

ANCHORAGE DEPENDENT ANIMAL CELL CULTURE IN PACKED BEDS WITH AIRLIFT DRIVEN LIQUID CIRCULATION:

A Theoretical Analysis of Oxygen Transfer and Comparison with Stirred Tank Microcarrier Culture System

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Airlift-packed bed combination reactors are evaluated for large scale culture of anchorage dependent animal cells. Based on the analyses, for cells with specific oxygen consumption rates in the range $0.05\text{--}0.5 \times 10^{-9}$ mmol O₂/(cell h), a 1.5 m³ airlift-packed bed reactor containing a 4 m deep bed of 3×10^{-3} m diameter glass beads is expected to support a monolayer of cells over the entire surface area of the packing while ensuring above critical oxygen concentration in the bed, so long as sufficient liquid circulation is maintained through the bed. The satisfactory level of liquid circulation depends on the oxygen demand of the cells, but is always within the capability of the airlift drive. Such an airlift-packed bed reactor is predicted to support 2.76-fold the cell concentration that could be achieved in a best case stirred tank microcarrier culture system having the same volume as the airlift device.

Keywords: animal cell culture; anchorage dependent cell culture; airlift bioreactors; packed beds; oxygen transfer

INTRODUCTION

Anchorage dependent animal cells are commonly cultured on spherical microcarriers ($200\text{--}400 \times 10^{-6}$ m diameter) suspended in culture fluid in stirred tank bioreactors similar to the ones described for non-anchorage dependent suspension culture¹. The microcarriers are typically made of glass or polystyrene. Some commercially available examples are the glass microcarriers produced by Sigma Chemical Company (St. Louis, MO), the glass-coated polystyrene carriers supplied by SoloHill Engineering, Inc. (Ann Arbor, MI) and the radiation sterilized polystyrene NUNC microcarriers marketed by Cole-Parmer Instrument Company (Chicago, IL). Concentrations of microcarriers are kept low to minimize damage to cells due to bead-to-bead collisions. The range of concentrations typically used in stirred tanks is $1\text{--}5 \text{ kg m}^{-3}$, as noted by Reuveny², but the most commonly used concentration is 3 kg m^{-3} . Higher solids loadings, up to 30 kg m^{-3} , have occasionally been employed.

Although more expensive carriers made of softer materials such as collagen and dextran are available to potentially reduce collision associated damage to cells, these carriers are usually not reusable and have not gained industrial acceptance. Interactions between the impeller and the beads are other causes of damage to cells in these systems. In addition, the reactor is usually

oxygenated by bubbling air (or other gas mixtures) through the microcarrier suspension and interactions between gas bubbles and the cells on the surface of beads cause further damage to cells. In view of these shortcomings, an alternative method of culturing anchorage dependent cells has been investigated. This scheme utilizes packed beds of relatively large ($2\text{--}4 \times 10^{-3}$ m) glass or ceramic spheres irrigated by continuous, recycle flow of separately oxygenated, pH-adjusted and temperature-controlled culture medium³. The cells grow on the beads in a monolayer. This scheme of cell culture is best implemented in airlift reactors in which the packed bed is contained in the downcomer region and the aerated riser drives the cell-free culture fluid through the bed without a pump (Figure 1). This airlift-packed bed combination has been successfully tested up to 0.1^3 for the production of foot-and-mouth disease vaccine⁴ using cells supported on 3×10^{-3} m beads⁵. Similar reactors have been used to grow hybridoma cells for the production of monoclonal antibodies⁶.

The riser of the hybrid airlift reactor shown in Figure 1 offers a better mixed zone than the packed section; the pH, temperature and the dissolved oxygen levels of the recirculating fluid can be easily controlled in the riser. Hence, the hybrid design overcomes some of the shortcomings of packed bed bioreactors. Airlift-driven liquid circulation is an important feature of these systems and the ability to design these reactors with predictable

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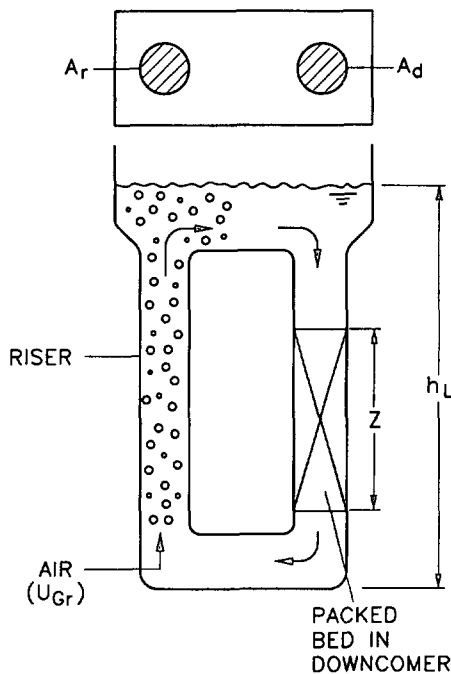


