SCALE-DOWN STUDIES FOR ASSESSING THE IMPACT OF DIFFERENT STRESS PARAMETERS ON GROWTH AND PRODUCT QUALITY DURING MAMMALIAN CELL CULTURE

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Abstract. Fed-batch cultures have been undertaken, one looking at the impact of spatial variations in pH and nutrients on performance, the other, fluid dynamic stresses. The first was unsuccessful because the use of a peristaltic pump led to a fall in cell density and product titre which masked 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. In none of the cases studied was product quality affected compared to controls. It is suggested that because of the possibility of cell line specific behavior 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: Animal cells, pH and mechanical stress, peristaltic pumps, cell viability, protein quality.

1. INTRODUCTION

Freely suspended animal cells have been grown commercially in agitated, stainless steel bioreactors since at least 1965 [1] 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\textsuperscript{3} scale at around the same time for the production of interferon from Namalwa cells [2]. More recently, bioreactors of 20 m\textsuperscript{3} or so have been installed at Lonza and Genentech [3]. 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 [3]. 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, $\overline{\varepsilon}_T$ W/m\textsuperscript{3}) of the order 10 to 20 W/m\textsuperscript{3} (the latter being used for comparison here), a wide variety of cells grew to the
same cell density when subjected to $\mathcal{E}_r$ values up to 250 W/m$^3$. It was 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 [4] and an experimental scale-down model [5] indicated that this pH deviation could lead to a significant reduction in cell density when growing GS-NSO cells. Work with bacteria has also shown a poorer performance on scale-up due to nutrient surface addition [6].

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 $\mathcal{E}_r$ values, product quality, especially glycosylation, may be. To further explore such issues, Chalmers and co-workers [7, 8] have recirculated cells though a flow device in which cells are subjected to a converging, mainly extensional, flow at very high local laminar $\mathcal{E}_l$ values suggesting comparability with a stirred bioreactor where $(\mathcal{E}_r)_{\text{max}} >> \mathcal{E}_r$. Using $\mathcal{E}_L$ 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 with $(\mathcal{E}_r)_{\text{max}}$ around 2.5 x 10$^4$ W/m$^3$ (assuming $(\mathcal{E}_r)_{\text{max}} = 100\mathcal{E}_r$); but in one case cell growth was inferior and in the other it was product quality.

The work reported here covers two projects, one (at the University of Birmingham) on the impact of stresses due to local high pH and glucose levels arising from surface feeding using a CHO cell line from MedImmune and based on a successful technique with microbial systems [6]. The other (at Genentech) used the method developed by Chalmers [7, 8] to study the impact of laminar stresses on two Genentech production CHO cells lines, supplemented by further studies in a turbulent bioreactor at $\mathcal{E}_r$ of 1000 W/m$^3$. In each case, cell growth, product titre and antibody quality were all assessed.

2. EXPERIMENTAL

3L and 2L working volume, fed-batch, bench scale stirred bioreactors (STRs) were used for the stresses associated with inhomogeneities in glucose and pH and mechanical stress study respectively. Such bioreactors were operated under standard low $\mathcal{E}_r$ conditions as controls; and alongside them another stirred bioreactor operated in the same way and had cells and media 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. For the latter, the loop was essentially as described earlier [6, 7] with a Pharmed BPT tubing loop plus the extensional flow device with constrictions of 227 µm and 762 µm to give $\mathcal{E}_l$ values of 2.9 x 10$^5$ W/m$^3$ and 1.9 x 10$^3$ W/m$^3$ respectively. The two pumps utilized were a Cole Parmer peristaltic pump and a Pump 33, Harvard Apparatus dual syringe pump. In addition, a run was undertaken with one bioreactor as control and one with dual Rushton turbines at $\mathcal{E}_r$ of 1000 W/m$^3$.

For the inhomogeneity study with the loop (PFR), sodium bicarbonate (pH 9.7) was introduced on demand in order to control the pH in the bioreactor at pH 6.95 and glucose was fed to maintain the concentration at 4.5 g/L. The volume of the PFR (made of neoprene) mimicked the proportion of a commercial bioreactor (~ 5%) occupied by the feed plume [4, 8]; and the rate of circulation through the loop by a Watson Marlow 505 S peristaltic pump was based on the estimated circulation time at the 20 m$^3$ scale [8].
Further details of the media and the fed-batch and other operating conditions in the bioreactor for the pH [9] and mechanical stress [10] study are given elsewhere.

