Scale-Up Using the Celligen™ Fixed-bed Bioreactor

The use of a Celligen™ Fixed-bed Bioreactor in the Production of Alkaline Phosphatase (AP) from Recombinant CHO Cells using Sigma-Aldrich CHO, Protein-Free Medium.

Recently, ECACC has added a Celligen™ fixed-bed Bioreactor (New Brunswick Scientific (UK) Ltd) to its scale-up and process development. This is in addition to it's range of standard stirred tank bioreactor (1L to 100L). As part of an on-going commission to test the effectiveness of the Celligen™ fixed-bed bioreactor, the process development group at ECACC used the AP recombinant CHO cell line from Sigma-Aldrich to demonstrate the capabilities of the Celligen™ fixed-bed bioreactor.

The Celligen™ fixed-bed bioreactor (Figure 1) has a proven record over many years in the production of monoclonal antibodies. More recently, fixed-bed bioreactors have been successfully used in the production of secreted proteins from recombinant cell lines.

ECACC is now in a position to offer the services of its' pilot-scale Celligen™ fixed-bed bioreactor for non-GMP production runs, and as a process development tool.

Fixed-bed perfusion offers a number of advantages:

- Constant and controllable pH, temperature, dissolved oxygen (DO) and constant metabolites
- The product is removed from the bioreactor as soon as it is secreted. This is a distinct advantage if the product is labile or sensitive to degradation by proteolytic enzymes
- The cells are protected from shear forces in the matrix of the Fibracell™ packed bed.

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In this study, ECACC's 1.4L pilot scale Celligen™ fixed-bed bioreactor was used.

The cell line, reagents and AP assay kits used in this study were provided by Sigma-Aldrich (Poole, UK).

The innoculum was grown using conventional cell culture techniques in Sigma-Aldrich CHO, Protein-Free Medium (Product Code: C5467) supplemented with 4mM L-glutamine and puromycin as a selective agent to maintain a secreting population of cells. Puromycin was omitted in the production run.

The Celligen™ fixed-bed bioreactor was seeded with 1 x 10^9 of the AP recombinant CHO cells in 1L of CHO Protein-Free Medium in a packed bed of 100g of Fibracell discs. Daily samples were taken from the reactor vessel to ascertain AP productivity, glucose and lactate levels. The bioreactor was left for approximately 66 hours post-seeding before perfusion commenced. The perfusion rate was adjusted daily in response to the glucose consumption rate and to maintain lactate at a sub-inhibitory level. Product (perfusate) was collected into autoclave-sterilised carboys and chilled to +4°C using an insulated ice bath.

The DO and pH in the bioreactor was controlled by a 4 gas mix (O_2, N_2, CO_2 and compressed air). Later in the run, the periodic addition of sodium bicarbonate was required to prolong pH control. The Celligen™ controller in conjunction with AFS Biocommand software (New Brunswick Scientific (UK) Ltd.) constantly monitored reactor conditions. The bioreactor was operated for 309 hours (13 days) before the run was electively terminated.

The summary table 1 shows the AP, glucose and lactate level in samples taken at daily intervals from the reaction; and the perfusion rate of the medium. From these figures we were able to calculate the glucose concentrate rate of the system and the AP productivity in both units per hour and units per litre. The volume of product per day was measured and from this the daily yield of AP was estimated (Figure 2).

Figure 1. Celligen™ fixed-bed bioreactor

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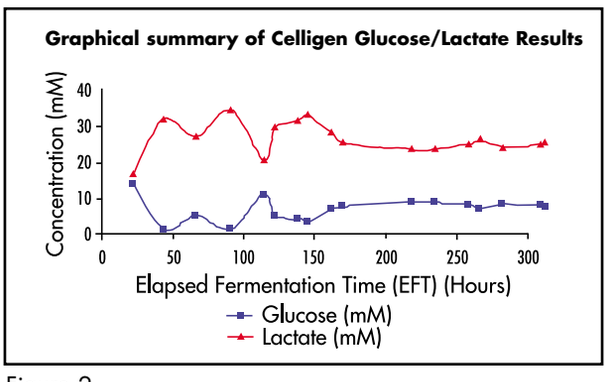
Results

