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Abstract

Perfusion processes have traditionally been used for the generation of unstable proteins in cell culture systems. The use of perfusion for production of stable proteins has been limited by low product concentration, media costs, and system complexity. However, with the advent of new single-use technology, cell culture media specifically formulated to support high density perfusion, and high-producing cell lines, perfusion processes are gaining widespread industry attention. Additionally, perfusion processes are considered an integral part of the "Factory of the Future" (FOF) vision through enabling continuous processing while delivering a product effluent with consistent product quality and concentration.

In this study, we evaluate the ability of a long duration perfusion process to deliver a consistent product stream. Although rarely reported, a reduction in protein sieving/transport through the microfiltration-based cell retention device is associated with many perfusion processes. To better understand this observation, we have investigated the impact viable cell density (VCD) and cross-flow rate on protein sieving through a microfiltration-based (MF) cell retention device connected to a Mobius® 3L single-use bioreactor operated in a 30+ day perfusion process. It has also been reported that perfusion processes can be exploited to deliver a consistent product with more uniform product quality attributes. To support this observation, we also present product quality data (glycosylation profiles) for a long duration mAb perfusion process and compare the results to a more traditional fed-batch process.

Purpose

- **Develop a long duration (LD) perfusion cell culture process**
- **Evaluate effect of VCD and cross-flow rate on protein sieving**
- **Compare perfusion product consistency to fed-batch over time**

Materials & Methods

CHO cells expressing a therapeutic mAb were grown and expanded first in shake flasks until sufficient cell density for inoculation of the bioreactors. Mobius® 3L single-use bioreactors were used for perfusion and fed-batch runs with working volumes of 1.7L. The ATF™ perfusion device was connected to the harvest line of the bioreactor. Detailed process parameters for perfusion bioreactor runs are shown in Table 1.

Table 1. Experimental Process Parameters

PROCESS VARIABLE	VALUE
Mobius® Bioreactor	3L
Cell line	CHO-S-C6
Cell Culture Media	CellVento™ CHO-100 + Methionine Sulfoximine + Antifoam C (as needed)
Total Working Volume	1.7L
Agitation	250 rpm
Temperature	36.8 ° C
pH	7.0 ± 0.05
Dissolved Oxygen	50%
Cell Retention Device	ATF™2 System
Circulation Rate	1.0-1.5 L/min
Hollow Fiber Cartridge	1mm lumen, 1300 cm²

Results

Develop a LD Perfusion Cell Culture Process

- Initial studies successfully demonstrated application of a baseline perfusion process to Mobius 3L® single-use (SU) bioreactor and subsequent scalability to the Mobius ® 50L single-use bioreactor (Figure 1).
- The implementation of bioreactor volume automation, reduced heating blanket size, and improved PID tuning improved process control variability (Figure 2A&B: temperature example).
- Improved cell growth consistencies with durations of 30+ days were obtained in Mobius 3L® bioreactors using improved perfusion process (Figure 3).

Figure 1A&B. Mobius® 3L & 50L SU Bioreactor Scalability

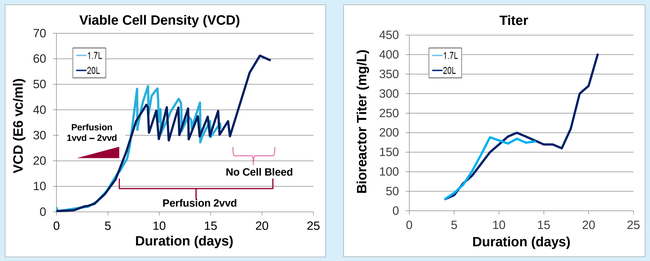
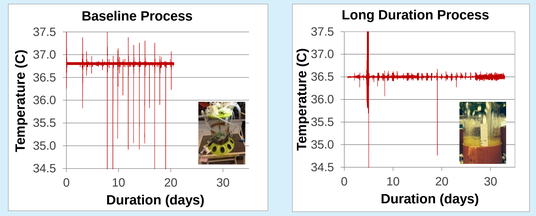
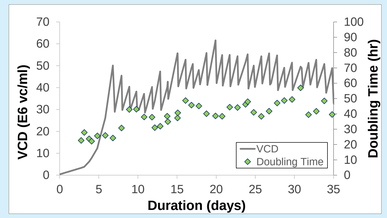


Figure 2A&B. Improved Temperature Control



- Media bleed/refeeds clearly visible
- Significant cell debris burn-on
- Tight temperature control (36.5±0.5C)
- No cell debris burn-on

