Dissolved carbon dioxide accumulation in a large scale and high density production of TGF/β receptor with baculovirus infected Sf-9 cells

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Abstract

Production of a TGF/β receptor with high density baculovirus infected Sf-9 cells (7 x 10⁶ cells ml⁻¹) served as a test run for a retrofitted 150 L microbial fermentor. The entire 110 L batch run was performed in serum free medium, with an addition of a concentrated amino acid and yeastolate mixture at the time of infection. This addition strategy has been proven effective at a small scale by enabling cultures to maintain maximum product yield. In the bioreactor however, while cellular growth was comparable to that of the smaller scale control, TGF/β receptor production was three fold below the control. To minimize the mechanical stress, low flow rate of pure oxygen was used to control the dissolved oxygen at 40%. As a consequence, it seems that this aeration strategy involved an accumulation of dissolved carbon dioxide that in turn inhibited the protein production. A model has been developed that estimated the CO₂ partial pressure in the culture to be in the vicinity of 0.15 atm. The effect of dissolved CO₂ at this concentration has been assessed at smaller scale for TGF/β receptor and β-gal expression, in controlled atmosphere incubators.

Abbreviations: CST – cumulative sparging time (s); dpi – days post-infection (d); DCO₂/H₂O/DO₂/H₂O – CO₂/O₂ diffusivities ratio (= 0.784 at 27 °C); DO – % saturation of dissolved oxygen (%); hpi – hours post-infection (h); He – Henry coefficient (HeCO₂ = 32.1 x 10⁻³ M atm⁻¹, HeO₂ = 1.23 x 10⁻³ M atm⁻¹ at 27 °C); kL,a – volumetric liquid-side mass transfer coefficient (h⁻¹); LDH – Lactate dehydrogenase; MOI – multiplicity of infection (pfu cell⁻¹); N – agitation speed (rpm); OTR – oxygen transfer rate (mole O₂ ml⁻¹ h⁻¹); OUR – oxygen uptake rate (mole O₂ ml⁻¹ h⁻¹); p – partial pressure (atm); P – total pressure (atm); pfu – plaque forming units; q – specific consumption or production rate (mole cell⁻¹ h⁻¹); QH,OUT – headspace outlet gas flow rate; QS,NOM – nominal volumetric sparged gas flow rate (92 ml s⁻¹ at bioreactor conditions); R – ideal gas constant (82.05 ml atm mole⁻¹ K⁻¹); SRVl – molar sparging rate per unit liquid volume (mole ml⁻¹ h⁻¹); SSR – specific sparging rate (mole cell⁻¹ h⁻¹); T – temperature (C or K); Vl – culture volume (ml); VVD – volume of feed per volume of culture per day (d⁻¹); X – cell concentration (cell ml⁻¹); Y – yield coefficient.

Indexes: CO₂, O₂ – related to CO₂ or O₂, respectively; glc – glucose; H – headspace gas phase or gas/liquid interface; L – liquid phase; lact – lactate; S – sparged phase or gas/liquid interface; T – total; V – viable.

Introduction

The growing needs for large and homogeneous quantities of recombinant proteins are a strong incentive for large scale production in unit processes. Moreover, cultivating animal cells is expensive. Operating both at large scale and high cellular density permits reduc-
tion of capital and operation costs. The work presented here combined the state of the art in both large scale and high density culture of baculovirus expression vector system (BEVS) in order to attain these objectives of product homogeneity and cost reduction.

A recent contribution to the large scale culture of insect cells is the work of Junker et al. (1994) who provided helpful guidelines to retrofit microbial fermentors for animal cell culture. They also presented multiple production run data with the baculovirus/insect cell system in a 75 L bioreactor, where per cell yields were maintained up to densities between $1 \times 10^6$ and $2 \times 10^6$ Sf-9 cells ml$^{-1}$ in EX-CELL 400 medium. Brown et al. (1995) produced a recombinant membrane-bound protein with baculovirus infected Sf-9 cells in a 100 L Chemap bioreactor. A fresh medium replacement 24h before infection allowed the maintenance of maximal r-protein yield at cell densities of $3 \rightarrow 4 \times 10^6$ cells ml$^{-1}$, in EX-CELL 400 supplemented with 1% FBS.

Looking towards high density culture, many reports have shown that it is possible to maintain maximum product yield by performing medium replacement. Caron et al. (1990) maintained VP6 specific productivity by infected Sf-9 at cell densities of $5 \times 10^6$ cells ml$^{-1}$ by replacing medium 1 hour post-infection (hpi). Using the same procedure, Tom et al. (1995) maintained EGF receptor per cell yield in Sf-9 at $1 \times 10^7$ cells ml$^{-1}$ in serum free medium (SF900-II). Similar results were obtained by Lindsay and Betenbaugh (1992) and Lazarte et al. (1992). Continuous medium replacement, perfusion, can yield even higher cell densities. Jäger et al. (1994) maintained HIV-1 gag-particles, interleukin-2 and interferon-β per cell yields while perfusing up to 2.2 volume of medium per volume of culture per day (VVD) of serum-free EX-CELL 401 medium in a baculovirus infected culture of $3 \times 10^5$ Sf-21 cells ml$^{-1}$. Nguyen et al. (1993) identified glucose and yeastolate additions as sufficient to maintain high β-gal specific productivity up to $5 \times 10^6$ infected Sf-9 cells ml$^{-1}$ in EX-CELL 400. In a systematic study involving experimental factorial design, Bédard et al. (1994) concluded that the only addition of a mixture of yeastolate and amino acids to SF900-II serum-free medium was sufficient to maintain β-gal production in $7 \times 10^6$ infected Sf-9 cells ml$^{-1}$. This last finding has been confirmed by many other protein productions in this laboratory.

In this report, the results of a 110 L test run in a 150 L retrofitted Chemap microbial fermentor for the production of the extracellular domain of the type 2 recombinant TGFβ receptor (rTGFβ-RII-ED) in baculovirus infected Sf-9 cells at $7 \times 10^6$ cells ml$^{-1}$ will be presented and discussed. The cell growth and r-protein production have been compared to smaller scale control culture and key-parameters have been thoroughly analyzed: oxygen transfer related parameters, glucose, lactate, intra- and extracellular LDH were monitored and kinetic parameters were estimated. The dissolved CO$_2$ level was computed using a model based on the rate data together with mass balances.

It appeared that in the bioreactor, while cellular growth was comparable to smaller scale control, TGFβ receptor production was three-fold below the control. The sparging of pure oxygen instead of air for dissolved oxygen (DO) control, while minimizing the mechanical stress in lowering the needed flow rate, was most probably responsible for that production loss by inducing dissolved carbon dioxide accumulation in the culture up to a partial pressure of 0.15 atm. This dissolved CO$_2$ effect has been assessed at the smaller scale for TGFβ and β-gal expression in controlled atmosphere incubators.