Time (hours)	AP (units/ml)	Glucose (mM)	Lactate (mM)	Glucose Consumption (g/day)	Perfusion ml/hr	Perfusate (AP) Productivity (units/hour) ($x10^3$)	Perfusate (AP) Units Per Litre ($x10^3$)	Litres Produced per day	Daily Yield (units) ($x10^4$)
21.62	16.22	14	16.9	3.02	0	0.0	0.0	0.0	0.0
43.05	44.90	1.32	32.4	3.6	0	0.0	0.0	0.0	0.0
65.87	45.10	5.25	27.3	4.5	65	2.93	45.1	1.56	7.04
90.13	67.80	1.5	34.8	8.71	77.7	5.27	67.8	1.86	12.6
113.95	31.03	11.3	20.7	8.5	188	5.83	31.00	4.51	14.0
121.78	36.32	5.08	30	15.7	188	6.83	36.3	4.51	16.4
137.85	29.49	4.29	31.7	16.85	187.5	5.53	29.5	4.50	13.3
144.53	29.69	3.45	33.4	18.04	187.5	5.57	29.7	4.50	13.4
161.42	21.44	7.07	28.6	21.33	292	6.26	21.4	7.01	15.0
169.2	22.72	7.97	25.5	21	292	6.63	22.7	7.01	15.9
217.86	15.01	9.17	23.7	21.8	326	4.89	15.0	7.82	11.7
234.05	12.46	8.97	24	21.2	308	3.84	12.5	7.39	9.21
258	11.32	8.3	25	26.21	365	4.13	11.3	8.76	9.92
265.37	11.05	7.3	26.5	28.54	365	4.03	11.1	8.76	9.68
282.27	8.57	8.64	24.5	20.74	304	2.61	8.57	7.30	6.26
306	7.64	8.24	25.1	25.75	358	2.73	7.64	8.59	6.56
309.08	7.57	7.9	25.6	24.47	358	2.71	7.57	8.59	6.50
Mean units/ day	24.6						Total	92.68 Litres	1.68E+06 Units
Final Concentration of AP in Pooled Harvest									18 Units/ml

Table 1. Summary of Celligen™ Run



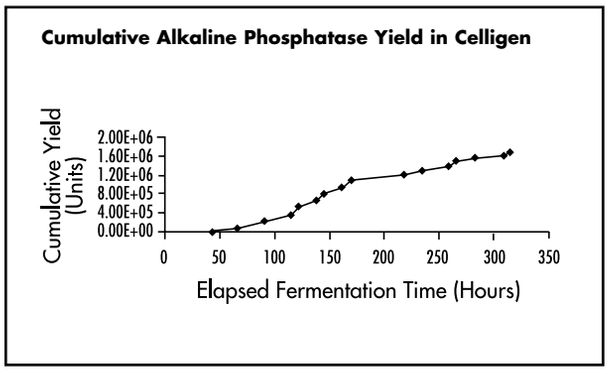


Figure 2.

Conclusions

The Sigma-Aldrich CHO Protein-free medium supported good cell growth achieving high cell densities along with good viability and productivity. The pilot scale Celligen™ (1.4L) can provide the equivalent yield of AP from the rCHO AP cell line as 136 x 300ml roller bottles (data not shown). The Celligen™ has the advantage that the reactor conditions can be maintained at steady state of dissolved oxygen and pH, and of critical metabolites such as glucose and lactate.

The Celligen™ fixed-bed bioreactor is scalable and suitable for use in cGMP manufacture. Fewer manipulations are required as compared to roller bottles, therefore contamination risk is reduced and the process is less labour intensive.

The system employed in this case was not optimised, and we are confident that with further work and trials, the yield could be increased and output sustained for a longer period.

Acknowledgements

ECACC appreciates the collaborative commitment between its team members to provide high quality through-put in the required time-scale.

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