Figure 1. Airlift-packed bed hybrid reactor.

performance depends on the ability to predict the rate of circulatory flow⁷. The mechanics of the airlift-induced circulation have been analysed and, based on the proposed design methods, industrial-scale airlift-packed bed devices have been determined to be capable of generating the necessary liquid flow for processes which require little or no oxygen⁷. Here, we combine the previously reported⁷ analysis of liquid circulation, with known oxygen consumption characteristics of animal cells to demonstrate the applicability of airlift-packed bed devices to large scale culture of anchorage dependent animal cells. In addition, productivities of the conventional microcarrier stirred tank culture system and the macrocarrier airlift-packed bed device are compared.

THEORY

As the cell free culture fluid, initially saturated with oxygen, percolates through the packed bed, oxygen is depleted axially down the bed due to consumption by the immobilized cells. The depth of packing up to which there is no oxygen starvation of the cells, the critical depth (Z_c), can be calculated by an oxygen balance on the bed. Thus,

Oxygen flow into bed—oxygen flow out of bed—
oxygen consumption in the bed = 0,

or

$$QC_i - QC_{crit} - q = 0, \quad (1)$$

where Q is the volume flow rate through the downcomer containing the packed bed, C_i is the oxygen concentration in the fluid flowing into the bed, C_{crit} is the critical oxygen concentration for animal cells and q is the total oxygen consumption in the bed. For animal cells, as for microbial cells, oxygen consumption is zero order in oxygen concentration for concentrations greater than or

equal to the critical oxygen concentration, C_{crit} ⁸. Thus, oxygen consumption is independent of axial location in the bed. The total oxygen consumption, q , can be calculated with the equation

$$q = NR, \quad (2)$$

where N is the number of cells in the bed and R is the oxygen consumption rate per cell. Assuming, conservatively, that a monolayer of cells develops on the entire surface area of the packing, we have

$$N = \frac{\text{Surface area of the packing}}{\text{Projected area of cell}}, \quad (3)$$

or

$$N = \frac{4SA_d Z_c (1 - \phi)}{\pi d_c^2}, \quad (4)$$

where A_d is the cross-sectional area of the downcomer, ϕ is the void fraction of the bed and d_c is the diameter of the cells. In equation (4), S is the surface-to-volume ratio of the packing; for spheres, $S = 6/d_p$, where d_p is the diameter of the particle.

Substitution of equations (2) and (4) in equation (1), followed by rearrangement leads to

$$Z_c = \frac{Q(C_i - C_{crit})\pi d_c^2}{4SRA_d(1 - \phi)}. \quad (5)$$

Because the flow rate of liquid, its superficial velocity and the cross-sectional area of the downcomer are related,

$$Q = U_{Ld} A_d, \quad (6)$$

equation (5) is modified to

$$Z_c = \frac{U_{Ld}(C_i - C_{crit})\pi d_c^2}{4SR(1 - \phi)}, \quad (7)$$

Equation (7) expresses the critical depth of packing in terms of the superficial velocity of liquid in the downcomer (U_{Ld}) and the specific oxygen consumption rate of the cells (R).

RESULTS AND DISCUSSION

For a large scale (1.5 m³) airlift-packed bed reactor containing a 4 m deep bed of 3×10^{-3} m spherical particles, the superficial liquid velocity in the downcomer (U_{Ld}) was calculated using the procedure described by Chisti and Moo-Young⁷. The geometric details of the reactor, the properties of the fluid and other parameters used in calculating the flow rate are given in Table 1. For a superficial riser gas velocity range of 0–0.12 ms⁻¹, the calculated superficial liquid velocity varied over 0–0.014 ms⁻¹. These velocities were used in equation (7) to calculate the critical depth (Z_c) of packing. Figure 2 shows a plot of critical depth against liquid flow velocity for three different oxygen consumption rates in the range 0.05–0.5 mmol O₂/(cell h). This is the range of specific oxygen consumption rates for most cultured mammalian cells as noted by Fleishaker and Sinskey⁹. In addition, the calculated critical depths are shown in Figure 2 for the commonly used anchorage dependent CHO cells for which Griffiths¹⁰ reported an specific oxygen consumption rate of

Table 1. Parameters used for calculation of the circulation velocity.

