles formed as a result of agitation and air sparging (Yang et al., 1992).

The exact process conditions at which damage begins will depend on many factors including reactor geometry and resistance of the specific cell line to shear. Cherry (1993) reported that for a hybridoma cell line, agitation speeds of up to 700 rpm did not harm cells when no air was present. Similarly, Chalmers (1994) reported that hybridomas can withstand speeds of 100–450 rpm in 1 L bioreactors, and it was only when air was introduced that cell damage occurred and indeed, when the air liquid interface was removed from the top of vessel, speeds of up to 700 rpm could be used with no cell damage. Leist (1990) gives tolerances of several cell lines to shear; human melanoma cells in serum-containing medium (5%FCS) were found to exhibit stress effects at >1.5 ms^{-1} stirrer tip speed, whereas CHO cells in serum containing medium (10%FCS) could only tolerate tip speeds of <1 ms^{-1}. Nelson (1988a) reported operation at 13000 L with agitator speeds of 500 rpm (6 m s^{-1} tip speed) with no measured adverse effects on a hybridoma cell line; he claimed that shear damage is generally overstated. There have been many other reports of the effect of tip speed and sparging on cell damage including those reviewed by Reuveny and Lazar (1989) and Gardner et al. (1990).

It is clear that shear sensitivity of cells varies from one cell line to another. However, several researchers including Bliem and Katinger (1988b) have found that a number of industrially relevant cell lines used at large scale are relatively insensitive to shear, including

e.g. BHK 21, Namalwa, Vero, and some CHO lines and a range of hybridoma lines.

Various designs of both aeration method and impellers have been proposed to reduce damage to cells. As discussed above, bubble free aeration is possible at a small scale, however, as scale increases, other means of introducing gas into the reactor are needed. Impellers have been specifically designed in an attempt to overcome this problem i.e. to give reduced mixing times at low agitation rates and low shear stress Moreira et al. (1995) (this is discussed further below).

The phase of growth can also be important in terms of the effect of shear. In viscometric and bioreactor studies it has been found that: i) the state of the inoculum (i.e. whether it is actively or slowly growing) influences a cell's ability to withstand shear forces, ii) cells are more fragile in stationary and lag phases, with the robustness of cells increasing during the exponential growth phase, iii) cells grown in well agitated cultures are less sensitive to shear than cells grown in stationary or slowly agitated cultures; iv) cells are more shear sensitive when exposed for long periods to an inhibitory environment e.g. high ammonia concentrations and v) low pH makes cells more shear sensitive (Reuveny and Lazar, 1989; Leist, 1990; Bleim and Katinger, 1988b). For agitated tanks there have been several reports that the time at which agitation is increased has an effect on the shear sensitivity of cells (Papoutsakis and Kunas, 1989). Other factors that may have an influence on cell damage include i) fluid temperature ii) extent and nature of container surfaces iii) concentration of cells and iv) deformation and stress history of the sample (Cherry and Papoutsakis, 1990).

Several equations have been proposed for cell death resulting from contact of cells with bubbles which are unsaturated with surfactants e.g.:

$$k_d = \frac{4FX'}{\pi D^2 H} = \frac{F}{V}X' \qquad (5)$$

where k_d is the death rate, F is gas flow rate, V is reactor volume, X' is a hypothetical killing volume and H is the reactor height (Tramper et al., 1987). From this equation, it can be seen that cell death should increase as F/V increases and therefore it is important to maximise mass transfer from bubble to liquid to minimise gas flow rate per unit volume.

Relationships have also been derived for cell death rates in bubble columns, for example: Jobses et al. (1991) proposed the following equation

$$k_d = \frac{Q_G}{T^2 H} \qquad (6)$$

where Q_G is the volumetric gas flow rate, T is impeller diameter and H is reactor height.

Kioukia et al. (1992) developed this further to take account of bubble diameter

$$k_d \, \alpha \, \frac{Q_G^{4/3}}{T^{1/3} H \, d_b^3} \qquad (7)$$

where d_b is bubble diameter. This latter equation indicates that death rate should increase with aeration rate; whilst increases in diameter and height should reduce cell damage and that smaller bubbles cause more damage; this has implications for reactor choice. Other researchers have also proposed correlations and models for cell death rates (notably Wang et al., 1994); however, a detailed discussion is beyond the scope of this review. Recently, there have been reports of research aimed at establishing the mechanism of cell death in bioreactors. For example Al Rubeai et al. (1995) have reported two mechanisms of cell death namely apoptosis and necrosis, dependent on the energy dissipation rates to the system.

Work on mechanical properties of the cells is important e.g. choice between different cell lines which can produce the same product, could be made on a basis of cell strength. Born et al. (1992) have proposed that the use of micromanipulation of animal cells in laminar shear stresses can be used to predict susceptibility of cells to shear damage in complex flows found in bioreactors. Measurements which can be made include: mean bursting tension, mean compressibility and mean diameter. Zhang et al. (1993) give such values for 3 cell lines (murine hybridoma, NS1 myelomas, SF9 insect cells). Zhang and Thomas (1993) describe the use of micromanipulation for these measurements and a theoretical model to calculate properties characteristic of cells. Damage is considered by Zhang and Thomas (1993) to be disruption of cells, not other possible effects of shear e.g. reduction in DNA synthesis and cell division are not considered.

It is also important to remember that shear may not only cause cell death but may also effect, for example, fluid mixing, cell suspension, mass transfer, product formation, cell growth and cell to cell or cell to substrate adhesion (Bliem and Katinger, 1988b).

When considering the effect of shear, it is important to be able to measure and quantify the shear that

cells are subjected to. Tramper et al. (1993) give a good review of the quantification of a range of relevant shear rates for bioreactor studies including e.g. time averaged shear rate, maximum averaged shear rate, maximum shear stress etc. for stirred tank reactors and provides details of shear stresses at the critical stirrer speed for insect cells in suspension. Air lift bioreactors are also considered with estimates given for local shear. Tramper et al. (1993) found it difficult to find methods for calculating shear stresses in an air lift reactor that allowed correlation with viability of insect cells, a particular case they considered in this reactor type.

The above discussion regarding the effect of shear on animal cell culture mainly applies to freely suspended cultures. Cherry and Papoutsakis (1990) have written a good review of fluid mechanical stresses for anchorage dependent cells.

Pluronic surfactants (polymers of poly(oxyethylene) and poly(oxypropylene)) are often used as protective agents in serum free medium and can also be added to reduce foaming and provide additional protection in medium containing serum. Leist (1990) discusses the effect of serum and polymers in protecting against damage to cells by agitation and/or sparging. Serum was found to provide protection against sparging, but also enhanced foaming. Cherry and Papoutsakis (1990) have shown that concentrations of up to 10% foetal bovine serum reduce cell death and allow growth at much higher agitation rates in bioreactors with surface aeration; it is not clear if this is a protective effect or a result of faster cell growth stimulated by the presence of serum. Indeed protective effects of a large number of surfactants have been investigated recently (e.g. Van der Pol, 1992, 1993, 1995). Zhang and Thomas (1993) considered how medium additives are effective in preventing cell damage. One factor which may be relevant in this case is foam formation which may protect cells from adverse effects of bubble bursting. Kioukia et al. (1992) showed that Pluronic improved culture performance; Pluronics are also known to interact with cell membranes which could directly affect cell strength. The exact mechanism for cell protection by such additives is still not fully understood, but these additives are likely to protect cells from direct exposure to the gas liquid interface when bubble bursting occurs. The exact effect of protective agents depends on the cell line, cultivation conditions and medium composition (Papoutsakis, 1991).

Attempts have been made to correlate surface properties with protective effects. It is clear that the static properties e.g. surface tension and bulk viscosity will not be relevant; dynamic surface tension and surface dilational modulus will be much more important. Indeed first measurements of dynamic properties indicate that different protective mechanisms may exist for different shear protection additives (Michaels et al., 1994).

Handa-Corrigan et al. (1989) and Handa-Corrigan (1990) considered oxygenation and cell damage, giving the following recommendations to minimise damage: i) to design stirred tank reactors considering mixing and oxygen transfer independently (with the impeller designed to give good mixing but to minimise bubble disruption and dispersion) ii) to sparge with very small micron sized bubbles so that stable foams are formed and iii) to formulate media to contain surface active components which result in stable foams. Also highlighted was the general lack of understanding of the importance of surface active components and their exact interfacial effect on foam stability, protective effect etc. Other researchers have also suggested that small bubbles are beneficial in terms of minimising cell damage (e.g. Michaels et al., 1996), however, as discussed below, it is now realised that the use of micron sized bubbles must be considered carefully as larger bubbles and higher gas flow rates may be needed to tackle another issue; that of removing dissolved carbon dioxide.

There are still a number of important questions that need to be answered regarding effect of hydrodynamic conditions on cell viability. The most important include: how do fluid mechanical and related stresses affect cell growth and the cell cycle as opposed to simply cell death; how do fluid mechanical forces affect the physiology, product expression, protein synthesis and other molecular processes of the cells; how do cells adapt to a high mechanical stress environment; are shear effects a result of intensity or frequency (or a combination of shear forces) (Cherry and Papoutsakis, 1990). Also, Bliem and Katinger (1988b) stress the need to understand micro turbulence if we are to find correlations between shear and cell damage since as scale increases superficial velocity and impeller speed usually increase thus increasing impeller shear and resulting in higher levels of macro turbulence and micro turbulence.

To summarise, to date, the main conclusions from research concerned with shear damage of animal cell cultures are i) many suspension cells particularly hybridomas are not as shear sensitive as first thought

and can tolerate relatively high levels of agitation; ii) most cell death is caused by bubble bursting in stirred vessels; iii) cell death in suspension increases when turbulence Kolmogorov length scale approaches the size of cells; iv) there will be other effects of shear apart from that of cell death and v) the potentially damaging effects of sparging combined with agitation leave a limited range of impeller speeds available for altering mixing.

