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Small Scale Perfusion Using an ATF Cell Retention Device with DASGIP® Parallel Bioreactor System

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Abstract

Experimentation at small scale is crucial for the cost-efficient development of bioprocesses, which then can be transferred to larger production volumes. To facilitate perfusion cell culture process development at small scale, we tested the feasibility of connecting an alternating tangential flow (ATF) filtration device with a DASGIP Parallel Bioreactor System. In a working volume of 1 L we reached a peak viable density of 60 x 10^6 cells/mL. Cell growth, peak density, and antibody production at the 1 L scale were comparable to those in a previously performed perfusion process with a working volume of 3.75 L. Our results demonstrate the feasibility of ATF-based perfusion at small scale using the DASGIP Parallel Bioreactor System.

Introduction

Upstream bioprocessing in perfusion mode holds great promise for the industrial production of cells and biologics. In perfusion, fresh medium is constantly added to the bioreactor and used medium is harvested, while the cells are retained in the bioreactor. As a result, the composition of the cell culture medium stays quite constant during the process. This has several advantages. In perfusion, higher cell densities can be reached than in batch and fed-batch processes and therefore the volumetric productivity will be enhanced. As the medium composition may influence the cell metabolism and therefore product characteristics, more constant process conditions can lead to a more consistent product quality. Products which pass the cell retention device are constantly harvested. The time they reside in the culture medium is reduced, which is advantageous for the production of less stable products.

Apart from being used for the production of secreted proteins or viral vectors, perfusion is used in the earlier sub-steps of a bioprocess, as in the production of high-density seed trains, or to reach high cell densities before transfection/infection of cells for viral vector production. In all cases, perfusion bioprocessing requires a cell retention device within or attached to the bioreactor.

Cell retention devices based on ATF are filters that hold back the cells and let the liquid and small molecules pass. In tangential flow filtration (TFF) the liquid flows past the pores of hollow fiber filters tangentially, rather than being forced through them orthogonally, thus reducing the likelihood of clogging. ATF devices use the same principle of tangential flow, but reverse the direction of flow regularly to minimize fouling and reduce shear forces on the cells. ATF perfusion is suitable for perfusion processes with suspension cells.

ATF cell retention devices support a broad range of bioreactor scales from small to pilot/production. The objective of this proof of concept study was to demonstrate feasibility of utilizing ATF for small scale (1 L) perfusion of CHO cells in the DASGIP Parallel Bioreactor System.

Material and Methods

Connection of DASGIP Parallel Bioreactor System with ATF cell retention device

We used a DASGIP Parallel Bioreactor System with Bioblock. The system was equipped with a DASGIP MP8 Multi Pump Module, a DASGIP TC4SC4 temperature and agitation control module, a DASGIP PH4PO4 module for pH and DO monitoring, and a DASGIP MX4/4 gas mixing module, supporting gas flow rates of up to 250 sL/h. High maximum flow rates of air and oxygen are beneficial for cell culture perfusion bioprocesses, because high cell densities can be reached and the oxygen demand of the culture may become high.

We used a DASGIP Bioblock Stirrer Vessel with a working volume of 200 mL – 1 L equipped with a pitched-blade impeller. The connection of a DASGIP Parallel Bioreactor System with ATF cell retention device is shown in Figure 1. For medium harvest the bioreactor was equipped with a dip tube (length 250 mm, diameter 12 mm, Repligen®, USA). The dip tube was connected to a XCell™ ATF 2 (Repligen) via silicone tubing and a CPC™ AseptiQuik® fitting (3/8" hose barb fitting).

We installed the dip tube opposite of the gas sparger as recommended by the manufacturer, ensuring no bubbles from gassing would enter the ATF filter. The filter had a pore size of 0.2 µm. The device was connected to an ATF controller (C24 Controller, Repligen). The ATF circulation rate was set to 0.5 L/min.