3. RESULTS AND DISCUSSION

3.1 Impact of stresses generated by pH and glucose excursions

In the two runs without circulation through the loop, the fed batch culture ran for approximately 18 days producing a maximum cell density of ~ 8.5 x 10^6 cells/mL. With the loop (PFR) (10 runs), compared to those runs without recirculation, the average maximum cell density was very similar regardless of where (into the STR or PFR) and at what concentration the bicarbonate was added but the culture duration was always reduced by similar amounts (~ 48 hours) and similar drops also occurred in the maximum antibody production rate per cell (~ 25%) and the overall titre (~ 20%). Fig 1 shows the impact of using the PFR loop whilst Table 1 summarizes the difference of the key parameters both without the loop and with it for each addition modes. On the other hand, in spite of the changes as a result of recirculation through the loop, the protein quality (mass and charge heterogeneity and glycosylation pattern) was the same for all cases (data not shown), with or without recirculation.

Thus, though this technique has provided much insight with bacteria, unfortunately, it was not possible to conclude anything concerning the impact of bioreactor heterogeneities with this cell line. It appears that the use of the peristaltic pump itself induced the changes in growth and productivity; but did not impact on product quality.

![Graph showing cell density and viability](image)

Figure 1. Comparison of control experiments without continuous recirculation (closed) with continuous recirculation (open): Viable cell number (square), dead cell number (triangle) and viability (circle). Each point is the mean and error bars are 1 standard deviation. (Experiments with recirculation, N = 10; without recirculation, N = 2).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>μmax (h⁻¹)</th>
<th>VCNmax (10⁶ cells/mL)</th>
<th>q IgGmax (pg/cell/h)</th>
<th>IgG titre (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR</td>
<td>0.026</td>
<td>84</td>
<td>1.57</td>
<td>1020</td>
</tr>
<tr>
<td>Control (Recirculate)</td>
<td>0.037</td>
<td>86</td>
<td>1.11</td>
<td>800</td>
</tr>
<tr>
<td>Recirculate (pH, 60s)</td>
<td>0.029</td>
<td>88</td>
<td>1.16</td>
<td>778</td>
</tr>
<tr>
<td>Recirculate (pH + glucose, 60s)</td>
<td>0.030</td>
<td>80</td>
<td>1.00</td>
<td>760</td>
</tr>
<tr>
<td>Recirculate (pH + glucose, 120s)</td>
<td>0.030</td>
<td>82</td>
<td>1.26</td>
<td>794</td>
</tr>
<tr>
<td>Recirc. (high pH (bicarb. x100) + glucose 120 s)</td>
<td>0.029</td>
<td>80</td>
<td>1.30</td>
<td>830</td>
</tr>
</tbody>
</table>

Table 1. Growth and productivity values (mean of duplicate experiments): maximum specific growth rate (μmax), maximum viable cell number (VCNmax), maximum specific rate of antibody productivity (q IgGmax); harvest antibody titre (IgG titre).
3.2 Impact of fluid dynamic stresses

Two cell lines were used, one of which (Cell A) was less robust to a single extensional flow experience at a much higher flow rate than the other (Cell B). In all cases, when recirculation was used, it was only begun on day 4 to check that cells were growing within the parameters expected from historical data.

Recirculation with the peristaltic pump. Control runs were always conducted without recirculation. In addition, in the first recirculation run, the cells went through the loop without the extensional flow device in place. Subsequently, runs with the device in place were then undertaken. Figure 2 shows some results for cell growth for the less robust cell A. As can be seen, the standard control is in good agreement with historical data but the recirculation runs with the 762 µm device and without it are significantly inferior. On the other hand, the protein quality and titre (data not shown) were statistically the same for all the runs. The differences with cell B were less clear but overall, it was decided to change to a syringe pump as in the previous work using the extensional flow device [7, 8].

Recirculation with the syringe pump. These experiments were conducted in the same way as those with the peristaltic pump. However, in this case, the control with and without recirculation in the absence of the device were equivalent. In addition, whether the 227 or 762 µm device was used giving \( \varepsilon_{L} \) values of \( 2.9 \times 10^5 \) W/m³ and \( 1.9 \times 10^3 \) W/m³ respectively, the performance with respect to growth and titre (normalized for commercial confidentiality) was the same as the controls for both cell lines. Figure 3 shows the results for the more robust cell B at \( 2.9 \times 10^5 \) W/m³.