Pressure

Pressure will affect the solubility of oxygen and carbon dioxide, size of gas bubbles and may also effect cell viability and physiology. There have been very few detailed studies of the effects of pressure on animal cell culture. However, Takagi et al. (1995) considered the effect of hydrostatic head (0.1–0.9 MPa) on hybridoma cell metabolism and found little effect on cell growth or specific glucose consumption. Some changes in conversion rates from glucose to lactate and glutamine consumption rates were found and the specific production rate of monoclonal antibody rose by about 20% in proportion to the pressure increase.

Scale up criteria

There are typically two possible starting points for scale up: i) scaling up a process for use in an existing reactor and ii) design of a new fermenter i.e. developing a fermenter to meet process requirements.

Typically, the following parameters can be held constant during scale up (Ju and Chase, 1992):

- reactor dimensions,
- k_La,
- shear rate (maximum value),
- P/V,
- volumetric gas flow rate per unit volume of liquid,
- superficial gas velocity,
- mixing time,
- impeller tip speed (for stirred tanks).

Obviously all these parameters cannot be held constant simultaneously. For example, if geometric similarity and constant P/V are used, shear rate, mixing time and impeller tip speed will not all be constant with scale up. Also superficial gas velocity cannot be maintained constant with scale simultaneously with gas volumetric flow rate. Generally, geometry is used as a scaling criteria as is constant mass transfer coefficient. For microbial systems constant power per unit volume is usually the third criteria. Constant mixing time is not a frequently used scaling criteria (partly because it is not a directly measurable variable). If constant mixing time is not used as a scaling criteria, mixing time is likely to increase progressively with scale e.g. if P/V is used as a scaling criteria. However, kinetic time constants will remain more or less constant.

Scale Down

It is increasingly being recognised that scale down of existing processes is important for understanding phenomena seen on the large scale and for considering process improvements. Sola and Gadia (1995) explain the importance of regime analysis in this process. Essentially this involves determination of the rate controlling mechanism so that this can be used as a scale down variable. In order to determine the rate controlling mechanism the time constants for, e.g., hydrodynamics and mass transfer must be compared. An example of the outcome of such studies is that in stirred tanks, under certain conditions, a well mixed and a less well mixed zone can be identified. An appropriate scale down experiment may be to have two separate but connected stirred tanks, with different mixing regimes; to effectively investigate the effect of cycling through these two regions. This was further emphasised by Kossen (1995) who pointed out that in scale down studies, geometric similarity is not necessarily paramount, of greater importance is the need to realise at the small scale time constants found for the full scale process. Jem (1989) provides a basic description of how scale down can be used to optimise a process for scale up and prevent operational problems including scale down to consider cell and product resistance to shear, oxygen utilisation rates, minimum oxygen tolerance and maximum carbon dioxide tolerance.

Reactor Types

A wide range of reactor types have been suggested in the literature for animal cell culture including: stirred tanks, air lift reactors, bubble columns, packed bed reactors, fluidised beds (e.g. Keller and Dunn, 1994) and membrane reactors. For very small scale simple systems, reactors used include roller/shake flasks, spinner flasks and hollow fibre systems (Kearns (1990)

give details of smaller scale bioreactors). A number of small scale hybrid type reactors, containing elements of e.g. an air lift and a stirred tank have also been proposed (e.g. Sucker et al., 1994).

Suspension culture is currently regarded as the preferred scale up method for animal cell culture. Attachment systems are likely to be used only when they are the only option; systems used range from hollow fibres or glass beads to microcarriers. Inhomogeneities in particular in solution pH and DOT, are a potential problem with attached systems (Leist, 1990). A range of attachment reactors have been described elsewhere; a detailed consideration being beyond the scope of this review (e.g. Glacken et al., 1983; Mizrahi, 1989; Hofman et al., 1989; Karare et al., 1985). Suspension culture is generally operated in fed-batch mode, but increasingly with examples of perfusion culture (Martin et al., 1987; Tolbert et al., 1985; Tokashiki and Takamatsu, 1993; Peshwa et al., 1993).

Despite the diversity of reactor types and modes of operation presented in the literature, for large scale operation (>100 L), stirred tank reactors and to a lesser extent air lift fermenters operated, generally as suspension cultures, in fed batch or perfusion mode are almost exclusively used for large scale animal cell culture. Therefore the remainder of this review will consider stirred tank and air lift reactors only.

Large Scale Stirred Tanks

Large scale stirred tanks are used widely throughout the chemical and biochemical industry up to scales of 20 000 m^3 (for sewage treatment). There is a wealth of information published in the literature regarding general design of stirred tanks, impellers, gas spargers etc. (e.g. Leng, 1991). Advantages of stirred tanks include wide scale use and availability albeit for applications other than animal cell culture and relative flexibility in terms of volume of culture broth that can be processed in any one batch (i.e. with one vessel it is possible to process a number of different size batches).

Design issues

The major design decisions for large scale animal cell culture in stirred tanks include: impeller speed, impeller geometry including diameter, clearance of impeller from base, single or multiple impellers, distance between impellers; ratio of impeller diameter to tank diameter; aspect ratio of tank; sparger details; gas flow rate; position for feeds, alkali, sample points; position of pH and DO probes; design of base of tank; design of impeller shaft, type and construction of motors and seals; design and position of baffles and temperature control.

Design guidelines developed for microbial fermentations are available for e.g. baffle design, clearance from base, impeller spacing for more than one impeller, geometry of base etc. For example, Reuss (1995) gives typical dimensions for a microbial fermenter at 100 m^3 scale and for a range of impellers (Figure 1). However, these designs were developed for processes with higher aeration rates and higher power inputs; typically 1000 W/m^3, rather than 10 W/m^3 which is more realistic for animal cell cultures. Therefore these designs are not likely to be optimal for animal cell cultures.

Overview of hydrodynamics

When designing stirred tanks, it is important to understand the hydrodynamics of these systems. There are three well defined flow regimes (Figure 2). For constant gas flow rate and increasing speed (N), at speeds below a certain value (N_F), the bubbles rise only in the middle of the tank (above the impeller) and the impeller is said to be flooded. As N increases, at a certain speed N_{CD}, the gas is driven out horizontally from the impeller. Although there is very little gas re-circulation below the impeller, the impeller is said to be loaded. For $N>N_{CD}$ there is good gas circulation throughout the vessel. The same regions occur, but in reverse, if N is held constant and gas flow rate increased. There are many flow regime maps and correlations available to predict the transitions between flow types in terms of e.g. power input per unit volume (P/V) (e.g. Nienow et al., 1985). It is important to realise that, for conditions currently used for large scale animal cell culture gas dispersion will generally be in the flooding regime.

Impeller choice

An important consideration in stirred tanks is to minimise mechanical stress damage. However, it is also important to minimise mixing times. To achieve these aims, an impeller with a high flow number (Fl) and a low power number (N_P) should be used; thus maximising flow for a given power input (this follows since for a given value of impeller diameter (D) and speed (N), mixing time is inversely proportional to Fl, while power is directly proportional to N_p). To maximise pumping capacity and minimise mixing times, it can

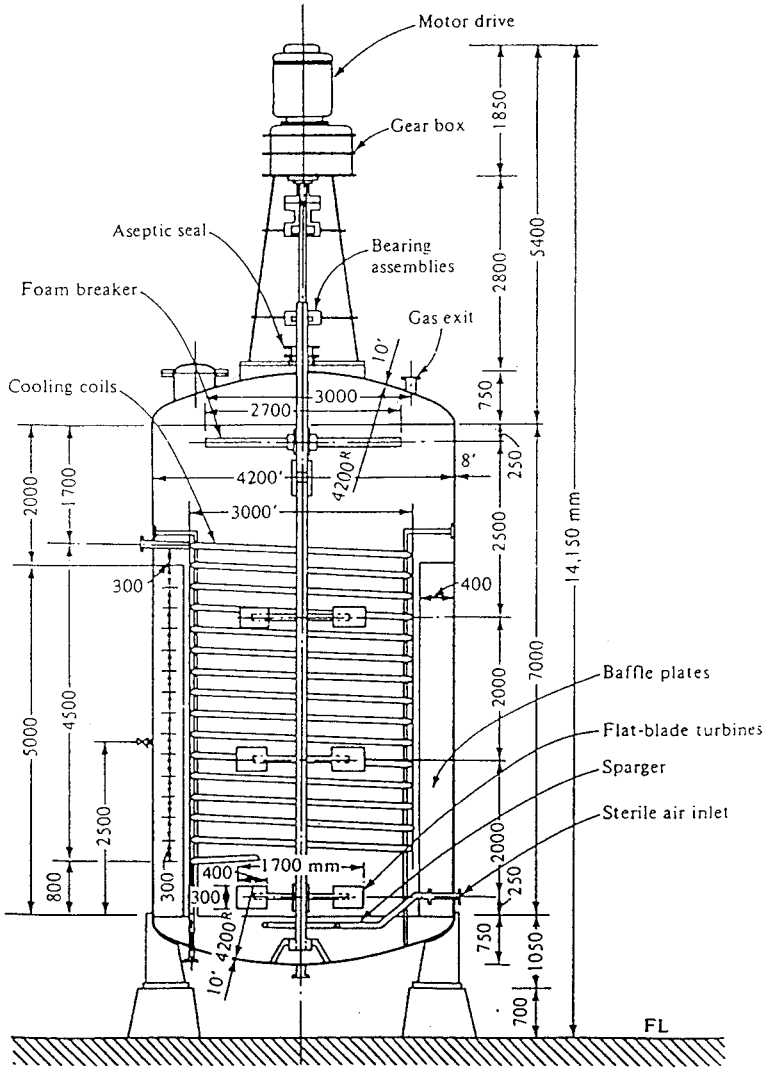

Figure 1. A typical 100 m^3 fermenter for microbial systems (from Reuss 1995 with permission).

