A TUNABLE AND SCALABLE PLATFORM FOR PRECISE FUCOSYLATION CONTROL TO ENHANCE EFFICACY OF THERAPEUTIC ANTIBODIES: APPLICATION TO CLINICAL MANUFACTURING OF BUDOPRUTUG

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Attenuation of fucosylation on monoclonal antibodies enhances their potency and has been applied to multiple marketed immunotherapy products. While several bioproduction platforms exist for generating afucosylated antibodies, it remains challenging to precisely control the fucosylation level for optimal pharmacological outcome. Here we describe a manufacturing process capable of targeting any predefined fucosylation content in the final product. The platform is based on the CHO DG44 GlymaxX® cell line (ProBiogen AG) in which the endogenous fucose biosynthesis pathway is genetically abolished. Fucosylation content can be fine-tuned by supplementing submillimolar concentration of fucose to the cell growth medium. The system was adopted for clinical-grade production of Budoprutug, an anti-CD19 antibody being developed for B cell immunotherapy. The producer cell line was switched from the original rat myeloma cells (YB2/0) to GlymaxX® to improve yield. Importantly, product fucosylation content from the new cell line needs to closely match the original material to ensure consistent safety and efficacy. A Design of Experiment (DoE) approach was implemented in small scale bioreactors to evaluate the combinatorial effects of fucose concentration, medium feed and other bioreactor parameters on product yield and quality. The study established process parameters that lead to >10 X increase in titer and fucosylation level mirroring the rat cell line. The process was further verified at 250 L and 1000 L scale, demonstrating highly comparable product attributes, such as glycan and charge profile as well as antibody-dependent cell-mediated cytotoxicity. Collectively, the approach provides a scalable solution for producing biologics with tunable glycan profile, which could unleash the full potential of immunotherapies.

Figure 1. Working Principle for GlyMaxX ® Technology

This is a CHO DG44 cell line stably expressing GDP-4-dehydro-D-rhamnose reductase (RMD) that diverts the endogenous fucose biosynthetic pathway to a dead-end and blocks fucosylation. This can be rescued by supplementing fucose in the cell culture medium and the fucosylation level on the protein product can be tuned by adjusting the exogenous fucose concentration.

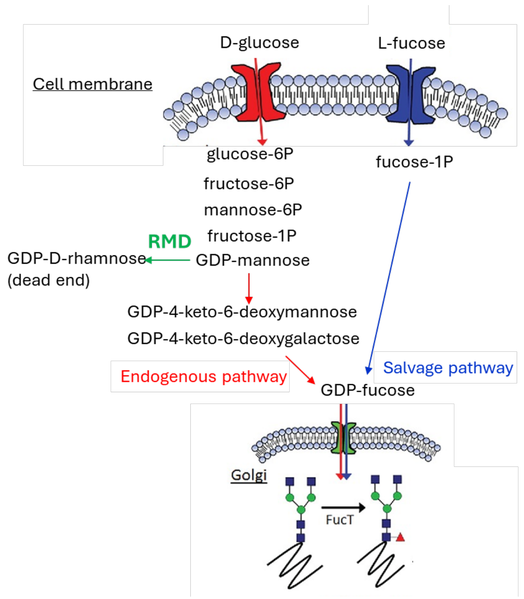


Table 1. Process Development at 1 L Scale

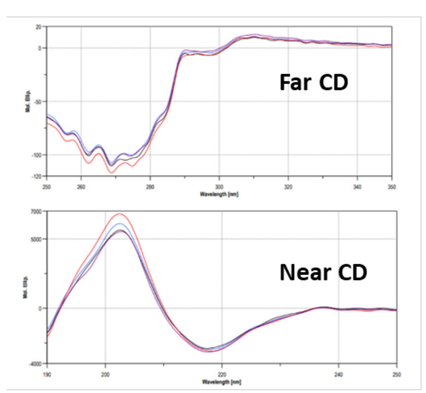
Process parameters including pH profile, feeding regimen, seeding density, fucose concentration, and dissolved oxygen were systematically evaluated for their impact on cell growth and productivity, as well as fucosylation level and potency.