Figure 3. Long Duration Perfusion: Improved Growth

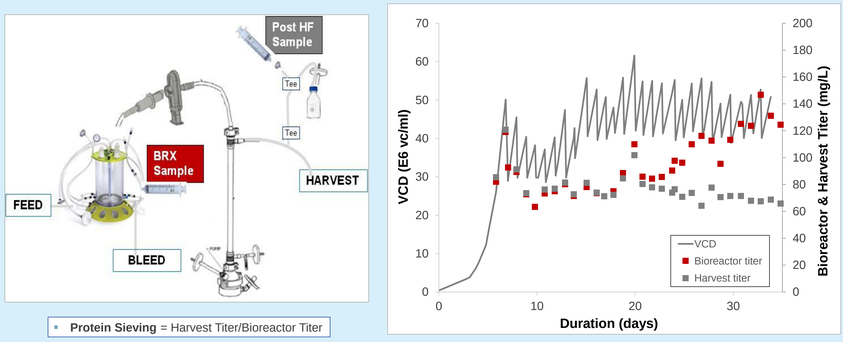


- **3L Mobius® SU Bioreactor can support long duration perfusion runs**
- 35+ day perfusion run using ATF™2 system

Evaluate Effect of VCD & Cross-flow on Protein Sieving

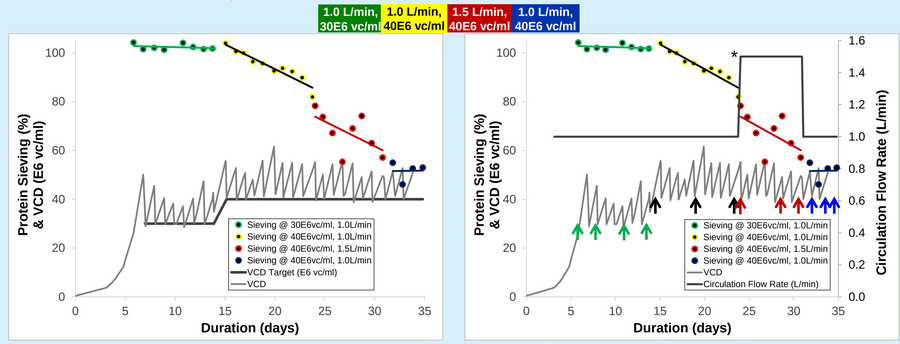
- A schematic of the experimental setup used for evaluation and the resulting differences in titer over time are shown in Figure 4A&B.
- A reduced sieving profile was observed when the VCD target increased to 40E6 vc/ml (Figure 5). An increase in ATF™2 cross-flow rate did not improve the reduced protein sieving trend (Figure 5B).

Figure 4A&4B. Protein Sieving: Titer in Bioreactor and Harvest



- **Protein Sieving** = Harvest Titer/Bioreactor Titer

Figure 5A&B. Protein Sieving: VCD and Cross-Flow



* Max cross-flow corresponded do 3400/s average wall shear rate.
Arrows indicate specific harvest stream sample points for product quality analysis in Figure 7.

Product Quality Consistency (Perfusion vs Fed-batch)

- Glycosylation profiles obtained from the harvest stream of perfusion presented in Figure 6 are more consistent over time compared fed-batch (Figure 7).
- For perfusion, equivalent glycosylation profiles were observed after VCD bleed target stabilized on Day 19.

Figure 6. Perfusion: Glycosylation Species %

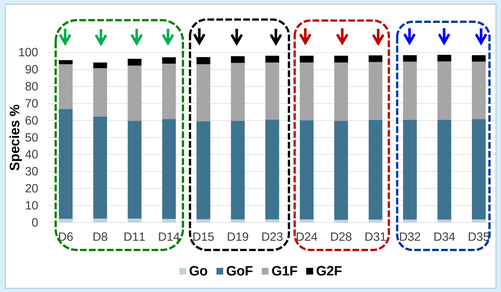
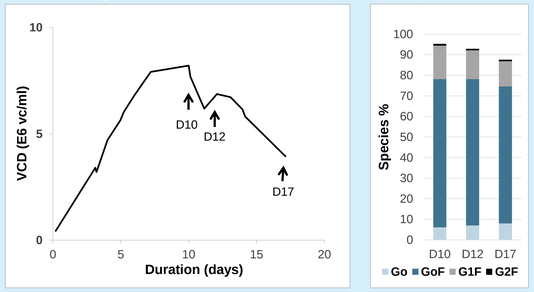


Figure 7A&B. Fed-Batch VCD & Glycosylation



Summary

- **Long-duration perfusion (30+ days) was successfully demonstrated in Mobius® 3L single-use bioreactor.**
- **An increase in VCD resulted in decreased protein sieving. Increased circulation flow did not improve the reduced protein sieving trend.**
- **Consistent product quality over time was observed in long duration perfusion compared to fed-batch.**

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