also be shown that it is better to use large agitators agitating slowly rather than small agitators agitating fast (at the same power input). Since, as discussed above, it is known that for animal cell cultures, tip speed is a critical parameter, the effect of impeller choice on this parameter must be considered. Low power number impellers are generally better at dispersing air effectively and values of aerated power/unaerated power are greater than for high power number impellers. Much effort has been focused on impeller design specifically for use in animal cell cultures. It is generally accepted that turbine impellers are damaging to many cell lines so the marine impeller has become the impeller of choice. Many modifications of the marine impeller have been proposed to provide more efficient mixing at lower impeller tip speeds (Nienow et al., 1996). A number of high flow, low power number impellers have been developed to provide improved performance; these include Intermig, Lightnin, Prochem Maxflow and Scaba 6SRGT impellers (Reuss, 1995). Further details regarding impeller choice and transitions between flow regimes (including effect of scaling up) are given by Nienow (1990). As the scale of vessel increases it may become necessary to have multiple impellers: there are a number of reports of hydrodynamics of multiple impeller systems (e.g. Whitton, 1988; Nienow, 1990; Cronin and Nienow, 1989). In particular, Whitton (1988) describes the existence of

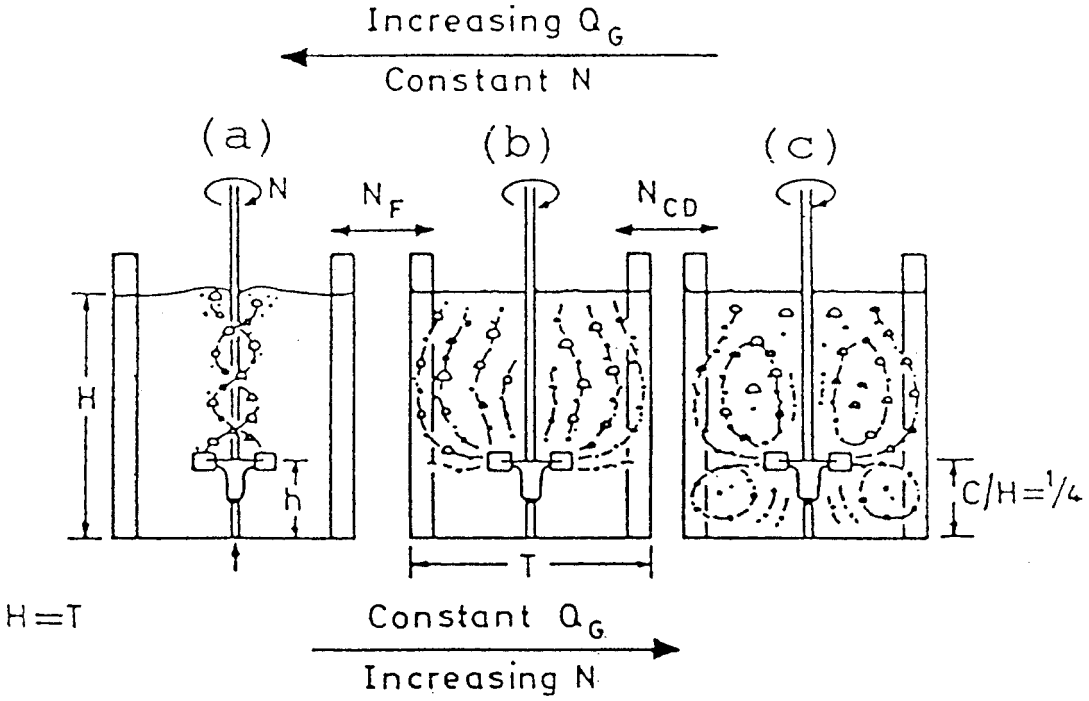

Figure 2. Gas liquid dispersion in an agitated vessel (from Nienow et al. (1985) with permission).

dissolved oxygen gradients in a large vessel (vessel diameter 0.95 m; vessel height 3.0 m; N = 5.33 rps; Q_G = 0.0127 m^3 s^{-1}) with 3 disc turbines (a single disc and twin axial turbines), highlighting a potential problem of such systems.

Aspect ratio

Another important, but much overlooked, design parameter is the aspect ratio for the vessel. Most large scale stirred vessels used for animal cell culture are either retrofitted microbial fermenters or are based on geometric designs for such systems and are typically between 1:1 and 3:1. However, these values are not based on a fundamental consideration of the effect of aspect ratio on the various phenomena occurring during an animal cell fermentation. For animal cell culture there appears to be an upper limit for the tip speed (albeit dependent on a particular cell line). Whilst improvements in impeller design are continuing, the existence of a critical tip speed, imposes a limit on the balance between impeller diameter and speed (since tip speed = π N D). As indicated above, animal cell cultures are typically operated in the flooding regime, where gas dispersion is poor; it is preferable therefore to have a wide impeller (relative to the vessel diameter) to ensure gas is dispersed across the diameter of the vessel. This then places a restriction on the diameter of the vessel and may mean that higher aspect ratios are preferable.

As the height of the vessel increases, the number of impellers needed is likely to increase; this may lead to compartmentalisation of the fluid and subsequent difficulties in pH measurement, control etc. However, it may be possible to address these problems by adopting appropriate injection and monitoring strategies. If scale and height increase significantly, hydrostatic pressure in parts of the fermenter will also increase and this may have implications for cell damage, physiology or concentration of dissolved gases.

Mixing

Recently, it has become increasingly apparent, that mixing times in large scale animal cell culture are significant and may have an adverse effect on the process. There are a number of accounts of mixing time measurements in stirred tanks at large scale. Also Bodenstein numbers have been determined for stirred vessels as a function of e.g. geometry of vessel and impeller speed. It has been reported that Bo number is independent of impeller speed for a given geometry and is proportional to the ratio of tank diameter to impeller diameter (Vonken et al., 1964). Most such

studies have been for P/V values significantly higher than those found for animal cells; the exception being research reported by Nienow et al. (1996), who, presented data and a correlation for mixing times at very low power inputs. Nienow et al. (1996) provided quantitative information for large scale animal cell culture in an 8000 L fermenter (vessel diameter = 2 m; maximum liquid height = 2.6). Several impellers were considered including a Rushton turbine (power number = 5; impeller diameter = 0.44 m) and Intermig impeller (power number = 0.3; impeller diameter = 0.78 m). An impeller speed of 60 rpm was given for the Rushton impeller. Some interesting observations can be made from the data presented by these authors. Power input which is important for both mixing and mass transfer in gas/liquid agitated tanks can be calculated (using $P = N_p \rho N^3 D^5$) as 82 W giving a power input per unit volume of = 10 W/m^3 (for the Rushton impeller). This is for liquid flow only. For gas liquid dispersions, power is likely to be approximately = $0.35 \times$ power in ungassed system. Therefore P/V will be closer to 3.5 W/m^3 in the large scale aerated vessel. This is an extremely low power dissipation rate. In fact the sparged air must act rather like a bubble column since air will not be dispersed effectively. Also graphs of tip velocity versus gas flow rate indicate that for conditions likely to be used for stirred tanks, gas circulation will be incomplete i.e. poor (the impeller will be flooded). This also has implications for sparger design; if the impeller does not break up or disperse the bubbles, sparger design and positioning will need to be considered in more detail. It would appear from the data presented that the tip speed at which the fermenter was operated was 1.38 ms^{-1} (calculated from tip speed = π N D (where N is in revs s^{-1})). This is just below the value of 1.5 ms^{-1}, above which it is generally thought that cell damage may begin. Finally, Nienow et al. (1996) presented a correlation and limited data for mixing time; mixing time was typically 100 s (ranging from just greater than 200 s to approx. 70 secs).

Amongst others, Cronin and Nienow (1989) have shown for agitated vessels that mixing time depends on position of injection point for addition of, for example, alkali for pH control. Cronin and Nienow (1989) considered a vessel of 0.72 m diameter with an aspect ratio up to 2:1. The longest mixing times were obtained with addition at or near the surface; whilst the shortest mixing times were obtained with addition into the lower circulation loop of the upper impeller (for dual impeller system), intermediate mixing times being found for injection at any other point. This strongly indicates that, if mixing times are to be minimised, addition should be made into each impeller region.

Mixing must not only be sufficient to provide a homogeneous environment in terms of pH, dissolved oxygen concentration etc. but must also keep solids in suspension. Despite the importance of keeping solids in suspension; there is no universal scale up rule to determine the power input required to keep particles in suspension as a function of suspension properties, tank size and geometrical conditions (Geisler et al., 1993).

Predictive models which could be used in design/scale up of bioreactors would be very useful. There have been some attempts to model mixing in large scale tanks. For example, Magyr et al. (1994) developed structured mixing models to predict mixing behaviour in large stirred tank and Reuss (1995) presented models in which the reactor is compartmentalised to allow for various types of fluid circulation within the reactor. However, despite the extent of published literature for stirred tank reactors, the development of models for large scale systems is constrained by the lack of reliable measurements carried out on large scale vessels with tank diameter >1 m. This applies particularly to multiple impeller systems where problems may occur because of poor exchange of flows between one agitator and another. A model for oxygen transfer in a production scale bioreactor has been proposed by Oosterhuis and Kossen (1984). This model is based on empirical correlations for k_La for oxygen transfer and uses a structured model for gas and liquid flow. Local oxygen concentration profiles in the reactor, local gas phase concentrations and oxygen transfer capacity are predicted as a function of stirrer speed and gas flow in a dual impeller stirred tank (volume = 19 m^3). Results indicate that oxygen gradients often exist, even in non viscous systems, because of the relatively high overall mixing times of the liquid phase as compared to the time constant for oxygen consumption and for oxygen transfer. There has been little attempt to couple circulation mixing models with reaction kinetics as has been undertaken for microbial systems (Bajpai and Reuss, 1982) and which would be useful for understanding operation at different scales and in different reactor configurations.

