Perfusion culture using TFF or ATF as cell retention method

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Perfusion culture using TFF or ATF as cell retention method

This work demonstrates perfusion culture of an antibody-producing Chinese hamster ovary (CHO) cell line, using either tangential flow filtration (TFF) or alternating tangential flow filtration (ATF) as cell retention method. In both setups, identical Xcellerex™ XDR-10 bioreactor systems and hollow fiber filter cartridges were used.

Introduction

Perfusion processes have many advantages such as being favorable for product quality and supporting efficient facility utilization by offering the possibility of using smaller bioreactors. Compared with batch or fed-batch processes, perfusion processes also allow cells to be maintained in exponential growth phase for an extended period of time and reach higher viable cell densities.

There are several cell retention methods available for perfusion cultures, of which one is TFF. In contrast to normal flow filtration (NFF), where the medium is pumped through a membrane filter, a peristaltic pump is used to recirculate the cell culture supernatant over the permeable membrane surface, which reduces the risk of filter fouling. In TFF, liquid and compounds with molecular weights less than the membrane cut-off can pass through the membrane (permeate), whereas larger molecules are retained (retentate). ATF uses the TFF technique, but in ATF, a diaphragm pump is used to alternate the flow direction over the membrane surface.

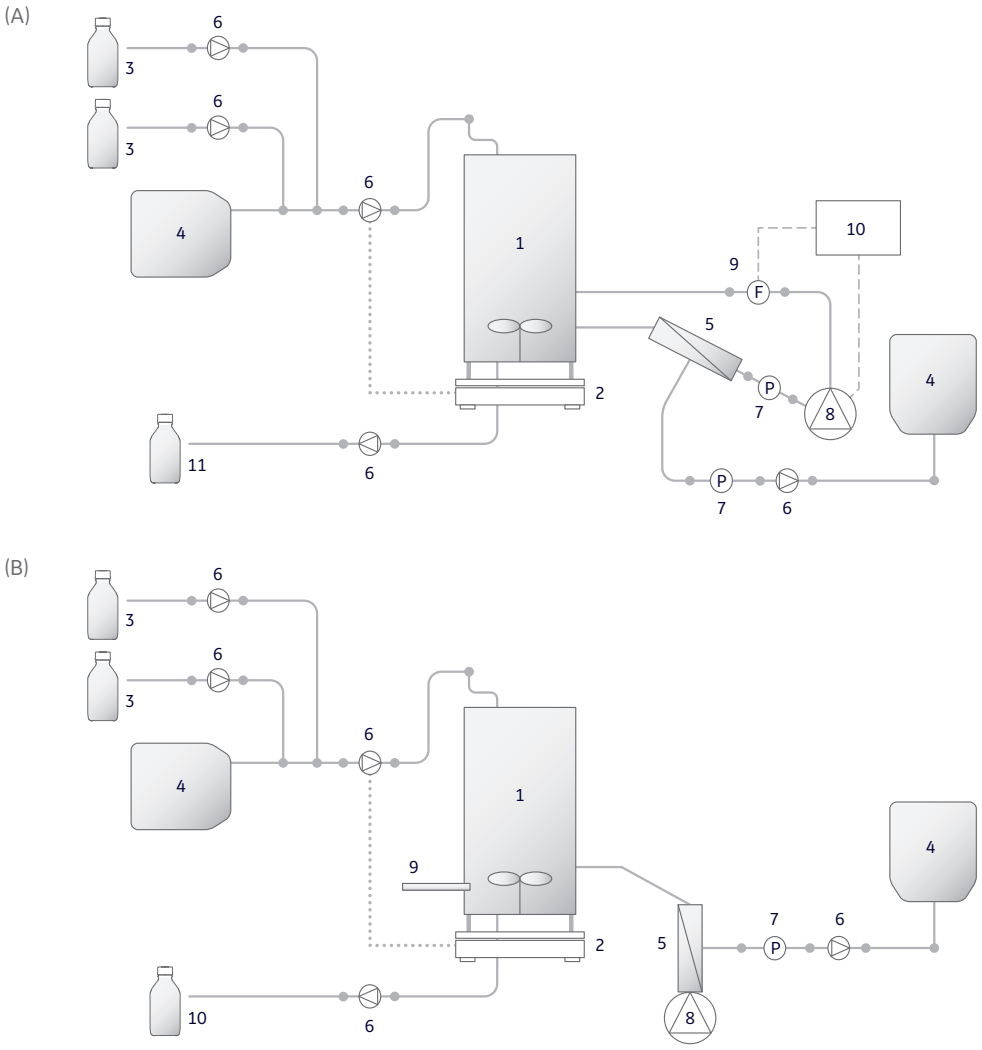
Similar performance in terms of cell growth and productivity has been achieved with ATF and TFF when used as cell retention methods in perfusion culture conducted in rocking bioreactor systems (1, 2). This work demonstrates perfusion culture conducted in the single-use XDR-10 stirred-tank bioreactor system (Fig 1), using either TFF or ATF as cell retention method. Process goals were cell densities above 25 × 10^6 cells/mL and cell-specific perfusion rate (CSPR) within 25–40 pL/cell/day and a steady state perfusion for a minimum of five days.