Medium was added and used medium was harvested using the system's integrated pumps. Depending on the pump head tube diameter, the pumps support feed rates of

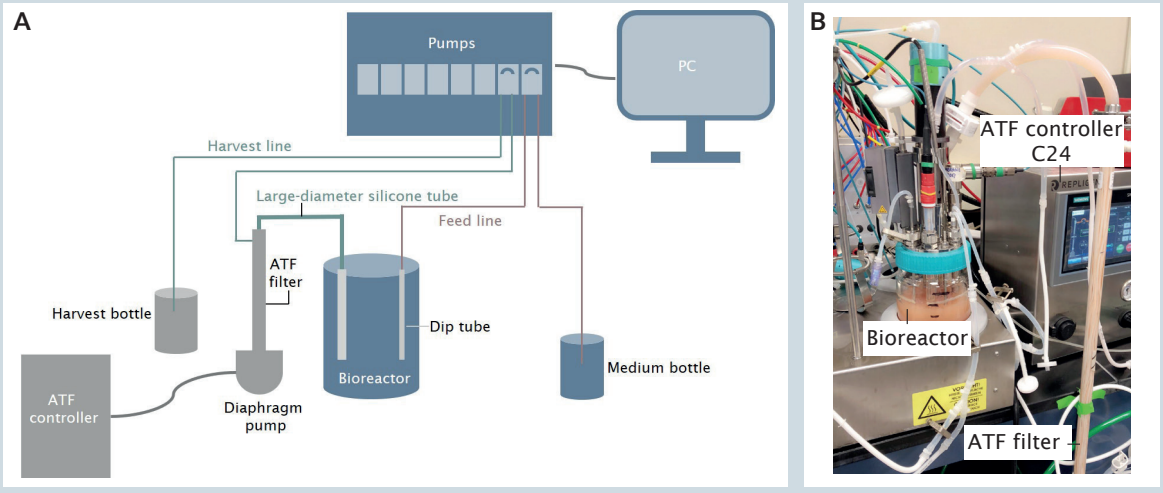

Fig. 1: Connection of DASGIP Parallel Bioreactor System with ATF cell retention device.
A: Schematic overview
B: Photo of the setup

Attention: In case of a rupture of the ATF membrane the pressure inside the vessel can increase.
> To avoid rupture of the vessel, maintain an unrestricted air vent.
> This can be done by installing a pressure relief valve on the head plate of the bioreactor.
> It needs to be ensured that there is no filter between safety valve and vessel to enable an unrestricted air vent.

0.3 to 9.5 mL/h or 13 to 420 mL/h. The broad feed rate range allows the adjustment of the perfusion rate during the run. In the experiments here we used pump head tubing with an inner diameter of 0.5 mm, supporting feed rates of 1.3 to 42 mL/h. Feeding and harvest volumes can also be matched using two integrated scales, one for feeding and one for harvesting. This allows for tight control over liquid additions and the removal of wastes from the vessel, maintaining exact vessel volume.

Cell line and medium

We used a suspension CHO cell line from TPG Biologics, Inc., expressing an hMAb. We cultivated the cells in Dynamis™ AGT™ Medium (Thermo Fisher Scientific®, USA). The medium was supplemented with 8 mM L-glutamine and 1 % Gibco® Anti-Clumping Agent (Thermo Fisher Scientific).

Cultures were perfused with Dynamis AGT Medium supplemented with 2 mM L-glutamine and 1 % Anti-Clumping Agent. Glutamine feeding concentration was decreased to reduce ammonia production during the run.

Inoculum preparation

We prepared the bioreactor inoculum by cultivating the cells in single-use baffled polycarbonate shake flasks in a New Brunswick™ S41i CO_2 incubator shaker set at 125 rpm and 8 % CO_2 with passive humidification. Cells from a cryopreserved stock vial were inoculated at a density of 0.3 x 10^6 cells/mL in a 125 mL flask with a 20 % fill volume.

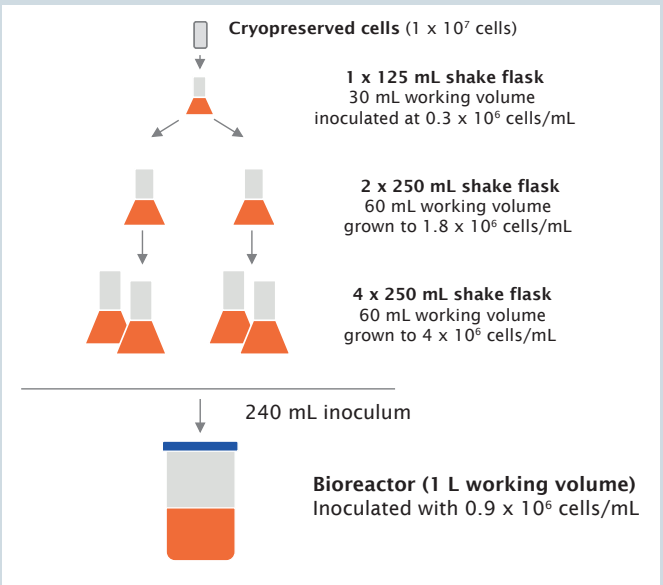

Fig. 2: Cell expansion from initial thaw to bioreactor inoculation

After one week of passaging every other day, we scaled-up the culture volume by increasing the flask size from 125 mL to 250 mL, while keeping the inoculation density, percentage fill, and all other parameters constant. A flow chart representing the cell expansion process from initial thaw to inoculation of the bioreactor is shown in Figure 2.