Mass transfer

Correlations for mass transfer for more general systems are discussed above. There are very few published correlations for k_La for oxygen transfer in

animal cell culture, especially for large scale systems. Measurements for k_La in small scale systems have been reported. For example, Lavery and Nienow (1987) measured oxygen k_La using an unsteady state technique at impeller speeds ranging from 1.6–5.8 s^{-1} in a mechanically agitated reactor of 1.5 L, for water, basal medium, basal medium supplemented with 5% foetal calf serum and addition of silicone antifoam using sparged and surface aeration. Moreira et al. (1995) considered the effect of method of aeration (surface, sparging and membrane) and power input on mass transfer, (k_La measured using the dynamic gassing method) and bubble size (using high speed video) in animal cell culture media in a 2 dm^3 working volume stirred vessel. Aunins et al. (1989) measured power inputs and surface oxygen transfer rates in 500 mL spinner vessels. For application at large scale, data is very limited. Indeed the only published data is that of Nienow et al. (1996) who provide values for k_La, as a function of energy dissipation rates (for one gas flow rate 6.3×10^{-4} m^3s^{-1}); for cell culture medium, values are in the range of 1–10 h^{-1} (for P/V of 10–100 W/m^3) in an 8000 L fermenter.

Recently, it has become apparent that build up of dissolved carbon dioxide in culture fluids can have adverse effects in terms of productivity. Gray et al. (1996) investigated experimentally the effect of pCO_2 in a 10 L perfusion stirred tank reactor with CHO cells and also from simulations predicted the effect of pCO_2 at 500 L scale. They found that productivity was maximised when pCO_2 was between 30 and 76 mm Hg and they recommended that bubble sizes should be between 2–3 mm to ensure an adequate balance between oxygen mass transfer and carbon dioxide stripping. This highlights that it is not appropriate to consider oxygen and carbon dioxide transfer separately. It may for example be necessary to increase gas flow rate to achieve the desired value for pCO_2 even though gas flow rates are traditionally kept as low as possible to minimise shear effects. Additives may then be necessary to counter any adverse effects on cell viability.

Previously reported stirred tank fermenter systems for animal cell culture

There are many descriptions of animal cell cultures in small stirred tank reactors, which give details of reactor design and process conditions (e.g. Leist et al., 1986; Moreira et al., 1995; Chisti, 1993). However, no indication is given of the design criteria used for these vessels or indeed if the designs are optimal. Nelson (1988b) gives very basic design and scale up for stirred tank bioreactors giving mass transfer correlations for small scale systems, with calculation of agitation rate and power input based on constant Kolmogorov length scale, to minimise shear effects. Brief details are also provided for sterile design and containment. Chisti (1993) describes a 300 L fermenter, scaled up from 20 L for growth of hybridoma cells giving geometric details including impeller design (one marine impeller). In particular the suitability of a number of spargers is discussed. Porous metal spargers (180–200×10^{-6} m) are found to produce foams that are difficult to control. Aeration with larger (>0.001 m) multi hole spargers is recommended. Backer et al. (1988) provide details of 150 and 1300 L scale vessels for monoclonal antibody production. The vessels used were retrofitted microbial fermenters. Maximum agitation speeds generally used were 140 rpm for 150 L and 75 rpm for 1300 L vessel; these speeds were not found to cause cell damage (for a hybridoma cell line). Agitation and air supply systems were able to provide aeration for cell concentrations $>5 \times 10^6$ cells mL^{-1}. For the 150 L vessel it was found that 170 rpm (corresponding to a tip speed of 2.03 m s^{-1}) was limiting and resulted in a reduction in oxygen uptake rate.

Zhou et al. (1996) describe production of recombinant mouse and rat growth hormone by fed-batch for GS-NSO cell cultures at a range of scales including 150 and 250 L, giving particular attention to balancing oxygen supply and carbon dioxide removal as scale increases. There are some details of impeller design and fermenter internals although there are clear differences between the 150 and 250 L reactors considered. There are also differences in cell growth obtained at 150/250 L scale as compared to that at 36 L scale. Ray et al. (1997) describe an approach to scale up and then briefly describe the production of a humanised monoclonal antibody expressed in a GS-NSO cell line at 2000 L using an agitated vessel in fed batch mode (also refereed to is previous production at a similar scale using an air lift fermenter). There are very few details of reactor design. Junker et al. (1994) usefully describe the modification of several microbial fermenters (75 and 280 L) for animal cell use.

Pullen et al. (1985) and Phillips et al. (1985) reported the use of a 1000 L pilot scale facility, and production vessels at 2000, 3000, 8000 L capacity for large scale animal cell culture in agitated vessels (producing e.g. viral vaccines and human interferon (gram quantities per batch)). In these reactors, the

cell suspension was agitated using impellers driven by indirect magnetic drives (eliminates possibility of contamination associated with a direct shaft drive). Redox, pH, stirrer speed and temperature were recorded and controlled. For redox control, pre filtered air was introduced into the cell suspension. For pH control, CO_2 was added to lower pH and sodium carbonate to raise pH. In the production vessel, cells were maintained in repeated batch culture for as long as possible, normally for many months; cultures were eventually lost to a variety of causes e.g. bacterial or fungal, mechanical failure and operator error. However, little information for design of such large scale vessels is provided in the reports by Pullen et al. (1985) and Phillips et al. (1985). Nienow et al. (1996) also recently provided quantitative mixing and mass transfer data (discussed in detail above) in a 8000 L fermenter for cell culture medium.

Scale up criteria

Typical combinations of criteria commonly used for stirred vessels include (Ju and Chase, 1992):

- geometric similarity, constant k_La and constant Q_G/V (N determined from correlation for k_La)
- geometric similarity, constant k_La and constant maximum shear (constant impeller tip speed) (Q_G determined from k_La correlation).
- constant k_La, constant impeller tip speed and constant Q_G/V.

The problem with the first criteria for animal cell cultures is that impeller tip speed is not controlled and may therefore be in excess of that known to damage animal cells. For the second criteria, mixing time is likely to be much longer for large rather than small scale processes; this is also the case for the third criteria. If mixing times are to be adequate to achieve homogeneity and if tip speeds and shear stresses are to be kept below those known to damage cells, then a different approach is needed for scaling up animal cell cultures. The maximum mixing time in terms of additions must be determined from experiments (using e.g. scale down techniques); this together with knowledge of the maximum impeller tip speed should be used as a basis to determine the impeller speed, impeller diameter, number of impellers and aspect ratio (remembering that the ratio of diameter of impeller to diameter of tank needs to be sufficiently large to ensure good gas distribution). Impeller speeds must also

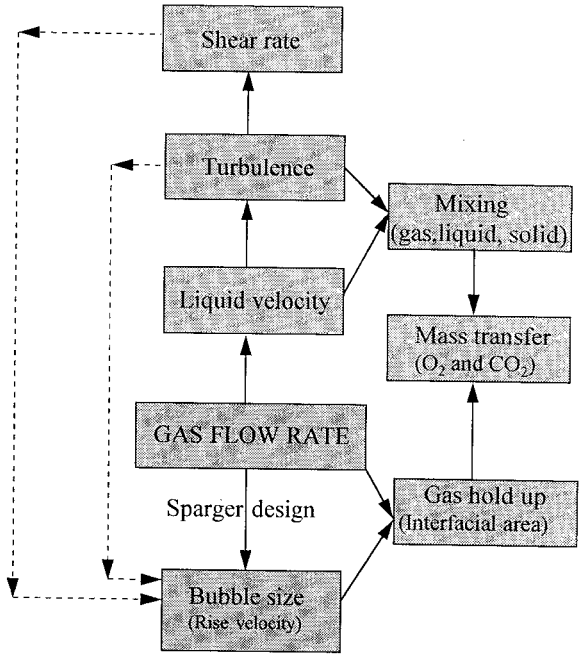

Figure 3. Interdependence of gas flow rate, mixing and mass transfer in air lift fermenters.

be sufficient to ensure that solids remain in suspension. Furthermore, oxygen mass transfer rates should be maintained similar on scale up, if similar oxygen demands of the cells are to be met (this will depend on the impeller speed chosen and hence P/V, and on gas flow rate). This will determine the minimum gas flow rate needed. However as discussed above higher gas flow rates may be required to ensure adequate stripping of carbon dioxide. Consideration of the issues outlined above is likely to lead to higher aspect ratio tanks with multiple impellers. As described above, this may lead to compartmentalisation of flow and therefore strategies for addition of nutrients and control agents (e.g. pH) need to be considered carefully e.g. addition to points other than at the surface will be required.

Large Scale Air Lift Fermenters

Air lift reactors have been widely used in, for example, chemical reactions, fermentation and biological waste treatment and have been operated successfully at very large scales. For Pruteen production and industrial waste water treatment air lifts of 1,500 m^3 and 17 000 m^3, respectively, have been reported.

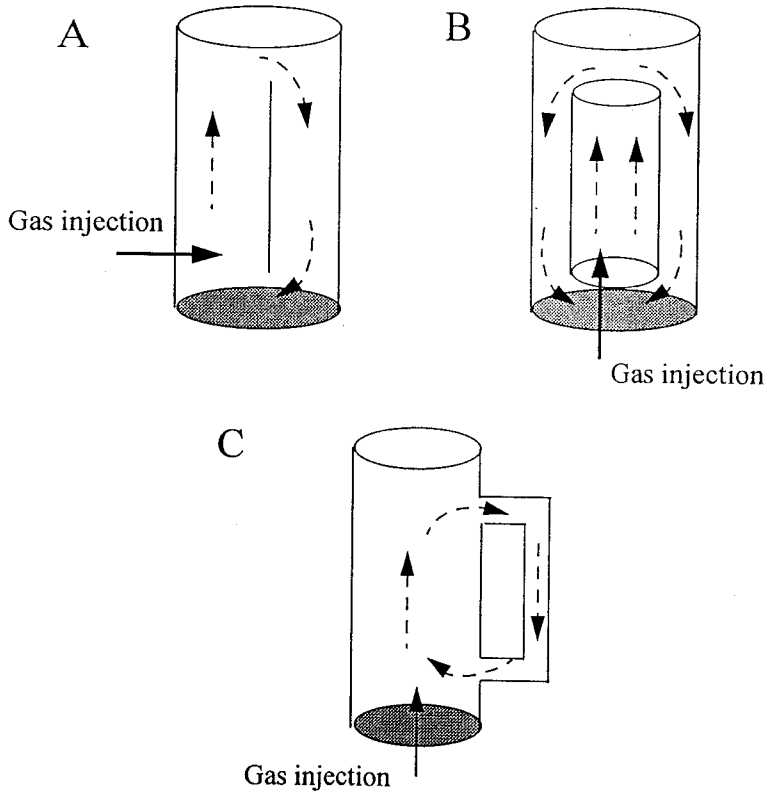

Figure 4. Geometric configuration of air lift reactors (A) internal loop with baffle, (B) internal loop reactor with draft tube and (C) external loop reactor; ↑ indicates direction of fluid flow.

