A streamlined single-use solution for an intensified high-density cell culture process

Peggy Lio1, Zhou Jiang1, Véronique Chateau2, Marie-Francoise Clinc6, Carin Mölleryd3, Ye Zhang4, Puneeth Samani4, Eric Fält1, Kieron Walsh1, Eva Lindskog1, and Christian Kaiser Mayer6

1 GE Healthcare Life Sciences, Westbrook, MA, USA; 2 The Royal Institute of Technology, KTH, Sweden; 3 GE Healthcare Life Sciences, Uppsala, Sweden

Introduction

Process intensification refers to the improvement of volumetric production capacity by the use of novel technologies. To maximize the biomanufacturing output, there is an emerging trend of implementing process intensification in upstream bioprocesses. Reported applications include intensified perfusion cultures, concentrated fed-batch cultures, and high-density seed trains. Compared with conventional batch and fed-batch cultures, significantly higher cell densities and/or product yields can be achieved with intensified processes. However, for successful implementation of intensified cell cultures, challenges such as increased hardware and operational complexity need to be overcome.

With advantages such as ease of implementation and hardware simplicity, single-use systems are often applied in batch and fed-batch cell culture processes. Given these benefits, we evaluated the feasibility of running a single-use perfusion cell culture using a streamlined single-use WAVE Bioreactor™ system. Either an internal floating filter or an external hollow fiber cartridge was used as a retention device. Evaluation studies were performed using multiple cell lines at various scales. The results demonstrate the simplicity and flexibility of this single-use solution for intensified cell culture, where high cell density (200 × 10^6 cells/mL) and volumetric productivity of up to 2 g/L/d were achieved.

Materials and methods

Cell culture studies were performed using a WAVE Bioreactor 20/50 system. Used CellBag™ Bioreactor, cell line, culture volume, culture medium, temperature, and perfusion rate are listed in Table 1. Cell retention was conducted using either an internal filtering system or an external hollow fiber cartridge. Alternating tangential flow filtration (ATF) was driven by an ATF membrane pump (Late) interfaced with an ATF-C24 Control unit.

Results

The perfusion CellBag bioreactor is equipped with an internal floating filter that separates the cells from culture fluid by a retentive membrane. The rocking motion of WAVE Bioreactor system results in alternating tangential flow of liquid medium, which sweeps up the cells from the filter surface and prevents blockage. The filtrate is transported by a harvest pump to a harvest bag. Fresh medium from a feed bag is delivered to the bioreactor by a feed pump.

The cell suspension on the retentate side of the membrane is constantly moving in one direction, tangential to the membrane. Only a relatively small portion of the fluid passes through the membrane and is withdrawn on the permeate side. The sweeping function of the tangential flow prolongs the filter lifetime. The ATF hollow fiber is disposable and can be replaced in the process. Intensified cultivation of CHO cells was performed according to the setup shown in Figure 3. The cell viable cell density was measured by 200 × 10^6 cells/mL, at a 10%/d perfusion rate. Cumulative IgG yield was 140 g total harvest over the 48-day culture, equivalent to 48.3 g/L volumetric productivity (Fig 4).

Pressures at three locations were measured: P permeate, P retentate, and P permeate. In Figure 5, measured pressures were plotted against viable cell densities. Dashed lines represent trends. A negative value of P permeate is indicative of filter fouling. Filter fouling occurred when cell density was above 150 × 10^6 cells/mL.

Materials and culture conditions of four intensified cell culture experiments

Table 1.

<table>
<thead>
<tr>
<th>Intensified Culture</th>
<th>CHO cell concentration</th>
<th>CHO cell concentration</th>
<th>CHO cell concentration</th>
<th>CHO cell concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell type</td>
<td>CHO</td>
<td>CHO</td>
<td>CHO</td>
<td>CHO</td>
</tr>
<tr>
<td>Culture medium</td>
<td>CHO-CD XP (Irvine)</td>
<td>CHO-CD XP (Irvine)</td>
<td>CHO-CD XP (Irvine)</td>
<td>CHO-CD XP (Irvine)</td>
</tr>
<tr>
<td>Culture media</td>
<td>CHO</td>
<td>CHO</td>
<td>CHO</td>
<td>CHO</td>
</tr>
<tr>
<td>Temperature</td>
<td>37°C</td>
<td>37°C</td>
<td>37°C</td>
<td>37°C</td>
</tr>
<tr>
<td>Perfusion rate</td>
<td>0.5–1.2 L/d</td>
<td>0.5–1.0 L/d</td>
<td>0.5–1.0 L/d</td>
<td>0.5–1.6 L/d</td>
</tr>
</tbody>
</table>

Conclusions

- Implementation of intensified cell culture processes can offer significant benefits in supporting extremely high cell densities and improvements in volumetric production capacities.
- Simple implementation of intensified cell culture was demonstrated using single-use WAVE bioreactor system, with an internal floating filter or an external hollow filter.
- The system was tested with multiple cell lines in various commercial media and in different scales. Top viable cell density was 214 × 10^6 cells/mL and the IgG productivity reached 2 g/L/d.