

Comparison of Growth And Productivity of a Monoclonal Antibody Producing Recombinant NS0 Cell Line in Laboratory Scale Airlift and Stirred Tank Fed-Batch Fermentations.

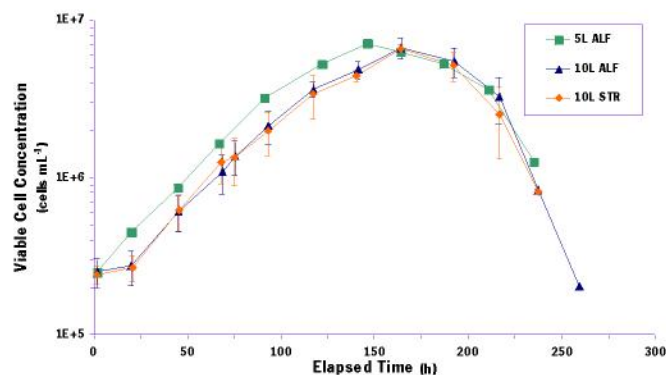
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Introduction & Aim

Large-scale mammalian cell culture for the production of proteins is performed in a variety of reactor formats. Suspension cell lines require reactors that can maintain a well mixed homogeneous controlled environment. The two main types of suspension cell reactors are stirred tank (STR), relying on an impeller system to mix the culture and airlift reactors (ALF) relying on a flow path driven by rising gas bubbles. Both systems use various probe sensors to monitor and control pH, dissolved oxygen concentration (DO) and temperature. Both types of reactor design are used for the commercial manufacture of mammalian cell products. The work below was performed to compare the performance of the two types of reactor designs using the same cell line, product and process carried out in parallel.

Cell Growth

After the initial fermentations, the 10L STR and 10L ALF fed batch fermentations were repeated in duplicate. The error bars on the graphs for these reactor type therefore represent the standard deviation of the average of all three fermentations that were run under identical conditions. All reactor types demonstrated similar specific growth rate (slopes of logarithmic plot) with an average doubling time of 29.6 h. All reactor types reach similar maximum cell concentrations with an average of 6.7×10^6 cells mL⁻¹. The reactors all demonstrated identical decline profiles.



Nutrient Profiles

The 10L STR and 10L ALF reactors showed very similar profiles of glucose consumption and lactate production whilst the 5L ALF reactors displayed slightly lower glucose consumption and lactate production. Amino acid analysis of all reactor types showed similar rates of utilisation between the different reactor types.

Methods

Cell Line: model production cell line: GS- NS0 myeloma transfected with GS linked genes for a B72.3 IgG antibody against TAG-72 (Bebbington, *et al.* 1992).

Inoculum Source: Cells were revived from cell bank and the culture expanded using shaker flasks. Inoculum for the fermentations was prepared using spinner vessels and pooled prior to transfer.

Medium: Protein-free production medium

Feed: Concentrated protein-free nutrient feed applied once cell concentration $> 1.5 \times 10^6$ cells mL⁻¹.

Control Parameters: Temp 36.5 °C, pH 7.3, DO 15% of saturation with air.

Product Concentration Assay: HPLC Protein A determination.

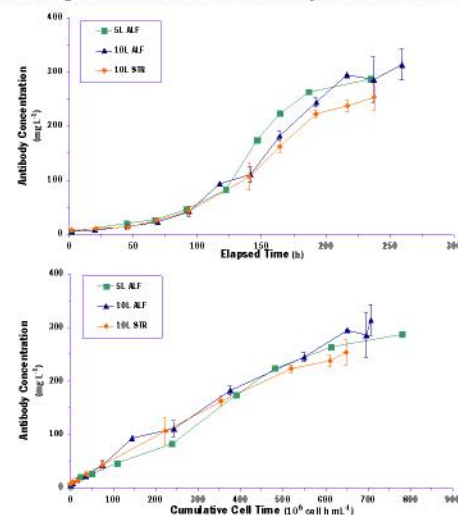
Product Quality Assay: reducing & non-reducing SDS PAGE and IEF analysis.

Productivity

All the laboratory scale reactors showed similar profiles of product accumulation through the fermentation.

All the reactors types reached similar harvest product concentrations with an average of 284 mg L⁻¹.

All reactor types showed similar specific production rates (shown by the slopes of the product concentration against cumulative cell time plot) with an average of 9.6 pg cell⁻¹ d⁻¹.



Conclusions

These experiments demonstrate that the performance of this cell line was similar between the two styles of reactor design. Rice, *et al.* (1993) used Sf9 insect cells in both airlift and stirred style reactors and showed similar good comparability between the reactor designs. Further developments of the fed fermentation process used for this model cell line has increased the productivity to over 200% of the levels demonstrated here.

Abstract

Large scale commercial manufacturing of pharmaceutical products from mammalian cells is performed using both airlift and stirred tank reactor designs. The effects of these two different methods of mixing on the performance of mammalian cell fermentations of a NS0 cell line producing a recombinant antibody was evaluated using a range of laboratory scale fermenters of both stirred tank and airlift design. Results of growth, productivity and product quality from these fermentations are presented showing that no significant difference in performance was observed.

Reactor Types



5L Airlift



10L Airlift



10L Stirred

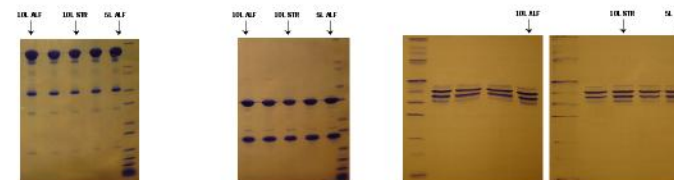
Product Quality

Preliminary analysis of protein characteristics was performed on the product from the end of the fermentations.

No significant differences were observed by SDS-PAGE (reduced and non-reduced) or IEF analysis.

More detailed characterisation is planned.

Unlabelled tracks are samples from other fermentations using this cell line operated under other conditions not covered in this presentation.



SDS-PAGE Non Reduced

SDS-PAGE Reduced

IEF

References

Bebbington, C.R., Renner, G., Thomson, S., King, D., Abrams, D. & Yarranton, G.T. (1992) *Biotechnology* 10, 169.

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