In general, advantages quoted for air lift reactors include: ease of scale up; low energy costs; low shear characteristics; no moving parts; greater reliability for sterilisation; high O_2 absorption efficiency and readily quantifiable flow patterns (Merchuk, 1990; Siegel and Robinson, 1992; Smart, 1984). Power dissipation levels are usually lower for air lifts than for stirred tanks. This offers an advantage and indeed, consideration of the enormous power requirements for mechanically stirred bioreactors first boosted the development of air lift fermenters (Sola and Godia, 1995). However, this also means that mixing is likely to be more critical in such systems (Fields and Slater, 1984).

Design issues

An important feature of an airlift reactor is that mass transfer and mixing are generally coupled i.e. the gas flow rate is often set to achieve a specific dissolved oxygen concentration; this gas flow rate will then determine the hydrodynamic conditions in the fermenter. The interrelationships between liquid velocity and other process parameters is shown in Figure 3. Figure 4 shows the two main geometrical configurations for air lift fermenters: internal or external loop design. The major design decisions for large scale air lift fermenters will include geometrical configuration of riser and down comer; aspect ratio; sparger details; gas flow rate; position for feeds, alkali, sample points; position of pH and DO probes; design of base of tank and temperature control.

Overview of hydrodynamics

In internal loop reactors the upflow (riser) and downflow (down comer) sections are split either by a vertical baffle (split cylinder) or by a cylindrical (concentric) draught tube, in an external loop reactor the riser and down comer are two separate cylindrical sections (see Figure 4). In either case, fluid flow is driven by the density difference, between fluid in the riser and down comer, which is a result of gas injection into the riser. In general, the hydrodynamic characteristics for these reactors are reported in terms of relationships between gas throughput, gas hold up and circulation velocity. For a given configuration, gas flow rate is the only in-

dependently controllable variable, other variables e.g. liquid velocity will depend on flow conditions and reactor geometry. There have been many detailed studies of the hydrodynamics of air lift reactors. There are a wealth of correlations for gas hold up, liquid velocity and mixing characteristics (Verlaan et al., 1986a, b; Clark and Flemmer, 1985; Chisti and Moo-Young, 1987; Chisti et al., 1988; Blenke, 1979; Ade Bello et al., 1985a, b, 1984; Merchuk and Stein, 1981a, b; Kubota, 1978; Jones, 1985; Merchuk, 1986; Lu et al., 1994; Orazem et al., 1979; Onken and Weiland, 1983; Merchuk and Siegel, 1988; Kawase, 1994; Hsu and Dudokovic, 1980; Wu and Jong, 1994). Kubota et al. (1978) describe the hydrodynamics of the ICI deep shaft aerator (depth 100–300 m) and a model to simulate operation of the aerator which is used to determine the air flow rate needed to ensure stable liquid circulation. Chisti and Moo Young (1987) give graphs of superficial gas velocity versus diameter of the column and indicate conditions for which flow is slug, bubbly etc. Chisti et al. (1988) considered other researcher's data and concluded that liquid circulation is much better in external loop air lifts, where there is little gas entrainment in the down comer (i.e. gas hold up in the down comer must be taken into account in liquid velocity predictions). Almost without exception, the hydrodynamic studies described above, have been carried out using non fermentation solutions. However, Russell et al. (1994) considered fermentation of yeast in a 90–250 L concentric tube air lift fermenter and measured liquid circulation velocity, gas hold up and liquid mixing as a function of gas flow rate, vessel height and top section height. Also, hydrodynamics in single and ten stage tower loop reactors have also been characterised for *E-coli* (50 L) (Adler et al., 1983; Lippert et al., 1983).

Hydrodynamic behaviour will to some extent depend on the configuration of the reactor i.e. external or internal loop, draught tube or split cylinder. There have been some comparative studies, for example, Ade Bello et al. (1984) found that external loop airlift contactors generated riser linear liquid velocities up to 3 times higher than those in concentric tube air lift contactors; however, mixing times were quite similar, although slightly shorter for the external loop reactor. A multiple air lift loop reactor has been proposed for scale up by Bakker (1993, 1995); comparison is made between a 3 compartment air lift reactor and a conventional internal loop reactor: however such designs have not been taken up for large scale production. Despite consideration in the literature of both types of reactor, to the authors knowledge only internal loop reactors have been used for animal cell culture; however there appears to be no real reason for not using external loop reactors, except perhaps that construction of internal loop reactors may be slightly simpler and more flexible.

Internal geometric details

Details of many aspects of air lift design have been studied. There have been several reviews of the design of pneumatically driven bioreactors (e.g. Blenke, 1979; Schugerl and Lubbert, 1995), as well as many more research papers dealing with specific design aspects. The importance of the ratio of area of down comer to area of riser (A_d/A_r) in relation to mixing times, liquid velocities and mass transfer has also been reported (Chisti, 1987; Ade Bello et al., 1984; Griffiths, 1988): the general conclusion for internal loop reactors being that in terms of mixing, the optimum configuration is to have the draft tube diameter fixed to give $A_d = A_r$.

For internal loop reactors, the height of liquid above the baffle or draught tube has also been shown to be important. Russell et al. (1994) for a yeast fermentation in a 90–250 L concentric tube air lift fermenter found that mixing time decreased as height of the top section increased up to a critical height; this led to the conclusion that above this critical height there was a two zone flow pattern in the top section of the fermenter. This has also been shown in research carried out by the authors. Mixing times (95%) were determined for a 30 L air lift fermenter with a central baffle, for a range of heights of liquid above the baffle. Mixing time was measured in aqueous solutions: a pulse of potassium hydroxide was injected and subsequent pH changes were measured. Results are shown in Figure 5. As the height of liquid above the baffle increases, mixing time initially decreases and then passes through a minimum before beginning to increase (gas flow rates were chosen to provide mixing times representative of those likely to be found at large scale >5000 L).

Sparger position and design have also been considered. Chisti and Young (1987) describe, for an internal loop reactor, the optimal position for the sparger as above the base of the baffle. They also consider gas injection into the down comer and higher up in the riser with the aim of reducing pressure drop and hence power consumption. A recommendation is given that the total cross sectional area just below the baffle should not exceed 1.65 times the down comer

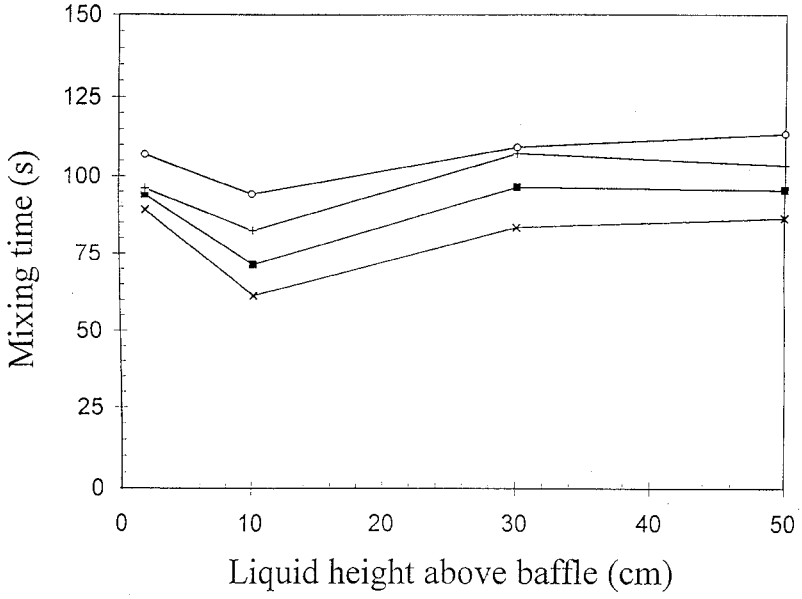

Figure 5. 95% mixing time a s a function of liquid height above the baffle, for a 30 L air lift reactor with central baffle. Gas injection just above baffle base, gas flow rate = 0.2 L min^{-1}. Mixing time measured after pulse injection of alkali (KOH): injection onto surface of down comer; onto surface of riser; just above gas sparger;2 simultaneous injections (i) just above gas sparger and (ii) onto surface of down comer.

cross section. Several possible design improvements are also considered, for example projecting baffles and insertion of static mixers (into liquid above draft tube/baffle). Merchuk and Stein (1981a) considered the effect of sparger geometry, for a 0.3 m^3 external loop air lift reactor. The two spargers considered were (i) 0.09 m diameter copper tube bent to form a 0.1 m diameter ring with 14, 0.025 m diameter holes drilled in the upper side of the ring (to give an even distribution of gas across the riser) and (ii) single orifice sparger of 0.09 m diameter. For sparger (i), the gas hold up increased almost linearly with distance up the riser, whereas for sparger (ii) gas hold up increased, reached a maximum and then tailed off. For sparger (i) with even gas distribution, there was little coalescence and there was an increase in gas hold up with height which was related to bubble expansion as pressure decreased. For sparger (ii) coalescence resulted in an increase in bubble size and hence rise velocity, which for high gas hold ups, led to a reduction in total gas volume. This highlights the importance of sparger design. However, other researchers have reported conflicting results e.g. Onken and Weiland (1983) found that, for their particular sparger, gas hold up was independent of bubble size at the sparger.

