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mAb Product Consistency Achieved in Long Duration Microfiltration-Based CHO Perfusion Process

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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 rates 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 batch-process.

Purpose
• Develop a long duration (LD) perfusion cell culture process
• Evaluate effect of VCD and cross-flow rates 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mobius® 3L</th>
<th>Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioreactor</td>
<td>2.8 L</td>
<td>CHO-68</td>
</tr>
<tr>
<td>Cell Culture Media</td>
<td>Cat# K71500-001 Methionine, Sulfate-free</td>
<td>1,7L</td>
</tr>
<tr>
<td>Total Working Volume (L)</td>
<td>1.7L</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Dissolved Oxygen (atm)</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Cell Retention Device</td>
<td>ATF™ 2®</td>
<td></td>
</tr>
<tr>
<td>Circulation Rate (L/min)</td>
<td>0.15 to 2.0</td>
<td></td>
</tr>
<tr>
<td>Mobius High-Capacity Cartridge</td>
<td>150,000 L</td>
<td></td>
</tr>
</tbody>
</table>

Results

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 6).

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 to fed-batch (Figure 7).

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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