APPLICATION NOTE No. 457

Integration of an ATF Device with a DASbox® Mini Bioreactor System for Cell Culture Perfusion at Small Scale

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Abstract

Bioprocess development usually starts at small scale, before the process is scaled-up to pilot production volumes. While process development at small scale is routinely done for batch and fed batch bioprocesses, small scale models of perfusion processes using tangential flow filtration are less well established. This application note describes the setup of a perfusion bioprocess system at small scale using a DASbox Mini Bioreactor System and glass bioreactors with a maximum working volume of 250 mL. The system was suitable for the cultivation of CHO and HEK293 cells in perfusion for a prolonged period and facilitated achieving cell densities of 80 × 10^6 cells per mL and more.

Introduction

Upstream bioprocessing in perfusion mode holds great promise for industrial production of cells and biologics. In perfusion, fresh medium is added constantly to the bioreactor, and used medium is harvested while the cells are retained in the bioreactor. This configuration allows nutrients to be replenished and byproducts to be removed. Consequently, in perfusion, higher cell densities can be reached than in batch and fed-batch processes and the process time can be prolonged. The increase in volumetric productivity allows the usage of smaller bioreactors, which saves lab space and potentially reduces capital expenditure compared to fed-batch processes. Furthermore, it has been reported that production in perfusion mode can improve certain critical quality attributes, such as protein glycosylation profiles [1, 2].

During bioprocess development the process is characterized and process parameters and medium composition is optimized. Small scale perfusion systems are desired to save resources and reduce costs, especially for culture media, which are a main cost driver in cell culture bioprocessing. If these bioreactor systems can be operated in parallel, there is additional potential to save time and minimize footprint.

The aim of this project was to develop a fully continuous, small scale perfusion bioreactor setup using alternate tangential flow filtration (ATF) for cell retention. A critical requirement of the system is that it should be suitable for achieving very high cell densities of up to 1 × 10^8 cells/mL [3].

Initial experiments were done with CHO cells. They are currently the most commonly employed cell type in perfusion processes. The current setup was subsequently adapted for the cultivation of HEK293 cells, which are less well established for biopharmaceutical applications. However, since they are of human origin, promise several advantages for the production of biologics.

Material and Methods

Perfusion bioreactor setup

For bioprocessing in perfusion mode, a DASbox Mini Bioreactor System equipped with DASbox glass bioreactors was used (Figure 1A). The bioreactors had a total volume of 350 mL and were used with a working volume of 230 mL.

Fig. 1A: DASbox Mini Bioreactor System.
For more technical information on the DASbox Mini Bioreactor System please visit www.eppendorf.group/dasbox

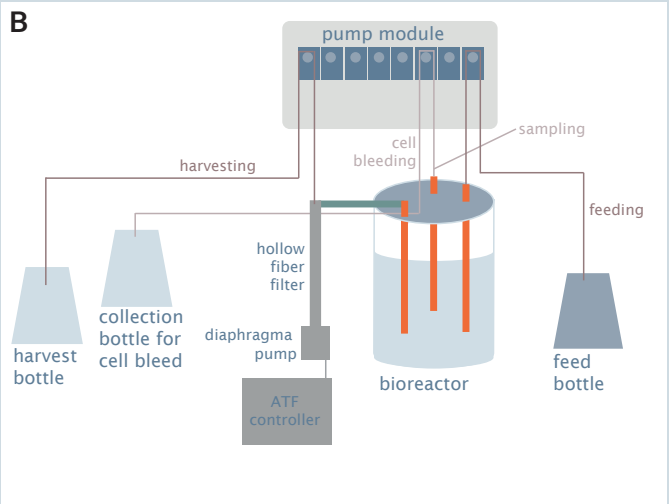

Fig. 1B: Setup of the perfusion system. Please refer to the text for details.

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 the safety valve and the vessel to enable an unrestricted air vent

For cell retention, a hollow fiber filter Xampler® CFP-2-E-3MA microfiltration cartridge (GE Healthcare®) was used. It had a pore size of 0.2 µm and a membrane area of 110 cm^2. To use it in ATF mode, the hollow fiber filter was connected to a XCell® ATF 2 system (Repligen®). The flow rate recirculation was set to 200 mL/min.