As for stirred tank reactors, base design is also important and should prevent stagnant zones (Chisti and Young, 1987; Chudacek, 1984).

Aspect ratio

In designing an air lift reactor, the choice of aspect ratio will always be important. Griffiths (1988) claimed that increasing height improves mixing time and mass transfer rates and that aspect ratios between 6:1 and 12:1 are normally used. The choice of aspect ratio will affect a number of process factors including mixing time; oxygen transfer rate and hence gas flow rate; liquid height above the baffle (and how it changes on additions during the process); carbon dioxide stripping and possibly cell damage. The effect of aspect ratio on these factors is discussed below: it is important to remember that none of these factors are independent.

Mass transfer and mixing

Mass transfer data has been reported for a wide range of air lift fermenters with different fluid types (e.g. Kawase, 1995; Stejskal and Potucek, 1985). Russell et al. (1995) give oxygen mass transfer data for a yeast fermentation in a concentric tube air lift fermenter. Adler et al. (1983) determined oxygen mass transfer in single and ten stage tower loop reactors for *E-coli*. Bakker et al. (1995) determined mass transfer in a multiple air lift loop reactor for a salt solution and found values similar to those in a bubble column Broad et al. (1989) give limited data for oxygen k_La versus superficial gas velocity for 100 and 1000 L air lifts and variation of k_La with aspect ratio (from 4–

12), for hybridoma cells. Attempts have been made to predict oxygen mass transfer coefficients as a function of gas hold up (and hence gas superficial velocity and A_d/A_r) in air lift reactors (e.g. Ade Bello, 1985). Mass transfer data for hybridoma cells in suspension in a 30 L air lift fermenter have been determined by Boraston et al. (1984); the specific respiration rate and effect of dissolved oxygen on growth and metabolism were also determined. Further details of mass transfer in air lifts can be found in e.g. Chisti and Moo Young (1987), Onken and Weiland (1983), Merchuk and Siegel (1988).

In the literature, relationships between k_La and gas superficial velocity (gas flow rate/cross sectional area of the riser) (U_{sg}) have been reported for air lifts and bubble columns; such relationships are generally of the following form:

$$k_La \; \alpha \; pU_{sg}^{q} \qquad (8)$$

where p and q are constants. This implies that for comparable volumetric cell densities, k_La should depend only on U_{sg}. Also, as scale increases, providing similar volumetric cell densities are achieved similar superficial gas velocities will be needed. Research results from LONZA Biologics illustrate the above relationship. Figure 6 shows a linear dependence of oxygen mass transfer coefficient on superficial gas velocity for two geometrically similar air lift reactors (20 and 2000 L): measurements were made in PBS (phosphate buffered saline) solutions.

As stated above, for air lift fermenters, mass transfer may be linked to mixing since in general the gas flow rate is determined by the oxygen uptake rate of the cells. Additional gas e.g. nitrogen can be used to increase mixing time; this is likely to have little effect on oxygen mass transfer (bubble size and rise velocity may increase, but this and the effect it has will depend on the absolute value of the gas flow rate).

Mixing characteristics for air lift reactors have been determined by a number of researchers. For example, Verlaan et al. (1989) determined the Bodenstein number (Bo) for individual sections of an external loop bioreactor (working volume 165 L). Verlaan et al. (1989) found that Bo = 30–40 for the riser, 40–50 for the down comer and 10 for the gas disengagement section, indicating that the first two sections were behaving like plug flow with superimposed circulation whilst the gas disengagement section behaves like a well mixed reactor. Lu et al. (1994) also determined Bo in an air lift reactor. They found that for an internal loop air lift reactor the degree of mixing in the riser was higher than in the down comer and that the degree of mixing in a two phase (gas/liquid) system was better than in a three phase system (gas/liquid/solid). Fields and Slater (1983) also measured mixing in laboratory air lift reactors using tracer dispersion techniques.

Mixing times in air lift reactors are typically 3–5 times the circulation time. Mixing times for the gas and liquid phase may be different and there are reports that gas phase takes longer to circulate than the liquid phase (Lubbert et al., 1988).

Chisti and Moo Young (1987) suggested having multiple injection ports for adding, e.g. substrates, to avoid substrate starvation at certain points in the air lift. Fields and Slater (1984) also found that two injection points, as opposed to a single point, led to markedly different mixing characteristics. The two addition points considered were (i) top of riser and directly below gas engagement section and (ii) the annular down comer. The method they used was to measure respiratory quotients for injection of methanol at these two points; the respiratory quotient being substantially greater for injection into the riser section. Research by the authors has also shown that mixing times can be improved by having two injection points and also that mixing time depends on position of injection point. 95% Mixing times were measured using pulse addition of KOH in a 30 L air lift reactor with central baffle (as described above). Figure 5 shows that mixing times are shortest when injections are made simultaneously onto the surface of the down comer and just above the gas sparging point in the riser. For single point injections mixing times were lowest for injection into the riser (just above the gas sparger) and longest for injection onto the surface of the down comer.

Previously reported air lift fermerter systems for animal cell culture

The largest published scale for animal cell culture in air lift reactors is 1000 L (Birch et al., 1995) although 2000 L reactors are routinely operated at LONZA Biologics, U.K. There are several published reports of the use of air lift reactors for animal and insect cell lines (e.g. Boraston et al., 1984; Chung et al., 1993; Katinger et al., 1979). Katinger et al. (1979) suggested that air lift reactors should be suitable for animal cell culture and they carried out hydrodynamic measurements of two internal loop (concentric tube) air lift fermenters (8 and 80 L) giving results for growth of

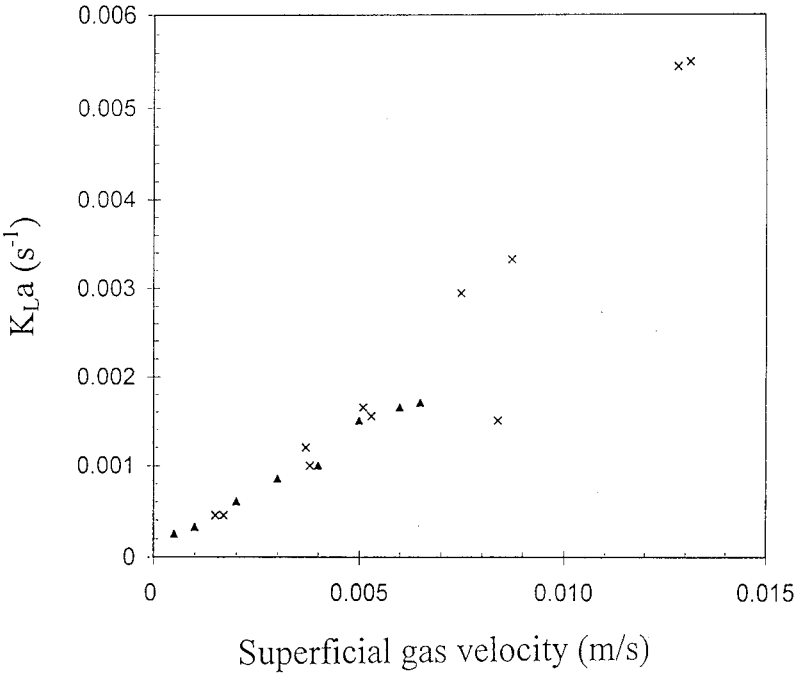

Figure 6. Oxygen mass transfer coefficient as a function of superficial gas velocity for geometrically similar air lift reactors, for PBS (phosphate buffered saline) solutions: 20 L reactor; 2000 L reactor.

BHK and Namalwa cells. Data presented in these reports includes mass transfer data, specific respiration rates and growth profiles for specific cell lines. However, there has been little consideration of design of such bioreactors at large scale.

Scale up

Despite a seemingly extensive literature on mixing and mass transfer in air lifts, it is important to remember that in most of the studies described above, superficial gas velocities were well above those used in air lifts for animal cells. Sola and Gadia (1995) and Choi (1990) both comment that comparison between literature values/correlations is difficult due to the large variation in experimental design of fermenters used in studies of air lifts and that there are currently no correlations effective for scale up. However, some recommendations for scale up can be made based on our understanding of the important scale up issues.

For air lift fermenters, scale up is generally based on geometric similarity, constant k_La and either constant superficial gas velocity, constant gas flow rate per unit volume of liquid or constant mixing time. For this type of fermenter, if dissolved oxygen level is used to control the air flow rate and air is the only gas used, there is an interdependence between mixing and mass transfer, and therefore it may not be possible to independently determine a third scaling criteria (i.e. the criteria of achieving constant k_La will determine gas flow rate per unit volume of liquid, superficial gas velocity and hence mixing time). If an additional gas (e.g. nitrogen) is used, this then allows oxygen mass transfer and mixing to be uncoupled. This may be advisable, particularly at large scale, for example, it may be beneficial to increase the nitrogen flow rate as cell numbers begin to decline to ensure good mixing in the later stages of the fermentation. Alternatively, total flow rate of gas could be held constant throughout the fermentation, with nitrogen flow varied in response to changes in air flow. This would ensure a constant mixing time throughout the fermentation. The choice of superficial gas velocity or volumetric flow rates of gas per volume of liquid as scaling criterion will be important, not only for mass transfer, but also for mixing when using systems without mechanical agitation. These two criteria cannot be met simultaneously. For geometrically similar vessels, higher superficial gas velocity will result at larger scale if gas volumetric flow rate per volume of liquid is used as a scaling criteria.

As it is becoming increasingly apparent that it is important to maintain mixing times at appropriate lev-

els it is recommended that mixing times should be kept as constant as possible when scaling up. There are several ways to reduce mixing times if required, for example:

a) improve mixing by altering positions of injection of alkali etc.
b) use multi point instead of single point addition of alkali etc.
(both these points have been discussed above).