Fig 1. The single-use XDR-10 stirred-tank bioreactor system.

Materials and methods

Cell culture

Monoclonal antibody (mAb) production in Chinese hamster ovary (CHO) cells was used as model process. Cells were grown in HyClone™ ActiPro™ basal medium supplemented with 6 mM L-glutamine and poloxamer 188. A 5 g/L poloxamer 188 concentration was selected, consistent with another study (3). Glucose was added as needed via a peristaltic pump and amino acids were added during the perfusion culture. The process was conducted in perfusion mode using the setups shown in Figure 2. Recirculation flow rates were 1 L/min, with a pulse rate of 10 pulses/min (each 100 mL) for the ATF 2 system setup. Culture parameters are listed in Table 1.



Setup of TFF experiment

1 XDA-10 Pro Plus bioreactor bag
2 Scale
3 Feed addition bottle
4 ReadyToProcess™ 50 L pillow bag for feed/harvest
5 Hollow fiber filter 0.65 µm 990 cm²
6 Peristaltic pump
7 Pressure sensor
8 PuraLev™ i30 pump (Levitronix)
9 Flow sensor
10 Flow control
11 Bleed container

Silicon/C-Flex® tubings
Feedback loop from scale to feed pump
Feedback loop from flow sensor to pump

Setup of ATF experiment

1 XDA-10 Pro Plus bioreactor bag
2 Scale
3 Feed addition bottle
4 ReadyToProcess 50 L pillow bag for feed/harvest
5 Hollow fiber filter 0.65 µm 990 cm²
6 Peristaltic pump
7 Pressure sensor
8 XCell™ ATF 2 System with diaphragm pump (Repligen)
9 Incyte probe (Hamilton)
10 Bleed container

Silicon/C-Flex tubings
Feedback loop

Fig 2. XDR-10 bioreactor process setups using either (A) TFF or (B) ATF as cell retention method.

Table 1. Process conditions

Parameter	Settings/comment for XDR-10 bioreactor system
Production medium	ActiPro basal medium supplemented with 6 mM L-glutamine and poloxamer 188. Glucose was added as needed. Amino acids were added during the perfusion.
Poloxamer 188	5 g/L
Starting viable cell density	0.6×10^6 viable cells/mL
Operating volume	4.5 to 5.0 L
Impeller speed	150 rpm
Temperature	37°C
pH set point	7.1
pH PID settings	Optimized PID
DO set-point	30% (controlled by oxygen enriched air)
DO PID settings	Factory default settings
Spargers	1.0 mm: air, CO_2 20 µm: air, O_2
Oxygen flow	PID controlled
Carbon dioxide flow	PID controlled

Results

Perfusion process using TFF as cell retention method

The perfusion setup with a TFF filter as cell retention devise is shown in Figure 3. In this setup, a peak cell density of 35 × 10^6 cells/mL was reached on Day 6 (Fig 4). An average CSPR of 33 pL/cell/d was achieved during steady state and the average volumetric production was 0.27 g/L/d during the five days of stable perfusion (Fig 5). Bleeding of the culture was started on Day 6, when reaching peak density. After five days of stable perfusion, the culture was ended.

