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Scale-down studies for assessing the impact of different stress parameters on growth and product quality during animal cell culture

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ABSTRACT

Two series of reproducible fed-batch bench scale cultures have been undertaken, one series simulating the impact of spatial variations in pH and nutrients as found at commercial scale on performance, the other, the impact of fluid dynamic stresses associated with agitation. The first was unsuccessful because, somewhat surprisingly, the use of a peristaltic pump to circulate cells and medium through different spatial environments always led to a similar reduction in culture time and resulting product titre compared to uncirculated controls. This fall was sufficient to essentially mask other effects. In the second, even at maximum specific energy dissipation rates up to ~160 times > with laminar extensional flow and ~25 times > with turbulent flow compared to typical commercial conditions, no significant effects were observed on cell growth and viability. Most importantly, in all of the cases studied, product quality was unaffected compared to controls. In addition, it is suggested that because of the possibility of cell line specific behaviour and the relationship between damage to entities and the Kolmogorov scale of turbulence, sensitivity to fluid dynamic stresses is best studied in turbulent bench scale bioreactors.

Keywords: CHO cells; Mechanical stress; Kolmogorov scale of turbulence; Environmental stress; Product quality; Glycosolation; Process performance

1. Introduction

Freely suspended animal cells have been grown commercially in agitated, stainless steel bioreactors since at least 1965 (Telling and Elsworth, 1965) when it was reported that one of 30 L was being used for the production of inactivated foot and mouth disease vaccine from BHK cells; and similar bioreactors were built at the 8 m^3 scale at around the same time for the production of interferon from Namalwa cells (Pullen et al., 1985). More recently, bioreactors of 20 m^3 or so have been installed at Lonza and Genentech (Nienow, 2006). In spite of these successes, since the beginning, there has been concern that because animal cells lack a cell wall, they were very prone to damage due to turbulent fluid dynamic stresses (often called shear 'sensitivity'), particularly due to impeller agitation and this concern was discussed in depth in a review (Nienow, 2006), In particular, it was shown that whilst the oxygen transfer requirements of the cells could be met by agitation intensities (expressed as mean specific energy dissipation rate, $\bar{\varepsilon}_T$ (W/m^3)) of the order 10–20 W/m^3 (the latter

Abbreviations: Ip, isoelectric point value; STR, stirred bioreactor; PFR, loop acting as an approximate plug flow reactor; FC, flow cytometer; HC, haemocytometer; VCN, viable cell number; DCN, dead cell number; sub, proprietary glucose feed as substrate.

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Nomenclature

ε_L	laminar specific energy dissipation rate (W/m^3)
ε_T	turbulent local specific energy dissipation rate (W/m^3)
$(\varepsilon_T)_{max}$	maximum turbulent local specific energy dissipation rate (W/m^3)
$\bar{\varepsilon}_T$	mean turbulent specific energy dissipation rate (W/m^3)
λ_K	Kolmogorov length scale (m)
μ	dynamic viscosity (Pa s)
ρ	density (kg/m^3)
τ_R	mean residence time in the loop (s)

being used for comparison here), a wide variety of cells grew to the same cell density when subjected to $\bar{\varepsilon}_T$ values up to 250 W/m^3.

It was also shown that this perception of fragile cells had led to problems because the low agitation intensity could lead to poor spatial and temporal homogeneity. The latter was especially poor with respect to pH close to the surface of the medium where base for control was added (Langheinrich and Nienow, 1999) and an experimental scale-down model (Osman et al., 2002) indicated that this pH deviation could lead to a significant reduction in viable cell density when growing GS-NSO cells. Work with bacteria has also shown a poorer performance on scale-up due to inhomogeneities associated with nutrient addition in fed-batch fermentations and with pH control chemicals due to surface addition (Hewitt and Nienow, 2007).