Mixing times are also likely to be reduced to some extent by reducing the aspect ratio with scale up (i.e. relaxing geometric similarity). The two major potential problems with reducing the aspect ratio are that (i) cell damage has been shown to increase as the gas flow rate increases, which is a consequence of decreasing the aspect ratio and (ii) radial mixing may become less effective which may in itself lead to inhomogeneity in the reactor. Finally, it is worth emphasising that, as discussed above, it is important to consider dissolved carbon dioxide levels when determining how gas flow rate will change with increases in scale (increases in gas flow rate are likely to be beneficial in terms of carbon dioxide stripping).

Bubble Columns

Bubble columns have no zones without bubbles (as compared to the down comer in an air lift reactor which generally has few bubbles), but flow is compartmentalised (see Figure 7). In general this leads to poorer mixing in bubble columns as compared to air lift reactors. Chisti (1987) gives a graph showing superficial liquid velocity versus superficial gas velocity for air lift loop reactors and bubble columns (Figure 8). Air lift reactors are shown to provide much better mixing in terms of superficial liquid velocity than bubble columns. Wu and Jong (1994) measured dispersion coefficients for air lift reactors with internal draft tubes and in a bubble column (13 cm diameter and 200 cm high); dispersion coefficients in air lift reactors were much higher than those in bubble columns under the same operating conditions. Verlaan et al. (1988) emphasised that the air lift reactor concept had evolved from the bubble column and that a special feature of an air lift reactor is the circulation of liquid down the down comer which provides efficient mixing with a controlled liquid flow in the absence of mechanical agitators. In extreme cases, where flow in the riser is restricted by e.g. a small down comer, flow will become less and less like plug flow until flow in the riser itself becomes compartmentalised, as in a bubble column. Novel bubble column designs have been considered, for example, Lu et al. (1995) considered an inclined bubble column (at 45 degrees from the vertical). Liquid circulated well and exposure of cells to bursting bubbles was minimised. At an inclination of 30 degrees, the antibody titre was almost twice that for a vertical column as a result of a prolonged stationary phase. However, this type of reactor does not seem to have been developed further. As a result of poorer mixing in bubble columns as compared to air lift and agitated reactors, bubble columns are not used for large scale production from animal cell culture, however, for further information on bubble columns see review by Merchuk and Niranjan (1994).

Comparisons Between Systems

Applications described in the literature

There are only a very limited number of comparisons between performance of reactor types, particularly at large scale or for animal cell cultures. Those comparative studies that are available are mainly restricted to microbial systems. Gunzel et al. (1991) compared production of 1,3-propanediol from glycerol by *Clostridium butyricum* up to a scale of 2 m^3, using a stirred tank reactor and an air lift fermenter and found no differences in product formation. On the other hand, Shin et al. (1994) found for a sisomicin fermentation (supplemented with $MgSO_4$) that cells were in a better physiological state and produced improved antibiotic yields in the air lift reactor. This was thought to be a result of the high shear stress due to strong agitation in the stirred fermenter (1.5 L air lift fermenter and 5 L jar fermenters were used in this study). Again Byun et al. (1994) compared microaerobic production of 2,3-butanediol by *Enterobacter aerogens*, at constant oxygen transfer rate in a bubble column, an air lift reactor and a stirred tank reactor. Although differences were found at 50 L scale, at 1.5 m^3 biomass and product concentrations were similar. Ohta et al. (1995) compared neomycin production from *Streptomyces fradiae* using soybean oil as the sole carbon source in an air lift bioreactor and a stirred tank (3 L air lift, 5 L stirred tank) and found that the final product concentration in the air lift reactor was less than one half that in the stirred tank (the consumption of

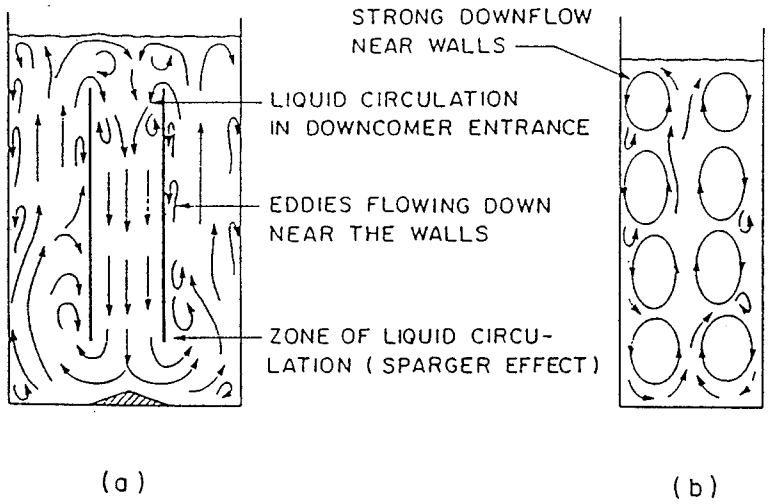

Figure 7. Flow patterns in a) an air lift fermenter with central draft tube and b) a bubble column (from Chisti and Moo-Young (1987) with permission).

soy bean oil was also significantly less in the air lift reactor). It was concluded that the soy bean oil consumption was suppressed in the air lift reactor due to a low degree of mixing. Also, in this case, shear stress due to mechanical agitation caused changes in morphology of mycelia and this was found to beneficially affect neomycin levels. Rice et al. (1993) compared large scale Sf9 insect cell growth and protein production in a stirred vessel and an air lift reactor: no differences in cell growth or protein expression were apparent between the two systems (8 L stirred tank, 6 L air lift fermenter).

Moo Young and Chisti (1988) considered bioreactors for a shear sensitive system: production of a biopolymeric material produced by *Acetobacter* (also being viscous/viscoelastic). They considered a stirred tank, airlift (draught tube) and a hybrid reactor. The stirred tank had a high shear environment and mixing was poor (due to gas channelling and stagnant zones away from the impeller) The airlift reactor provided low shear but poor circulation and hence poor mixing for the highly viscous liquid. The hybrid reactor provided gentle agitation which enhanced mixing, but kept shear rates low (1000 L vessel with H/D = 3, Ad/Ar = 1 and the draught tube split vertically into 3 sections to ensure that increased H/D did not impair mixing). k_La performance was similar (as a function of P/V) for all three reactors as were aeration capacities and biomass productivities. The quality of biopolymer produced in the hybrid reactor was superior to that in other reactors. They also compared single cell protein production and found that an air lift reactor, under otherwise identical conditions, performed better than a stirred tank (50 L: agitated tank with 2 impellers).

Comparative data for air lift and agitated reactors is very limited and generally confined to microbial systems which are not directly relevant as it is well known that for the higher mixing rates needed in microbial systems air lifts are not ideal.

Scale up

It is clear that several issues are very important in terms of scale up:

i) shear rates,
ii) mass transfer of oxygen and carbon dioxide,
iii) mixing (gas, liquid and solid phases),
iv) mode of operation,
iv) scale up experience,
vi) flexibility.

Constraints imposed by these factors mean that similar approaches may need to be taken for design of both air lift and stirred tank reactors at large scale. Agitation rates are limited for stirred tanks meaning that higher aspect ratio tanks are more favourable to achieve adequate mixing; geometries will be more similar to air lifts than is the case for microbial systems. Gas flow rates in both systems must be sufficient to prevent build up of carbon dioxide and this may lead to similar gas flow rates in both reactor types. For both reactor types, despite consistent worries about increasing the risk of contamination by having multipoint injection,

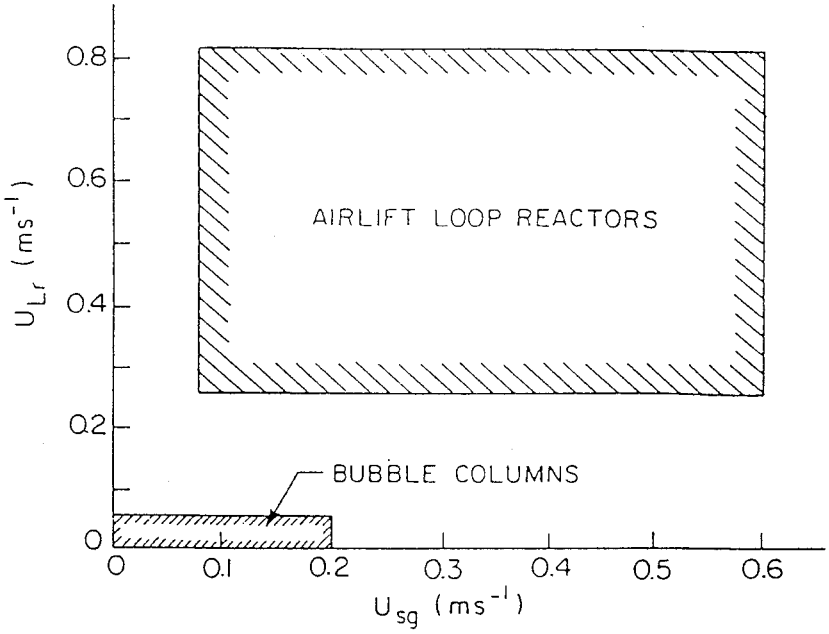

Figure 8. Operating ranges of gas and liquid velocities in bubble column and air lift reactors (from Chisti and Moo-Young (1987) with permission). U_{Lr} = superficial liquid velocity in the riser, U_{sg} = superficial gas velocity.

careful thought needs to be given to strategies for addition of alkalis, nutrients etc. It is likely that stirred tank reactors may offer more flexibility in terms of operation in perfusion mode, particularly with respect to incorporation of certain cell separation devices such as spinning filters. It seems feasible that provided these issues are considered, both stirred tanks and air lift reactors could be operated at levels significantly greater than those currently used industrially.