Bioreactor control and process parameters

The glass vessel was equipped with a pitched-blade impeller. The culture was agitated at 309 rpm. We measured DO using a polarographic sensor (Mettler Toledo®, Switzerland) and controlled it at 50 % by sparging air and/or O_2. The pH was controlled at 7.0 (deadband = 0.2) via a cascade to CO_2 (acid) and 0.45 M sodium bicarbonate (base). We inoculated the culture with a final cell density of 0.9 x 10^6 cells/mL. We cultivated the cells at 37 °C.

Sampling and analytics

We took two 3-mL samples from the bioreactor daily, one in the morning and one in the evening, to analyze cell density, viability, and the concentrations of glucose, ammonia, lactate, and IgG. The samples were taken through a dip tube connected to a swabable valve. To collect the highest quality sample from the growing culture, we connected a sterile 5 mL syringe to the sample port Luer Lock and removed and discarded a dead volume of 3 mL. We then collected a second 3-mL sample in a new syringe to provide a fresh, viable sample for analytics. The total bioreactor volume was readjusted to compensate volume lost through sampling after every sample.

We measured cell density and viability (via the trypan blue exclusion method) using a Vi-Cell® XR Viability Analyzer (Beckman Coulter®, USA), and pH using an Orion Star™ 8211 pH meter (Thermo Fisher Scientific). Using the offline pH value, we restandardized the controller pH calibration daily, if necessary, to prevent any discrepancy between online and offline measurements. Glucose, ammonia, lactate, and hMAb concentrations were measured using a Cedex® Bio Analyzer (Roche Diagnostics®, Germany).

Feeding and perfusion control

Our perfusion target was to keep the ammonia concentration < 4 mM. We also aimed to keep the glucose concentration > 3 g/L. We adjusted the perfusion rate and fed the culture with glucose based on the metabolite concentrations determined offline.

We turned on the ATF device a few hours prior to inoculation and began media circulation through the filter at our setpoint for the run (0.5 L/min), as recommended by the manufacturer. The filter could then be properly wetted by

media circulation and allow for any air bubbles to gradually work themselves out of the filter prior to adding cells to our vessel. As per manufacturer recommendations, we started the ATF circulation of media and cells 5 hours before it was needed to initiate perfusion so that our cells could adjust to the alternating tangential flow-related stress.

Results

Metabolite concentrations

We aimed to keep the ammonium concentration < 4 mM and the glucose concentration > 3 g/L in the course of the run. On day 2 the ammonia concentration exceeded 3 mM and we started perfusion at 0.2 vessel volumes per day (VVD). Based on the ammonia concentrations determined offline, we gradually increased the perfusion rate up to 1.4 VVD. The ammonia concentration stayed below 4 mM until day 15. The lactate concentration increased up to 1.9 g/L on day 2. With the start of perfusion on day 2 it decreased, and was kept below 1 g/L from day 6. When the glucose concentration dropped below 3 g/L, we fed the culture by pumping in the appropriate amount of 200 g/L sterile glucose into the culture twice daily, in addition to replenishing the glucose throughout the perfusion process (Figure 3).

Cell growth and antibody production

We had inoculated the culture at a density of 0.9 x 10^6 cells/mL. On day 13, the culture reached a peak cell density of 60.88 x 10^6 cells/mL. At that time point, 90 % of the cells were viable (Figure 4). The IgG concentrations in the bioreactor and harvest increased up to approximately 500 mg/L. As expected, IgG production steadily increased following the cell growth profile (Figure 5).

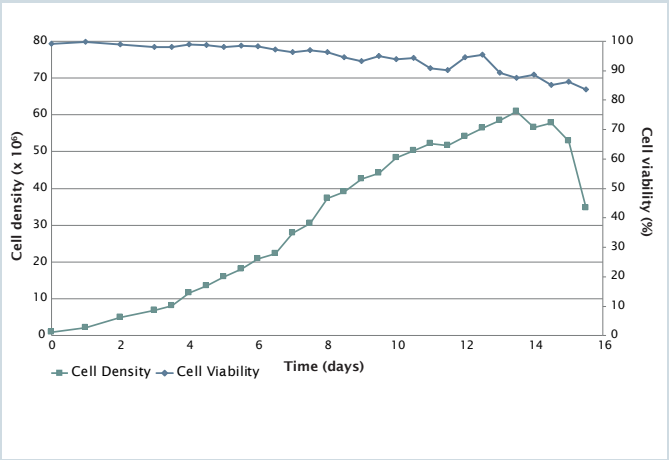

Fig. 4: Cell growth and viability. Samples were taken twice daily and cell number and viability were analyzed offline.