In spite of the earlier studies, the perception of cell fragility remains and recently, concern has been expressed that though cell viability may not be compromised at higher $\bar{\varepsilon}_T$ values, product quality, especially glycosolation, may be. To further explore such issues, Chalmers and co-workers (Godoy-Silva et al., 2009a,b) have recirculated cells though a flow device in which cells are subjected to a converging, mainly extensional, flow at very high local laminar specific energy dissipation rates, ε_L (W/m^3). Their justification for the very high values utilised was that in a stirred bioreactor the maximum local turbulent specific energy dissipation rate, which is found very close to the impeller, is much greater than the average, i.e. $(\varepsilon_T)_{max} >> \bar{\varepsilon}_T$. They assumed that $(\varepsilon_T)_{max} = 100\bar{\varepsilon}_T$; and that, more importantly, high specific energy dissipation rates in turbulent and laminar flow should produce the same (or a similar) impact on cells during cell culture. Using ε_L values of the order of 10^6 W/m^3 with two different cell lines, they found a reduction in performance compared to that found at standard conditions ($(\varepsilon_T)_{max}$ around 2.5×10^4 W/m^3 based on the assumption $(\varepsilon_T)_{max} = 100\bar{\varepsilon}_T$). However, in the first case, cell growth was inferior compared to controls without recirculation (Godoy-Silva et al., 2009a); and in the second, it was product quality (Godoy-Silva et al., 2009b).

The work reported covers two projects with different objectives. However, they are brought together here because one of the findings in both studies was surprising; and at the same time, important for gaining further insight into the robustness of animal cells and their products under different processing conditions. One was undertaken at the University of Birmingham and aimed to investigate the impact of environmental stress on cells due to local high pH and glucose levels arising from surface feeding during large scale fed batch culture. In this study, a CHO cell line from MedImmune was used; and MedImmune also assessed the quality of the protein product. The technique used was based on one that had been used successfully when applied to microbial systems (Hewitt and Nienow, 2007). In this technique, first introduced by Enfors and co-workers (Larsson and Enfors, 1988), the medium and cells are circulated through a plug flow loop with a frequency similar to the average with which they circulate due to agitation at the large scale, The volume of the loop is chosen to match the size of the zone (relative to the total volume in the stirred bioreactor) where high concentrations of nutrients or pH control chemicals are found when feeding to the top surface of the large scale bioreactor, as is common practice.

The other study was undertaken later at Genentech using the laminar extensional flow device developed by Chalmers (Ma et al., 2002). The aim was to clarify the earlier work of Godoy-Silva et al. (2009a,b) on the impact of laminar stresses on process performance and product quality using two Genentech production CHO cells lines. In addition, further studies with these two lines were conducted in a bioreactor agitated by dual Rushton turbines at turbulent mean specific energy rates, $\bar{\varepsilon}_T$ up to 1000 W/m^3. In both studies, cell growth, product titre and antibody quality were all assessed.

2. Experimental

3 L and 2 L working volume, fed-batch, bench scale stirred bioreactors (STRs) were used to cultivate CHO cells producing an IgG antibody with pH and dO_2 control; the first for the stresses associated with inhomogeneities due to glucose and pH and the second for the mechanical stress study respectively. Firstly, duplicate primary control runs were undertaken where cells were cultured in the bioreactor using standard conditions including low turbulent specific mean energy dissipation rates ($\bar{\varepsilon}_T = \sim$20 W/m^3). Secondly, duplicate runs were undertaken where each bioreactor was operated as in the primary control but cells and medium were taken from it and circulated through a loop, either by a peristaltic pump for both stress conditions; or by syringe pump for the mechanical stress study. In the secondary control runs for investigating the stress due to inhomogeneities due to nutrients or pH, circulation was undertaken without any additions into the loop. Subsequently, for the actual investigation of homogeneities, duplicate runs were undertaken where additions were made into the loop (Fig. 1). For the secondary control for the mechanical stress study, the laminar extensional flow device was not included in the loop whilst for the actual investigation, it was. In both cases, circulation was only begun at day 4 to ensure that both cultures started in a similar fashion.

The primary and secondary controls in the inhomogeneity study used a defined medium, including Pluronic F68 (Sigma–Aldrich, UK), with a temperature of 36.5 °C, the dO_2 being held at 30% using a fixed agitation speed and variable air flow rate. Sodium bicarbonate buffer (pH 9.7) and sparged CO_2 were introduced directly into the bioreactor on demand in order to control the pH in the bioreactor at 6.95. A proprietary glucose supplement (pH 2.5) was also introduced into it using exponential feeding, starting when either the viable cell number (VCN) had reached 1×10^6 cells/mL (measured by haemocytometer and Trypan Blue exclusion and/or flow cytometric analysis using a Coulter Epics Elite Analyser (Beckman Coulter, UK)) or after day 4 from inoculation of the STR, whichever occurred first. In this way, the concentration of glucose in the bioreactor was maintained at 4.5 ± 1.5 g/L. For the