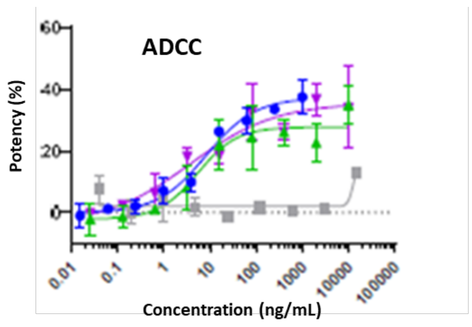
Run ID	pH Profile	Feeding Regime	Harvest Viable Cell Density (10^6/mL)	Harvest Cell Viability	Product Titer (g/L)	Fucosylation Level	ADCC
1	Multistep 2	Platform	24.0	86.2 %	3.4	56.1 %	68 %
2	Platform		22.1	91.6 %	3.7	57.5 %	76 %
3	Multistep 1	High	23.6	87.6 %	3.6	58.8 %	62 %
4		Low	22.4	84.3 %	3.3	46.8 %	97 %
5		Platform	25.4	86.1 %	3.4	51.2 %	81 %
6		CSF1	23.5	89.8 %	3.7	53.6 %	74 %
7		CSF2	24.7	85.9 %	3.6	52.7 %	71 %
8	Multistep 2	CSF1	24.1	88.7 %	3.7	55.4 %	na
9		CSF2	25.5	87.9 %	3.8	54.5 %	na

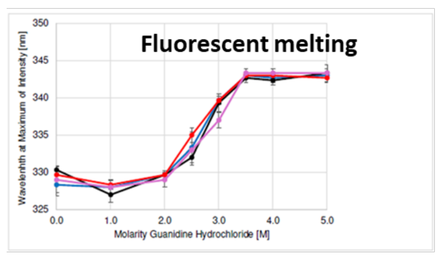
Run ID	pH Profile	Feeding Regime	Fucose Feed Concentration	DO	Seeding Density (10^6/mL)	Temp. Shift	Product Titer (g/L)	Fucosylation Level	ADCC Potency
10	Multistep 1	CSF1	Level 1	40%	0.6	Day 4	3.7	43.5 %	111 %
11						Day 6	3.8	46.7 %	109 %
12				10%		Day 5	3.4	36.2 %	124 %
13				40%	0.4		3.7	41.9 %	116 %
14	Elevated end-pH				0.6		3.8	43.2 %	117 %
15	Multistep 1		Level 2				4.0	38.6 %	105 %
16			Level 3				4.0	46.9 %	92 %
17							3.4	39.0 %	123 %
18							3.7	42.7 %	97 %
19							3.8	42.0 %	118 %

Figure 2. Product Comparability for Budoprutug

The product quality attributes for budoprutug produced from the GlyMaxX platform (pink) are compared with the previous YB2/0 cell line (blue). The new process increased the titer by more than 10X and maintains consistent product quality.







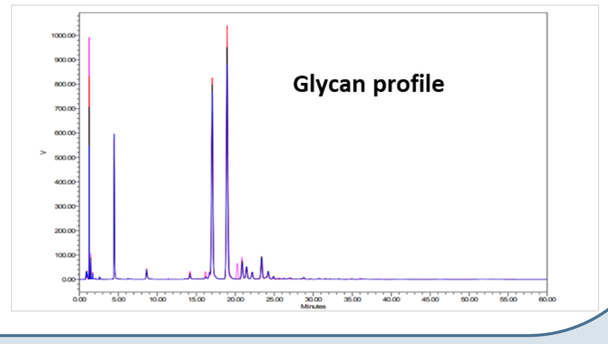


Figure 3. Process Implementation at 250 L Scale

The process parameters from the 1 L run were implemented at 250 L scale. The cell expansion and bioreactor parameters are shown.