Agitation with dual Rushton turbines at \( \bar{\varepsilon}_{T} = 1000 \) W/m³, \( (\bar{\varepsilon}_{T})_{\text{max}} = 5.0 \times 10^4 \) W/m³. The growth and productivity in the Rushton turbine studies was overall in the same range as with the extensional flow device for each of the two cell lines with small differences between the control runs and those with the Rushton turbines but without a specific trend (data not shown).

Product quality. Product quality is shown in Fig 4 for cell B for all the configurations and different stress levels studied; the % of the different glycoforms in Figs 4a and mAb % monomer and charge variants in Fig 4b. The values are consistent over the wide range of stresses employed. Similar results were found for cell A (data not shown).

![Graph showing viable cell count and % viability over time for different treatments](image1)

Figure 2. Growth of higher sensitivity cell line (Cell A): Historic data (dark blue); Control (blue); Recirculation, control (light blue), with 227 µm device (red), with 762 µm device (pink).
Figure 3. Cell B: Recirculation from day 4 by syringe pump without device (control-open symbols, n=2) and with it (closed symbols, n=2) to give $\varepsilon_L$ of $2.9 \times 10^5$ W/m$^3$. (Squares, viability; diamonds, viable cells; triangles, titre; the error bars indicate one standard deviation).

Figure 4. Cell line B: Product quality from different configurations and operating conditions: a) glycosylation; b) mAb % monomer and charge variant (% main peak).

Implications for Commercial Cell Culture. The consistency with which culture performance at these high stress levels gives results similar to each other and to historical data is contrary to those obtained earlier [7, 8], which suggests cell line specificity. However, the really important point is this: Are any cells (or their product quality) likely to be affected by the values of $(\varepsilon_T)_{\text{max}}$ required to operate cell culture bioreactors successfully, recognizing that the levels of $\varepsilon_T$ used in this work are up to ~50 times greater than those currently required. Even allowing for the increased cell density that will probably be achieved in future and therefore requiring a higher $\varepsilon_T$ to satisfy the increased oxygen demand, there is a considerable margin available before the levels tested here are reached.

The Suitability of Extensional Flow for Turbulent Stress Studies. The impact of turbulent stresses on entities suspended within it is usually assessed by comparing the size of the entity with the Kolmogorov microscale of turbulence, $\lambda_K$;

$$\lambda_K = \left( \frac{\mu^3}{\rho \varepsilon_T (\varepsilon_T)_{\text{max}}} \right)^{1/4} \quad (1)$$

where $\mu$ (Pas) is the viscosity and $\rho$ (kg/m$^3$) is the density. If the size of the entity is $< \lambda_K$, it should not be damaged. There are difficulties in applying this concept because values of $(\varepsilon_T)_{\text{max}}$ are not well established. Nevertheless, such approaches have worked well for many single cell biological entities (bacteria, yeast, animal cells [3, 6]) as well as drop breakage in liquid-liquid systems. Indeed, for analysis of the latter topic,
completely different theories are applied for turbulent and laminar flow; and under laminar flow conditions, drop breakage occurs under some conditions in extensional flow when it would not for shear flow [11]. Since Equ 1 applies independent of reactor scale, it is recommended that cell sensitivity to mechanical stress is studied in turbulent stirred bench scale bioreactors for scale-up purposes.

4. CONCLUSIONS

Two fed-batch studies have been undertaken. In the first on pH and nutrient excursions associated with poor homogeneity, the method employed was not successful because it required cells to be circulated for the duration of the culture by peristaltic pump which led to a poorer performance with respect to cell density and product titre. In the second, cells were subjected to elevated fluid dynamic stresses either by recirculating them through a laminar converging flow device or in a bioreactor agitated by a Rushton turbine. In this case, cell density and product titre were not affected by the stress type or level imposed. Very importantly, in every case studied, product quality was the same as the control regardless of the stress imposed. Use of the turbulent stirred bench scale bioreactor for fluid dynamic stress studies is recommended for predicting sensitivity to such stresses on scale-up.

5. REFERENCES