Riser-downcomer cross-sectional area ratio, $A_r/A_d(-)$	1.0
Diameter of riser, d_r (m)	0.4
Volume of reactor V (m ³)	1.5
Diameter of spheres, d_p (m)	0.003
Surface-to-volume ratio of particles, S (m ⁻¹)	2000
Void fraction of the bed, $\phi(-)$	0.4
Depth of bed, Z (m)	4.0
Frictional loss coefficient for the top zone, $K_T(-)$	8.0
Frictional loss coefficient for the bottom zone, $K_B(-)$	8.0
Height of gas-liquid dispersion, H_D (m)	6.0
Density of culture fluid, ρ_L (kg·m ⁻³)	1000
Viscosity of culture fluid, μ_L (Pa·s)	10 ⁻³
Superficial riser gas velocity range, U_{Gr} (ms ⁻¹)	0-0.12
Gravitational acceleration, g (ms ⁻²)	9.81

1.5×10^{-10} mmol O₂/(cell h). For calculation of Z_c using equation (7), the diameter of cells was assumed to be 20×10^{-6} m which is typical¹¹ for lymphocytes and many other animal cells. The inlet oxygen concentration, C_i , in the bed was assumed to be the air saturation value for aqueous media at 203.13 mmol m⁻³ (6.5 ppm). Considering the low flow rates and, consequently, long residence times of the liquid in the air-sparged riser, the liquid entering the downcomer will indeed be oxygen saturated as can be shown using the methods described by Chisti¹². The critical oxygen concentrations for animal cells are quite low, and a typical value of 0.5% of air saturation determined by Miller *et al.*⁸ was used in this analysis.

As shown in Figure 2, when, for a given liquid flow ($U_{Ld} \leq 0.014$ ms⁻¹) and oxygen consumption rate (R), the calculated critical depth (Z_c) is greater than or equal to the actual depth of 4 m, the airlift-packed bed combination can be successfully used for culturing the cells. Further, even for cells for which the oxygen demand is at the upper limit of 0.5×10^{-9} mmol/(cell h), sufficiently high rates of flow and liquid can be

generated to maintain a viable cell population throughout the packing. The aeration rates required (Figure 2) to maintain circulation of the medium are not expected to cause foaming in the increasingly common protein-free media. When serum containing media are employed, any foam formation may be controlled using chemical antifoam additives or mechanical foam breakers as recently described for industrial cell culture reactors¹. High rate aeration of bicarbonate buffered media can lead to unwanted pH changes; however, this problem is easily overcome by CO₂-supplementation of the aeration gas typically at 5% (by volume) level¹.

In addition to oxygen supply considerations, another important factor needed in establishing the merit of the airlift-packed bed combination relative to the conventional stirred tank culture system is the final cell concentration attainable in these devices for otherwise identical conditions. A comparison of these systems in terms of production of cells follows.

Airlift-packed Bed vs. Stirred Tank

For the 1.5 m³ airlift-packed bed combination detailed in Table 1, the total number of cells which can be physically supported in the reactor can be calculated using equation (4). For 20×10^{-6} m diameter cells the total number is 1.92×10^{12} cells, or 1.28×10^6 cells/ml of reactor volume which includes volume of the liquid and the packing.

For stirred tank microcarrier culture the typical diameter of microcarriers is 200×10^{-6} m and the density of the spherical particles is 1030 kg m⁻³. The most commonly used concentration of microcarriers (C_c) is 3 kg m⁻³, but the typical range is 1–5 kg m⁻³ as noted by Reuveny². We will use the higher limit C_c -value of 5 kg m⁻³ for the best case stirred tank system. For the same cells as were employed in the airlift, the total number of cells which can be accommodated on the available surface area as a monolayer is

$$N = \frac{4C_cVS}{\rho_p d_c^2}, \quad (8)$$

where ρ_p is the density of carriers and S is their surface-to-volume ratio. The total number of cells (N) works out to 6.95×10^{11} cells. Hence, the airlift-packed bed system can support 2.76-fold the cell concentration that may be supported in the stirred tank. The high productivity of the airlift-packed bed results from the high packing density of the macrocarriers in that system. These results can be attained without motors, agitators, gearboxes and mechanical seals. Moreover, unlike in the stirred tank, in the airlift device there is no contact between the gas phase and the cells during most of the operation because the gas leaves the liquid prior to its entering the downcomer¹³. Operations such as trypsinization of cells from stationary carriers are simpler than in stirred tanks. The carriers can be cleaned-in-place with the reactor, sterilized and reinoculated.

