

Continuous, Steady-State Protein Expression

Scott Waniger, Curt Gleiter, Meg Duskin, Liza Deiman; Minneapolis, MN 55433, USA



Abstract

Biopharmaceuticals require highly complex processes and are costly to manufacture. Therefore, biopharmaceutical manufacturers must maximize efficiencies to minimize costs and sell their products in an increasingly competitive world market. Traditional stir tank bioreactors require downtime for cleaning and re-evaluation, a labor-intensive, time-consuming, and costly practice. Perfusion bioreactors, on the other hand, provide an environment that promotes the steady-state production of monoclonal antibodies and recombinant proteins under long-term sustainable homeostatic conditions. In this poster, data from a case study are presented demonstrating long-term homeostatic pH conditions, metabolite levels (glucose and lactate), and monoclonal antibody (IgG) production in a hollow fiber perfusion bioreactor. The data show steady-state conditions are consistent with continuous protein expression for more than 100 days.

Hollow Fiber Promotes Uniform Cell Growth

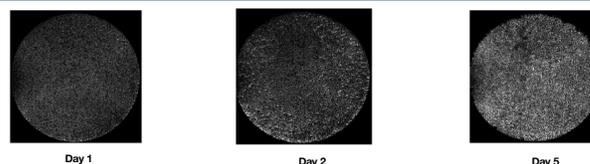


Figure 1. Nuclear Magnetic Resonance Images of Cell Growth in Hollow Fiber. A high-resolution, diffusion weighted image of the bioreactor at 1, 2, and 5 days post inoculation with a mammalian cell line. The white color shows homogenous cell mass distribution throughout the available space in the hollow fiber bundle. EC cycling (automated and cyclic cross-membrane ultrafiltrative flow) began on day 3.

Steady-State Metabolic Control

Stable pH Conditions Promote Cell Health

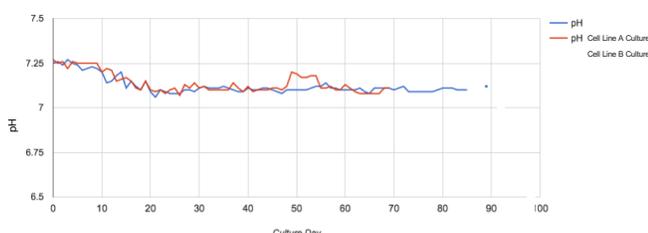


Figure 2. Stable pH. This graph represents the in-line pH value of two different perfusion bioreactor cultures from two different mammalian cell lines. The data demonstrate a consistent pH of 7.25 for the growth phase (culture days 0-10) followed by an equally consistent pH of 7.1 for the production phase (cultures days 10-85 for the one cell line and culture days 10-69 for the second culture). The in-line pH probe was calibrated every 5-7 days.

Nutrient and Waste Levels Remain Optimal and Constant

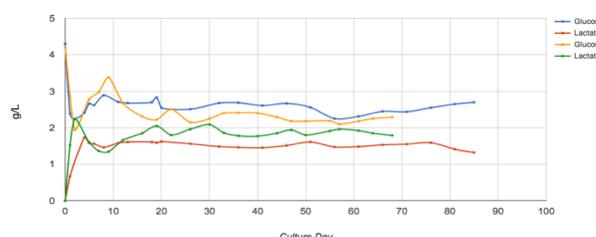


Figure 3. Consistent Glucose and Lactate Concentrations. This graph demonstrates glucose and lactate values from the same cultures represented in Figure 2. The blue and red lines are from the 85-day culture, and the green and yellow lines are from the 69-day culture. As seen in Figure 2, hollow fiber perfusion bioreactors maintain consistent levels of key metabolic components for long periods of time for continuous manufacturing operations.

Continuous, Consistent Protein Expression

Long Lasting Protein Expression

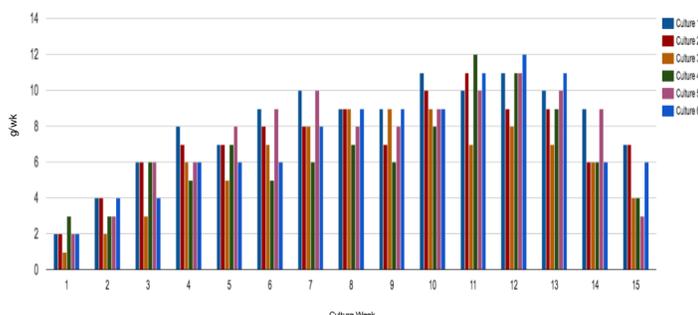


Figure 4. Monoclonal Antibody Continuous Production. This graph represents the amount of mAb collected each week from six different perfusion hollow fiber bioreactor cultures using the same mammalian cell line. The 105-day culture length is a validated continuous manufacturing process that has been shown to yield comparable high quality IgG over the entire length of the culture.

Predictable and Scalable Protein Expression

Cell Line	Bioreactor System	No. of 2.1 m ² Cartridges	No. of Culture Days	Grams of IgG	Titer g/L	IgG Production mgs/day/cartridge
A	AutovaxID	1	30	9.0	3.00	300
	Maximizer	2	60	40.8	2.55	340
	Xcellerator	10	37	111.0	2.58	300
B	AutovaxID	1	30	3.5	1.16	116
	Maximizer	2	62	13.0	0.81	105
	Maximizer	2	30	6.1	0.76	102
	Maximizer	2	60	17.0	1.06	142
	Maximizer	2	30	7.5	2.27	125
	Maximizer	2	60	14.0	0.88	117
	Maximizer	2	60	17	1.14	143
C	Xcellerator	10	60	70	1.40	117
	Xcellerator	10	47	8	0.25	18
	Xcellerator	10	94	21	0.31	22
	Xcellerator	10	51	11	0.31	22
	Xcellerator	10	51	11	0.30	21
	Xcellerator	10	55	10	0.27	19
	Xcell	6	82	12	0.32	23

Figure 5. Reliable, Scalable, and Predictable Protein Expression. Each bioreactor model (AutovaxID, Maximizer, and Xcellerator) contains a different number of the 2.1 m² cartridges connected in parallel. This configuration permits predictable and scalable manufacturing as represented by the data in the far right column. The two variables used to calculate the data in this column are the number of cartridges and length of cultures in days. A calculation to normalize these two variables shows data demonstrating that hollow fiber bioreactors provide reliable and predictable levels of protein yield.

Summary

- C3's, single-use, perfusion bioreactors enable cells to grow evenly surrounding the hollow fibers.
- pH remains stable for two cell lines cultured for 69 and 85 days, respectively, with minimal maintenance.
- Key metabolic components, such as glucose uptake and lactate production, remain constant for long periods of time for continuous manufacturing operations
- Protein expression lasts for months in C3's perfusion platform—compared to fed-batch cultures that express proteins for only 10-14 days.
- By running hollow fiber cartridges in parallel, manufacturing can be predictable and easily scalable.