CO₂ in large-scale and high-density CHO cell perfusion culture

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Received 12 August 1996

Key words: oxygen, carbon dioxide, CHO cell culture, inhibition, mass transfer coefficient, bubbles

Abstract

Productivity in a CHO perfusion culture reactor was maximized when pCO₂ was maintained in the range of 30-76 mm Hg. Higher levels of pCO₂ (> 105 mm Hg) resulted in CHO cell growth inhibition and dramatic reduction in productivity. We measured the oxygen utilization and CO₂ production rates for CHO cells in perfusion culture at $5.55 \times 10^{-17}$ mol cell⁻¹ sec⁻¹ and $5.36 \times 10^{-17}$ mol cell⁻¹ sec⁻¹ respectively. A simple method to directly measure the mass transfer coefficients for oxygen and carbon dioxide was also developed. For a 500 L bioreactor using pure oxygen sparge at 0.002 VVM from a microporous frit sparger, the overall apparent transfer rates ($k_{La}+k_{A}A$) for oxygen and carbon dioxide were 0.07264 min⁻¹ and 0.002962 min⁻¹ respectively. Thus, while a very low flow rate of pure oxygen microbubbles would be adequate to meet oxygen supply requirements for up to $2.1 \times 10^7$ cells/mL, the low CO₂ removal efficiency would limit culture density to only $2.4 \times 10^6$ cells/mL. An additional model was developed to predict the effect of bubble size on oxygen and CO₂ transfer rates. If pure oxygen is used in both the headspace and sparge, then the sparging rate can be minimized by the use of bubbles in the size range of 2–3 mm. For bubbles in this size range, the ratio of oxygen supply to carbon dioxide removal rates is matched to the ratio of metabolic oxygen utilization and carbon dioxide generation rates. Using this strategy in the 500 L reactor, we predict that dissolved oxygen and CO₂ levels can be maintained in the range to support maximum productivity (40% DO, 76 mm Hg pCO₂) for a culture at $10^7$ cells/mL, and with a minimum sparge rate of 0.006 vessel volumes per minute.

Nomenclature

- $A$ = volumetric agitated gas-liquid interfacial area at the top of the liquid, 1/m
- $B$ = cell broth bleeding rate from the vessel, L/min
- $CER$ = carbon dioxide evolution rate in the bioreactor, mol/min
- $[CO₂]$ = dissolved CO₂ concentration in liquid, M
- $[CO₂]^*$ = CO₂ concentration in equilibrium with sparger gas, M
- $[CO₂]^{**}$ = CO₂ concentration in equilibrium with headspace gas, M
- $CO₂(l)$ = dissolved carbon dioxide molecule in water
- $[C_T]$ = total carbonic species concentration in bioreactor medium, M
- $[C_T]_F$ = total carbonic species concentration in feed medium, M
- $D$ = bioreactor diameter, m
- $D_I$ = impeller diameter, m
- $D_b$ = the initial delivered bubble diameter, m
- $F$ = fresh medium feeding rate, L/min
- $H_L$ = liquid height in the vessel, m
- $k_A$ = carbon dioxide transfer coefficient at liquid surface, m/min
- $k_A^O$ = oxygen transfer coefficient at liquid surface, m/min

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Introduction

Previously published analyses of sparging strategies for animal cell culture bioreactors have focused on the requirement for adequate oxygen transfer rate to support high cell density culture, while minimizing cell damage through minimizing bursting bubbles at the liquid surface (Handa-Corrigan, 1989; Bavarian, 1991). One such approach to this end has been the use of ‘microbubbles’ (Db ≤0.1 mm) of pure oxygen to achieve high oxygen transfer rate (OTR) at low gas flow rate (<0.01 vvm) (Ingham, 1984). Microbubbles are generated through a sintered porous tube frit sparger with pore sizes in the 2–10 micron range. This approach results in high bubble interfacial area and enables high oxygen transfer rates. The inclusion of surfactants such as pluronic polyol F68, at less than 0.1% w/v, is standard in cell culture media and serves to minimize cell attachment to bubbles, thereby further reducing cell damage at the liquid surface (Murhammer, 1988).

Sparge systems employing porous sintered frits have been used for oxygen supply in small-scale animal cell perfusion culture reactors (Weiss, 1985) and have also been described for scale-up of cell culture reactors (Radlett, 1972). The drive towards increasing the total oxygen transfer rate to support high density cultures has led to methods where pure oxygen has been flushed through the headspace compartment of the reactor in addition to using oxygen in the sparger (Backer, 1988).

An important consequence of the small oxygen bubble approach is the low removal rate of CO2 from the bioreactor medium by bubble transport. The carbonic system is made up of the CO2(g) in mass transfer with dissolved carbon dioxide CO2(1) and the bicarbonate ion. In this article we refer to the concentration of CO2(1) as [CO2]. We refer to the combined dissolved species (CO2 and bicarbonate) as CT, the concentration of combined species as [CT], and the partial pressure of carbon dioxide gas in equilibrium with CO2(1) is referred to as pCO2 (mm Hg). In the bioreactor, the CO2 removal rate across the agitated liquid surface is much lower than the rate of transfer of O2 into solution by microbubbles. As cell density increases, cellular generation of CO2 combined with the bicarbonate entering the reactor in the culture medium feed can exceed the removal rate from the culture and [CT] will increase in the culture broth. As the liquid height increases with increasing reactor scale, we predicted that this problem will become more pro-

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k_{L} = \text{volumetric CO}_2 \text{ transfer coefficient, } \text{L/min}
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k^{O}_{L} = \text{volumetric oxygen transfer coefficient, } \text{L/min}
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k_{L} = \text{liquid-film CO}_2 \text{ transfer coefficient at any section, } \text{m/min}. \text{ It is predicted by Boussinesq equation (Aiba, 1973) for micro bubbles}
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Ka_{1} = \text{Equilibrium constant for HEPES acid dissociation, } 4.9 \times 10^{-8} \text{ M (Perrin, 1974)}
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Ka_{2} = \text{Equilibrium constant for CO}_2 \text{ hydration, } 5.2 \times 10^{-7} \text{ M (Perrin, 1974)}
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Ka_{3} = \text{Equilibrium constant for overall chemical reaction involved HEPES and CO}_2, 10.61
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[O_2] = \text{dissolved oxygen concentration in liquid, M}
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OTR = \text{oxygen transfer rate, M/min}
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OUR = \text{oxygen uptake rate of the cells, M/min}
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P = \text{medium withdraw rate from the settler, L/min}
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pCO_2 = \text{partial pressure of CO}_2 \text{ in the outlet of headsweeping gas, mm Hg}
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pCO_2 = \text{partial pressure of CO}_2 \text{ in gas phase in equilibrium with [CO}_2], \text{ mm Hg}
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P_g = \text{pressure of headsweeping gas, atm}
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pO_2 = \text{partial pressure of oxygen in gas phase in equilibrium with [O}_2], \text{ mm Hg}
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Q = \text{gas flow rate in the headspace of the bioreactor, m}^3/\text{min}
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Q_s = \text{sparger gas flow rate m}^3/\text{sec}
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q = \text{specific CO}_2 \text{ evolution rate, mol/cell/min}
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R = \text{gas law constant, } 8.2 \times 10^{-5}, \text{ m}^3\text{atm/mol/K}
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S_b = \text{surface area of a single bubble, m}^2
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T = \text{temperature of headsweep gas, K}
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t = \text{time, min}
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V = \text{liquid volume in bioreactor, L}
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V_b = \text{bubble rising velocity, m/sec}
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X = \text{viable cell density in bioreactor, cells/L}
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