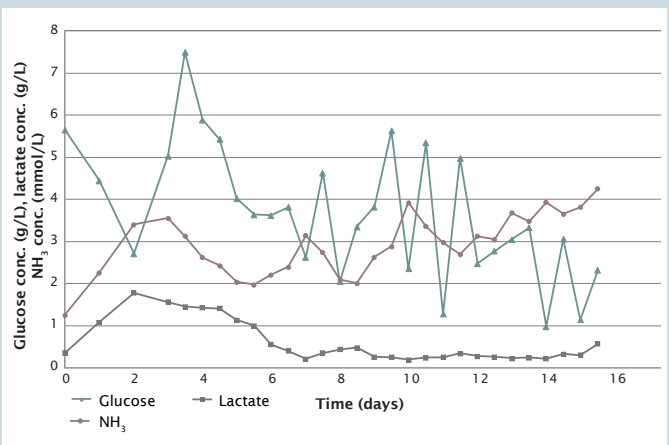

Fig. 3: Metabolic profile. Samples were taken twice daily and the concentrations of glucose, lactate, and ammonia were determined offline.

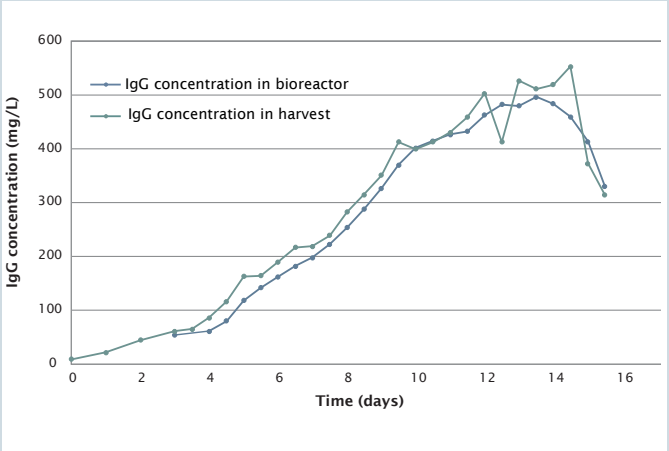

Fig. 5: IgG production. Samples were taken twice daily from the culture medium in the bioreactor and the harvest. The IgG production in culture medium and harvest were determined offline.

Conclusion

The results described above demonstrated the feasibility of cultivating CHO cells using perfusion in a DASGIP Parallel Bioreactor System equipped with an ATF cell retention device. The system allowed cell culture perfusion at small scale, which is well suited for process development. The system's pumps support a broad range of perfusion rates. The system's broad gas flow ranges help meeting the oxygen demand of high-density cultures.
In a previous study we had cultivated the same cell line in perfusion using a custom BioBLU c Single-Use Vessel (maximum working volume of 3.75 L) equipped with an ATF-2 device from Repligen and controlled with a BioFlo 320 bioprocess control station [1]. Likewise, an ammonium concentration of less than 4 mM and a glucose concentration higher than 3 g/L were targeted. The peak viable cell density was 74 x 10^6 cells/mL at day 15. The fact that we achieved similar cell densities in CHO cell perfusion processes with working volumes of 1 L and 3.75 L indicates the scalability of the system. Larger ATF filtration devices are available, which in principle support perfusion cultures with working volumes of more than 1,000 L.

Literature

[1] Willard S, et al. Comparing Culture Methods in Monoclonal Antibody Production. *Bioprocess International*. 15 (3): 38–46. 2017

Ordering information

Description	Order no.
DASGIP® Parallel Bioreactor System, for microbial applications, max. 250 sL/h gassing, 4-fold system with DASGIP® Bioblock	76DG04MBBB
DASGIP® Vessel, SR0700ODLS, 200 mL – 1.0 L	76SR0700ODLS
New Brunswick™ S41i, 170 L, CO_2 incubator shaker with inner shelf and touch screen control, 1 (2. optional) shelves, orbit diameter 2.5 cm (1 in)	S41I120010

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