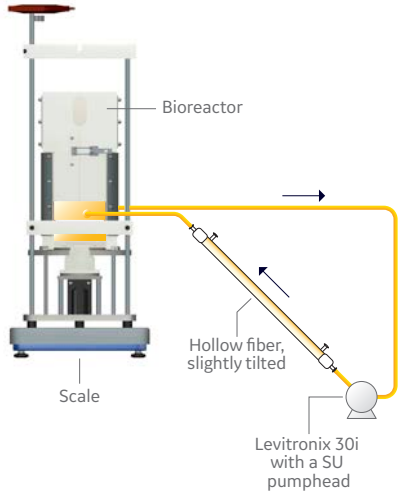


Fig 3. Perfusion setup with XDR-10 bioreactor system, using TFF as cell retention method.

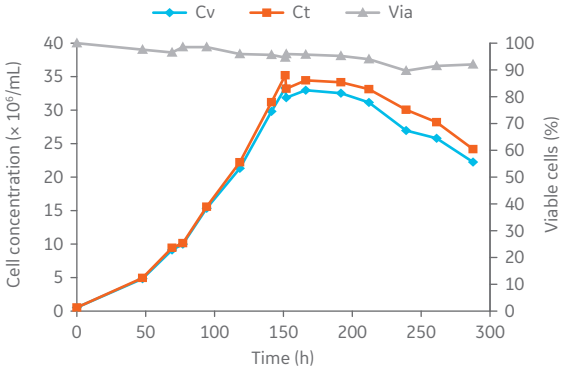


Fig 4. Cell growth and viability for perfusion culture using TFF as cell retention method.

Fig 5. CSPR, product titer, and vessel volumes per day (VVD) for perfusion culture using TFF as cell retention method.

Perfusion process using ATF as cell retention method

The perfusion setup with an ATF filter as cell retention devise is shown in Figure 6. In the AFT setup, a peak cell density of 47 × 10^6 cells/mL was reached on Day 6 (Fig 7), when bleeding of the culture was started. The Incyte sensor measuring the VCD was used to follow cell growth in-line in the ATF cultivation. The VCD sensor can also be used for automatic bleeding of cultures to keep them healthy. An average CSPR of 30 pL/cell/d was achieved and the average volumetric production was 0.38 g/L/d during the seven days of stable perfusion (Fig 8). No sign of filter fouling was observed during the process.

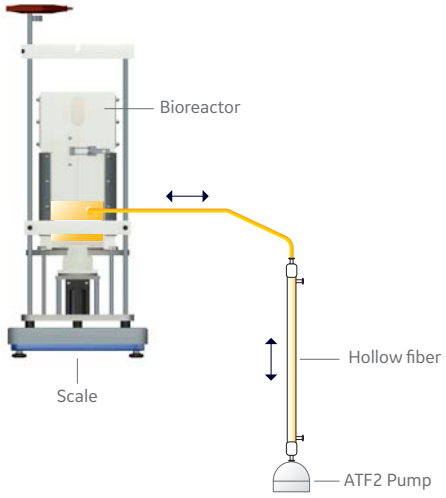


Fig 6. Perfusion setup with XDR-10 bioreactor system, using ATF as cell retention method.

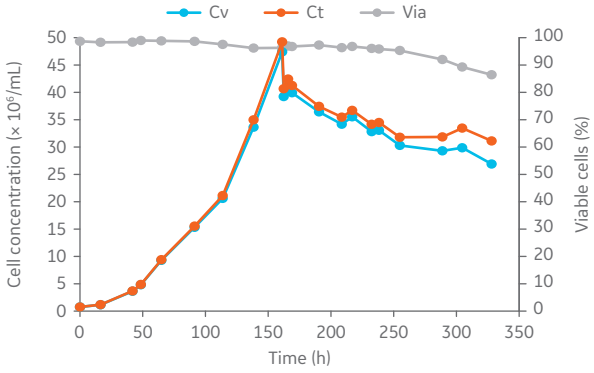


Fig 7. Cell growth and viability for perfusion culture using ATF as cell retention method.