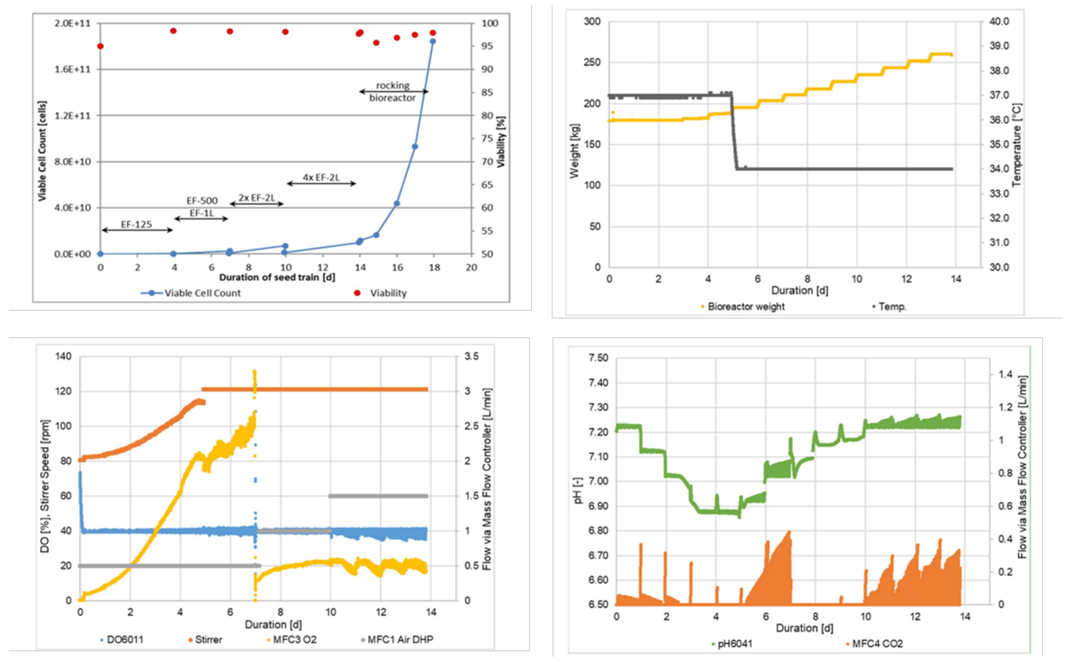
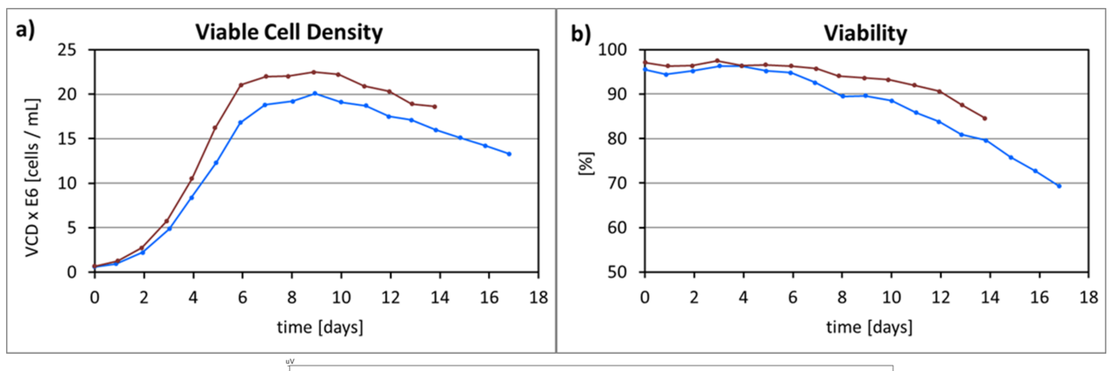
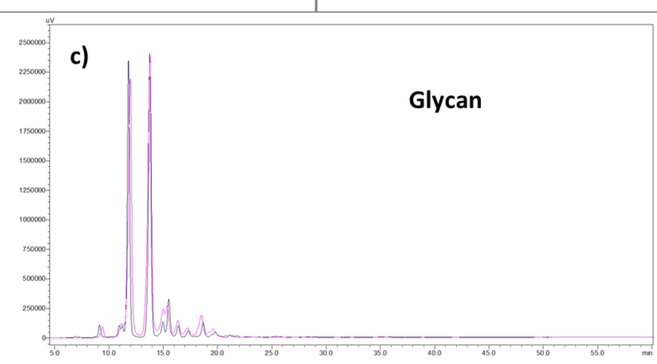


Figure 4. Process Scale-Up to 1000 L Scale

The process was scaled up from 250 L (brown/pink) to 1000 L (blue). Slight differences were observed in cell density and viability, but the glycan profiles were highly similar.





CONCLUSIONS

- **Budoprutug (an anti-CD19 mAb) was originally produced in a rat YB2/0 cell line with reduced fucosylation and enhanced ADCC activity**
- **The producer cell line was switched to CHO DG44 GlyMaxX ® cell line to increase yield**
- **Small-scale bioreactor runs established process parameters that led to 10X increase in productivity and consistent fucosylation level**
- **The process was scaled up to 250 L and 1000 L and achieved highly comparable product quality profile**
- **The platform can be broadly applied to the production of biotherapeutics with tunable glycan profiles**

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