The bioreactor was equipped with dip tubes (Figure 1b, orange) to transfer culture medium to add and remove culture medium and cell broth from the bioreactor through feed lines (Figure 1B, purple):

> Long dip tube, through which the culture broth was pumped into the hollow fiber filter. Hollow fiber filter and dip tube were connected with a silicone tubing with an inner diameter of 5 mm (Figure 1B, green).
> Long dip tube, through which feed was added using the peristaltic pumps of the DASbox MP8 module
> Long dip tube equipped with a T-connector, through which samples were taken and cell bleeding was performed using the peristaltic pumps of the DASbox MP8 module

The headplate assignment of the DASbox Mini Bioreactor is illustrated in Figure 2.

Cell lines

The following cell lines were used:

> CHO-M cells, producing IgG (Selexis®)
> HEK293 F cell line, producing recombinant human Erythropoietin (rhEPO) (KTH inhouse development)

Culture media

The following culture media were used:

> **CHO preculture:** BalanCD CHO Growth A medium (Irvine Scientific®) + 4 mM glutamine
> **HEK293 preculture:** BalanCD HEK293 medium (Irvine Scientific) + 4 mM glutamine + 50 mg/L puromycin dihydrochloride (Sigma-Aldrich®)
> **CHO perfusion culture:** BalanCD CHO Growth A medium (without glucose) + 100 mg/L streptomycin + 60 mg/L penicillin G (both Sigma-Aldrich) + glucose and glutamine according to cell's needs
> **HEK293 perfusion culture:** HEK293 feed medium A, consisting of BalanCD HEK293 medium + 5 % BalanCD HEK293 feed + glucose and glutamine according to cell's needs

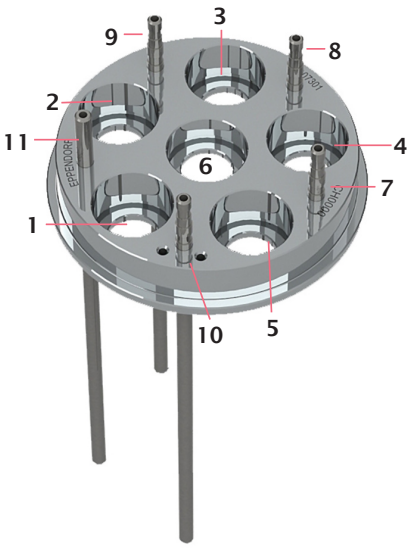

Fig. 2: Head plate assignment for the DASbox Mini Bioreactor used in perfusion bioprocess.

	Port	Port accessory	Associated device	Purpose	Connected to
1	**Pg 13.5**	Compression fitting	Exhaust condenser with silicone tubing and filter	Exhaust treatment	DASbox base unit
2	**Pg 13.5**	Compression fitting	L-sparger with silicone tubing and filter	Gassing	DASbox base unit
3	**Pg 13.5**	Compression fitting	4 mm dip tube with silicone tubing connected to hollow fiber cartridge for ATF	Cell separation for harvest removal	ATF2 system; harvest line to DASbox MP8-PHPO module
4	**Pg 13.5**	–	pH sensor	pH monitoring	DASbox MP8-PHPO module
5	**Pg 13.5**	–	DO sensor	DO monitoring	DASbox MP8-PHPO module
6	**Pg 13.5**	–	Lipseal stirrer assembly	Agitation	Overhead drive and DASbox base unit
7	**Dip tube short**	–	Silicone tubing connected to base bottle	pH regulation	DASbox MP8-PHPO module
8	**Dip tube short**	–	Silicone tubing with filter	Headspace aeration with oxygen, air, CO_2 for pH and DO regulation	DASbox base unit
9	**Dip tube long**	–	Silicone tubing with T-connector for sampling port and bleed line	Sampling and cell bleed	DASbox MP8-PHPO module
10	**Dip tube long**	–	Silicone tubing connected to feed bottle	Perfusion medium inlet	DASbox MP8-PHPO module
11	**Thermowell**	Thermowell	Platinum RTD temperature sensor (Pt100)	Temperature monitoring	DASbox MP8-PHPO module

Table 1: Process parameters

Parameter	Device/setpoint
Bioreactor system	DASbox Mini Bioreactor System
Bioreactor	Glass bioreactor (total volume 350 mL)
Working volume	230 mL
Inoculation density	5 × 10^6 cells/mL (CHO cell bioprocess) 1 × 10^6 cells/mL (HEK293 cell bioprocess)
Temperature	37 °C
pH	7.0 (controlled by adding 0.5 M Na_2CO_3 or CO_2 to the headspace)
DO	40 % (adjusted by headspace gassing with air enriched in O_2 or N_2; if headspace oxygenation becomes limiting, in addition sparging with 0.0035-0.07 VVM oxygen through L-sparger)

The feeding strategy in all perfusion runs aimed at keeping the concentration of lactate below 20 mM and the concentration of ammonium below 4 mM.

