Oxygen gradients in animal-cell bioreactors

J. Tramper
Food and Bioprocess Engineering Group, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Key words: Air lift reactor, bubble column, bioreactor, oxygen gradients, scale-up, stirred vessel

Abstract

An estimation is made of oxygen gradients in animal-cell bioreactors, using straightforward engineering calculations. Three types of bioreactor are considered: stirred vessel, bubble column and air lift, of sizes between 0.01 and 10 m³. First, the gradient is estimated in the stagnant layer surrounding a cell (15 µm), a microcarrier (185 µm) with 300 cells attached to it, a macroporous support (1.25 mm) containing 185,000 cells and one (6 mm) containing 4,250 million cells. It is assumed that oxygen consumption is $10^{-16}$ mole O₂ cell⁻¹ s⁻¹, while mass transfer coefficients are obtained from Sherwood relations. Circulation and liquid-retention times of the bioreactors are compared with the oxygen-exhaust times of suspensions with $10^{12}$, $10^{13}$ and $10^{14}$ cells/m³ to estimate if oxygen gradients are likely to exist in the bulk-liquid phase. Finally, the gradient in the liquid film surrounding air bubbles is estimated using $k_tA$-values obtained from empirical correlations. It is clear from all these estimations that in many situations severe gradients can be expected. The question remains, however, whether gradients should be avoided as much as possible, or may be tolerated to a certain extent or even created on purpose because of possible beneficial effects.

Introduction

It is well-known that in fermenters gradients may develop. In particular during large-scale microbial fermentation, oxygen gradients may be severe, due to the poor solubility of oxygen in aqueous media. Although, respiration rates of animal cells are relatively low, oxygen gradients are also likely to occur in animal-cell bioreactors. It is unknown, however, whether such gradients are always deleterious or positive effects might occur under certain conditions. In this paper an estimation is made of oxygen gradients which may be expected in various bioreactors.

The stirred vessel is the “workhorse” of biotechnology; also for animal-cell cultures. In the latter case 10 m³ is about the largest size in use and therefore this size is taken as the example to calculate gradients. As a reference a 0.01 m³ vessel is analysed as well, corresponding to the largest bench-scale bioreactors. Apart from stirred vessels, air lift fermenters are used to a limited extent; therefore, calculations are also given for these loop bioreactors. The author is unaware of bubble columns being used beyond the scale of 0.01 m³ for animal cells, but in this case study, for the sake of comparison, estimations of gradients in this type of bioreactor is performed on 10 m³ scale as well.

Theory

Figure 1 illustrates the process of oxygen transfer by i) molecular diffusion from gas bubbles to the bulk-liquid phase, ii) mixing in the latter phase by convection, and iii) again molecular diffusion from bulk-liquid phase to the surface of spherical particles through the stagnant layer surrounding them. A particle in this study is either the cell itself, a microcarrier with cells attached to the surface, or a macroporous support containing immobilized cells. In the latter case molecular diffusion in the porous support is involved as well, as it is in micro-colonies of cells if these exist (Wijffels et al., 1994). Assuming that heterogeneous cell growth
due to diffusion limitation, will eventually cause most of the cells to grow into a thin layer, just below the surface of the macroporous particles. An estimation of the number of cells per particle may be obtained from a mass balance, incorporating oxygen diffusion and consumption.

The equation describing molecular diffusion through the stagnant layer of oxygen-consuming particles reads for the steady state:

\[ \text{OTR}' = k_z \cdot A \cdot \Delta C = k_z \cdot A \cdot (C_{\text{bulk}} - C_{\text{surface}}) \]

\[ \approx \text{OUR}' \quad (1) \]

where OTR' (mole particle\(^{-1}\) s\(^{-1}\)) is the oxygen transfer rate through the stagnant layer of one particle, \(k_z\) (m \(s^{-1}\)) the oxygen transfer coefficient, \(A\) (m\(^2\) particle\(^{-1}\)) the surface area of a particle, \(\Delta C\) (mole m\(^{-3}\)) the oxygen concentration gradient over the stagnant layer, \(C_{\text{bulk}}\) and \(C_{\text{surface}}\) (mole m\(^{-3}\)) the oxygen concentration in the bulk liquid and at the surface of the particle, respectively, and OUR' (mole particle\(^{-1}\) s\(^{-1}\)) the oxygen consumption rate per particle, which in the steady state will equal the oxygen transfer rate. To calculate \(\Delta C\), the diameter of the spherical particle (to calculate \(A\)), the oxygen transfer coefficient, the number of cells per particle and the oxygen consumption rate per cell should therefore be known. For the latter the convenient value of \(10^{-16}\) mole O\(_2\) cell\(^{-1}\) s\(^{-1}\) is taken, which is a rough average of the values we usually find for insect cells (Spodoptera frugiperda) and at the middle/high side of the values found in literature (e.g. Spier and Griffiths, 1984; Aunins and Henzler, 1993) for other animal cells. This value, multiplied by the number of cells per particle yields the particle oxygen consumption rate, assuming that all cells consume oxygen at the same rate. Oxygen transfer coefficients are estimated from Sherwood-type relationships. For bubble columns and air lifts the calculation is performed at two air-flow rates: rather low and average. High air flows are not considered, since they are unlikely to be used in animal-cell cultures, because of shear sensitivity and foaming.

The bulk-liquid phase circulates through the bioreactor. In general, aeration occurs only locally and oxygen will therefore be exhausted in the non-aerated parts. Comparison of the times spent in the latter parts with the exhaust times calculated for three cell densities (one average, one rather high and one very high) will give an idea of the gradients which may occur in the bulk-liquid phase. Liquid flow rates and empirical correlations for circulation times are used in these estimates. Gradients are also likely to occur in the liquid stagnant layer surrounding the air bubbles blown through the aerated bioreactors. Due to the high partition coefficient and the high gas diffusivities, this is not the case in the stagnant layer at the gas side. Air-bubble size and hold-up are poorly defined and thus the calculation of the specific interfacial area \(a\) (m\(^2\) m\(^{-3}\)) is rather inaccurate. Therefore, empirical correlations for the product of the oxygen transfer coefficient \(k_z\) and specific interfacial area \(a\) have been used here to estimate the gradients in the stagnant liquid layer sur-