Mechanisms to Improve Productivity

Above, scale up of reactors is discussed as a means to increase product output, another approach is of course to improve productivities at current scales. There are a number of possible ways to increase productivity; these range from changes in reactor design, process conditions and addition or removal of compounds and include increasing biomass and specific productivity by for example nutrient supplementation or a reduction in toxic metabolites; addition of enhancers or selection of high producing cell lines (Broad et al., 1989). Many environmental factors including pH, temperature and dissolved oxygen are known to affect productivity and can therefore be manipulated to maximise productivity (Reuveny and Lazar, 1989; Wayte et al., 1997). Over the past few years there has been increasing interest in the effect of other parameters including osmolarity and use of osmoprotective agents (Oyaas et al., 1989; Schlaeger and Schumpp, 1989; Reddy and Miller, 1994; Ozturk and Palsson, 1991; Park and Lee, 1995; Kurano et al., 1990a; Oh et al., 1993; Oh et al., 1995; Oyaas et al., 1994) and lactate and, ammonia concentrations (Borys et al., 1994; Schlaeger and Schumpp, 1989; Ozturk et al., 1992; Ozturk and Palsson., 1991a, b; Miller et al., 1988; Kurano et al., 1990b), addition of glutamine (Flickinger et al., 1992) and sodium butyrate (Oh et al., 1993) on cell growth and productivity. Chang et al. (1995a, b) reported improved cell growth and antibody productivities for hybridoma cultures through nutrient enrichment and in-situ waste removal using electric fields. An improved understanding of these effects should allow optimisation of productivity in terms of these parameters, however this is not discussed further as it is outside the scope of this review paper.

Other Issues Related to Scale Up

As well as those issues discussed in detail above, there are a number of other issues which need to be considered when scaling up animal cell culture. As scale increases the number of fermenters in a given train increases and hence the generation number in the final

fermenter and the potential to select undesired variants, will increase. Also important for scale up is the choice of method for temperature control, methods for harvesting, materials of construction, and issues related to monitoring control and cleaning (fouling, equipment reuse etc. (Reisman, 1993; Hu and Peshwa, 1991)).

If anti foams were not routinely used, foaming would be encountered in most animal cell fermentations. Proteins which are surface active are generally believed to be the cause of foam formation, however, many other components e.g. lipids can contribute to foam formation and stability. Foaming can occur even at quite low levels of protein and, indeed, even in protein free media; protein secreted by cells may reach 500 mg L^{-1} which is sufficient to cause foaming, especially if gas sparging is used to control pH and DO (Bliem and Katinger, 1988b). Antifoam addition is common but is known to affect mass transfer and can also cause problems in subsequent downstream processing and may cause denaturation of some medium components. Foaming can be reduced by using pure oxygen in place of air which effectively reduces the total gas flow reaching the surface of the reactor (however this may not be feasible for reactors in which gas injection provides not only oxygen but also mixing requirements). The role of foams in cell damage is not yet understood: foams may protect cells from high shear rates associated with bubble bursting, but the cells may be damaged on prolonged adsorption to the gas liquid interface in the foam. It is currently not possible to predict the effect of scale up on foaming. Foaming will be affected by changes in gas flow rate, bubble size distribution, area of liquid surface/volume of liquid ratio, bubble residence times and liquid velocity/turbulence at the surface. All these factors must be considered during the scale up process.

Conclusions

Despite the recent increase in the number of feasible methods for producing recombinant proteins, the quantities of products and time scales of requirement mean that animal cell culture will be needed for the foreseeable future and indeed production via this route is likely to increase. A variety of approaches will be used to meet this demand. Undoubtedly there will be much continued effort aimed at achieving higher productivity on current scales through improved cell line development and media design coupled with an improved understanding of the physiological response of cells to changes in media composition.

It is inevitable however, that production will have to move to larger scale fermenters; the costs of simply operating larger numbers of smaller fermenters being prohibitive for this option. It is also likely that batch freely suspended cultures, either stirred tank or air lift reactors will continue to be used, although developments in high density perfusion cultures are likely to continue and may provide an attractive alternative mode of reactor operation.

Within the published literature it is now accepted that even the largest scales currently operated (approx. 10 000 L) do not operate optimally as a result of our inadequate understanding of key issues related to scale up of these particular cells. The crucial process engineering aspects which must be considered on scale up are mixing, mass transfer (particularly oxygen and carbon dioxide) and shear effects: these will not be independent and an understanding of the effect of each on cell physiology is paramount.

In terms of shear, it is now generally accepted that (i) many suspension cells particularly hybridomas are not as shear sensitive as first thought and can tolerate relatively high levels of agitation; (ii) most cell death is caused by bubble bursting in stirred vessels; (iii) cell death associated with bubbles is largely overcome by the use of protective polymers; (iv) shear will have effects other than simply cell death and (v) there is a critical impeller speed (albeit cell line dependent) as a result of the damaging effects of sparging combined with agitation. There are however, several areas which still need to be addressed including the effect of shear on cell growth, physiology, product expression and protein synthesis; improved understanding of the mechanisms by which cells adapt to a high mechanical stress environment and whether shear effects are a result of intensity or frequency of shear.

It is also clear that the importance of scaling on mixing time has been underestimated particularly at large scale. If inhomogeneities exist within the fermenter, cells will be exposed to fluctuations in e.g. pH, DOT, and dissolved carbon dioxide concentration. Since mixing time generally increases with scale, while kinetic time constants generally remain constant it is essential if we are to achieve successful scale up that we determine the relevant kinetic time constants and compare these to mixing times. Here scale down must play an increasing role, with geometric similarity being relaxed. Despite the fact that design guidelines developed for microbial fermentations were developed

for processes with significantly higher aeration rates and higher power inputs than those used for animal cell cultures, such guidelines continue to be used; but are likely to be far from optimal.

For large scale animal cell culture in stirred tanks the most important parameters are likely to be impeller design, number of impellers, sparger design and aspect ratio. Many modifications of the marine impeller have been proposed to provide more efficient mixing at lower impeller tip speed, as it is now accepted that tip speed has a critical effect in terms of shear. This limit on impeller speed leads to a balance between impeller diameter and speed and hence, if there is to be a reasonable value of ratio of tank diameter/impeller diameter, to a limit on tank diameter, which in turn results in the need to consider multiple impellers and higher aspect ratios.

The aspect ratio of vessels used for animal cell culture is a much overlooked design parameter: it generally lying between 1:1 and 3:1, as for microbial vessels. These values are not based on a fundamental consideration of the effect of aspect ratio on the various phenomenon occurring during an animal cell fermentation and are not necessarily optimal. It may be necessary to move to taller vessels, due to the restriction on impeller and hence tank diameter (as mentioned above). However, there are disadvantages of taller columns including an increase in the number of impellers needed which may lead to compartmentalisation of the fluid and subsequent difficulties in pH measurement, control etc. However, it is already acknowledged that such problems exist at current scales and there is therefore a need to change current injection and sampling strategies. Firstly since the longest mixing times are found for addition at or near the surface and the shortest mixing times for addition into the most turbulent region; addition points should be modified accordingly if mixing is it be optimal, and also for multiple impeller fermenters additions should be made into each impeller region, if mixing times are to be minimised

For air lift fermenters, if dissolved oxygen level is used to control the air flow rate, which is generally the case, there is an interdependence between mixing and mass transfer. To date, the approach to mass transfer in animal cell cultures has been very empirical and there has certainly been little attempt to consider mass transfer and mixing in an integrated manner; this needs to be addressed if optimal designs are to be achieved particularly for this type of reactor. This has important implications for operation of air lift fermenters since gas flow rates generally vary depending on the stage of the fermentation: it may be more appropriate to decouple mixing and mass transfer by for example having constant gas flow rates (determined by mixing time requirement), with the nitrogen flow rate adjusted in line with air flow rates to maintain overall gas flow rate constant. Mixing times for additions of alkalis, feeds etc. can also be improved in air lift fermenters by making additions to the most turbulent region rather than the surface of the fermenter, as discussed above for stirred tanks.

It is difficult to compare stirred and air lift fermenter systems but it should be remembered that animal cell cultures in stirred tanks are typically operated in the flooding regime, where gas dispersion is poor; therefore it is unlikely that differences in mixing are as great for animal cell culture as have been shown for microbial fermenters operating at much higher energy inputs.

There has been little attempt to couple circulation mixing models with reaction kinetics as has been undertaken for microbial systems and this has to be an area for future development. The use of for example computational fluid dynamics for prediction of flow patterns in air lift fermenters has been proposed (Lapin et al., 1996), and similar approaches have been proposed for stirred tanks. Such models could be very useful in determining the relevant time scales of mixing at large scale. It may then be possible to test at a small scale the effect of such mixing times on physiology of the cells. It will only be through coupling of engineering variables to physiological response of the cells and appropriate scale down experiments that successful scale up will be achieved.

Acknowledgements

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Notation

a	interfacial area per unit volume of liquid	m^{-1}
A_d	area of downcomer	m
A_r	area of riser	m
Bo	Bodenstein number	
D	tank diameter	m
D_1	axial diffusivity	$m^2 s^{-1}$
d_b	bubble diameter	m
d_{vs}	Sauter mean diameter = $\sum n_i\, d_i^3 / \sum n_i\, d_i^2$ (for n_i bubbles of diameter d_i)	m
d_o	orifice diameter	m
C	dissolved gas concentration	kg m^{-3}
C^*	saturation gas concentration	kg m^{-3}
F	gas flow rate	$m^3\ s^{-1}$
Fl	Flow number = $Q_G/N\ D^3$	
g	acceleration due to gravity	m s^{-2}
H	reactor height	m
k_d	death rate	s^{-1}
k_La	overall mass transfer coefficient	s^{-1}
L	characteristic length	m
N	impeller speed	revs s^{-1}
N_p	Power number = $P/\rho\ N^3\ D^3$	
P	power	W
Pe	Peclet number	
Q_G	gas volumetric flow rate	$m^3\ s^{-1}$
v	average velocity	m s^{-1}
v_o	gas velocity at the sparger	m s^{-1}
V	volume	m^3
T	tank diameter	m
U_{Lr}	superficial liquid velocity in the riser	m s^{-1}
U_s, U_{sg}	superficial gas velocity	m s^{-1}
X'	hypothetical killing volume	
σ	surface tension	Nm^{-1}
ρ	density	kg m^{-3}

Subscripts

F	flooding
CD	complete dispersion

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