CONCLUSIONS

For most anchorage dependent animal cells for which oxygen demands are $\leq 0.5 \times 10^{-9}$ mmol O₂/(cell H) (i.e.,

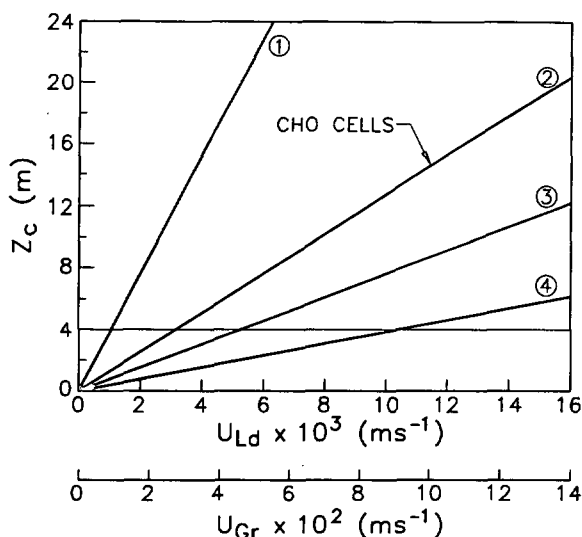


Figure 2. Critical depth of packing (Z_c) vs superficial liquid velocity in the downcomer for various specific oxygen consumption rates (mmol O₂/(cell h)): (1) 5.0×10^{-11} ; (2) 1.5×10^{-10} ; (3) 2.5×10^{-10} ; (4) 5.0×10^{-10} . The superficial air velocities required to generate a given level of liquid flow in the downcomer are also shown.

vast majority of cell lines) large-scale culture in airlift-packed bed combination reactors with beds up to 4 m deep is feasible even when the bed is made up of relatively small glass beads 3×10^{-3} m in diameter. Deeper beds of somewhat larger particles may also be successfully used. Because of the high packing density in the beds, these reactors can sustain more than twice as large a cell population as would be possible to culture in a stirred tank microcarrier culture system of equal volume. In addition, the retention of the packing in the reactor can ease cell removal by trypsinization; the packing can be cleaned and sterilized in-place and reused. During process start-up, slow, percolating flow through the bed may be used to favour attachment of cells to the carriers, as has been shown in small scale experiments. Other significant advantages of airlift-packed bed devices are an absence of contact between cells and air bubbles during most of the operation and avoidance of collisions between carriers or carriers and impeller.

NOMENCLATURE

A_d	cross-sectional area of downcomer, m^2
A_r	cross-sectional area of riser, m^2
C_c	concentration of microcarriers, $kg\ m^{-3}$
C_{crit}	critical concentration of oxygen, $mmol\ m^{-3}$
C_i	oxygen concentration at inlet of bed, $mmol\ m^{-3}$
d_c	diameter of cell, m
d_p	diameter of particle, m
d_r	diameter of the riser, m
g	gravitational acceleration, ms^{-2}
h_D	height of gas-liquid dispersion, m
h_L	height of gas-free liquid, m
K_B	frictional loss coefficient for the bottom
K_T	frictional loss coefficient for the top
N	total number of cells in reactor
Q	volume flow rate, $m^3\ s^{-1}$
q	total oxygen consumption in the bed, $mmol\ h^{-1}$
R	specific oxygen consumption rate of cells, $mmol/(cell\ h)$ or $mmol/(cell\ s)$
S	surface area per unit volume of particle, m^{-1}
U_{Gr}	superficial gas velocity in the riser, ms^{-1}
U_{Ld}	superficial liquid velocity in the downcomer, ms^{-1}
V	volume of reactor, m^3
Z	depth of the packed bed, m
Z_c	critical depth of the packed bed, m

μ_L	viscosity of the liquid, Pa s
ρ_L	density of the liquid, $kg\ m^{-3}$
ρ_p	density of the particles, $kg\ m^{-3}$
ϕ	void fraction of the packed bed

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ADDRESS

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