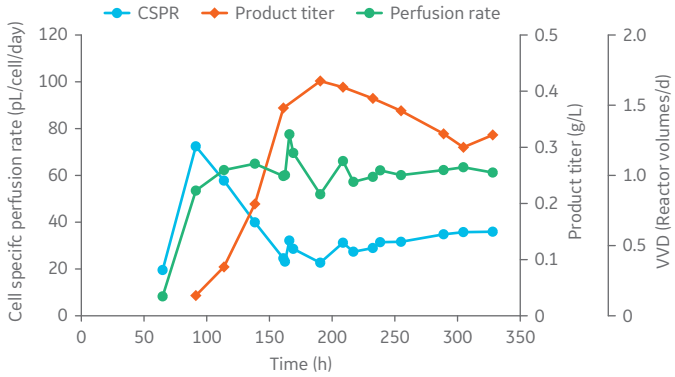


Fig 8. CSPR, product titer, and vessel volumes per day (VVD) for perfusion culture using ATF as cell retention method.

Discussion

The presented results show that perfusion culture can successfully be conducted in the single-use XDR-10 bioreactor system using either TFF or ATF as cell retention method. Ultimately, the characteristics of selected media and cell line will play a major role in the process performance. In addition, process performance will be significantly affected by the area and pore size of the selected filter.

Here, the bioreactor system was connected to the single-use filter unit through disposable tubing. For fully disposable solutions, single-use pump systems can be used in the setups. Single-use equipment avoided the need for cleaning and associated validation, reducing setup and changeover times. Single-use equipment also minimized the cross-contamination risk, as all process components that have been in contact with the process material, including the filter cartridge, can be conveniently disposed after use without the need for open handling of the product.

ReadyToProcess hollow fiber filter cartridges were used for cell retention as these filters are especially suited for processes where the process stream needs to be contained for health and safety reasons.

Conclusion

This work describes two perfusion process setups, using either ATF or TFF as cell retention method. The XDR-10 bioreactor system and a hollow fiber cartridge with a filter area of 990 cm^2 were used in both setups, differing only in cell retention unit. Though the filter fouling was slightly faster with the TFF compared with the ATF setup, the results show comparable performance between the two setups.

References

1. Clincke, M.F. *et al*. Very high density of CHO cells in perfusion by ATF or TFF in WAVE Bioreactor™. Part I. Effect of the cell density on the process. *Biotechnol Prog* **29**, 754–767 (2013).
2. Clincke, M.F. *et al*. Very high density of Chinese Hamster ovary cells in perfusion by alternating tangential flow or tangential flow filtration in WAVE Bioreactor. Part II: Applications for antibody production and cryopreservation. *Biotechnol Prog* **29**, 768–777 (2013).
3. Xu, S. *et al.* Impact of Pluronic F68 on hollow fiber filter-based perfusion culture performance. *Bioprocess Biosyst Eng.* **40**, 1317–1326 (2017).

Ordering information

Product	Description	Product code
XDA-10 Pro Plus bioreactor bag	10 L, pitch blade impeller, one disc each of 2 µm, 20 µm, 0.5 mm, and 1 mm spargers.	888-2-0396-C
ReadyToProcess pillow bag	50 L	12410228
ReadyToProcess hollow fiber filter cartridge	0.65 µm pore size, 990 cm^2 filter area, 60 cm cartridge length	CFP-6-D-4X2MA
HyClone ActiPro	Cell culture medium formulated to provide high yields of recombinant proteins in processes using CHO cell lines.	SH31037.05

To order the XDR-10 system, please contact your local sales representative.

gelifesciences.com/bioprocess

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