Perfusion process

Perfusion process parameters are summarized in Table 1. To prepare the bioreactor inoculum, cells were grown in shake flasks in a CO_2 incubator shaker at 37 °C and 5 % CO_2. After inoculation, the bioprocess was run in batch mode for one day. Subsequently, the perfusion mode was started by initially feeding and harvesting 0.5 bioreactor volumes per day using the peristaltic pumps of the DASGIP MP8 module. When the cell density increased after a couple of days, a cell specific perfusion rate of 50-60 pL/cell/day was applied. The estimation of the cell specific perfusion rate was based on a daily cell density measurement. The culture media used during preculture and perfusion culture are described above.

In some experiments, cell suspension was continuously removed from the bioreactor at a rate equivalent to the growth rate, to maintain constant cell density (cell bleeding).

Analytics

The viable and total cell density, viability, and pCO_2 were analyzed using a BioProfile® Flex Analyzer (Nova Biomedical®).

The concentrations of the products IgG and rhEPO were quantified through protein A affinity HPLC and an EPO human ELISA kit (Thermo Fisher Scientifc®), respectively [3].

The sieving coefficient was calculated as the ratio of product concentration in the harvest to product concentration in the bioreactor. It describes how much of the product is transferred from the bioreactor to the harvest through the hollow fiber filter. A low sieving coefficient indicates that the transfer of the product is impaired, for example caused by clogging of the filter membrane.

Oxygen transfer to the bioreactor

Different aeration strategies were tested to optimize oxygen transfer to the bioreactor. The strategies differed in the impeller configurations and the gassing strategy.

The following impeller setups were tested:

> One marine-type impeller placed at the bottom of the impeller shaft
> Two marine-type impellers, one placed placed at the bottom of the impeller shaft and one placed at the top at the liquid/headspace interface
> One Rushton-type impeller placed at the bottom of the impeller shaft
> One Rushton-type impeller placed at the top of the impeller shaft at the liquid/headspace interface and one marine-type impeller placed at the bottom of the shaft

Marine-type impellers are commonly used for cell culture bioprocesses that require gentle mixing. Marine-type impellers produce an axial flow, so their mixing efficiency tends to be slightly lower than those of impellers producing both radial and axial flows.

Rushton-type impellers produce a radial flow. Their mixing efficiency is higher than that of marine-type impellers, however higher shear forces are generated.

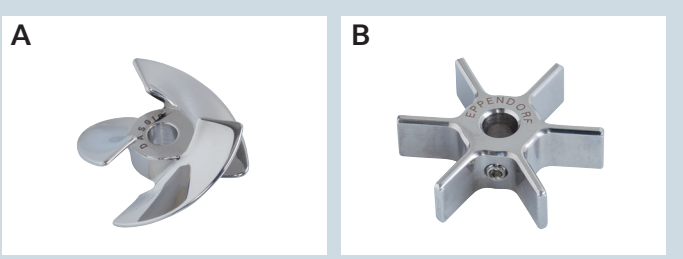

Fig. 3: Impeller types
A. Marine-type impeller **B.** Rushton-type impeller

For each impeller setup, two gassing strategies were compared

> Gassing into the bioreactor headspace (0.35 VVM air)
> Gassing into the bioreactor headspace (0.35 VVM air) and additionally through sparging using a L-sparger (0.035 VVM air)

Gassing into the bioreactor headspace is commonly used

for mammalian cell cultures. Gas transfer to the headspace is less efficient compared to gas sparging to the liquid, but gentler, as shear forces caused by sparging in gas bubbles to the medium and damaging effects from bubble rupture are avoided.

Oxygen transfer to the bioreactor at each configuration was assessed by determining the volumetric oxygen transfer coefficients ($\kappa_L a$) using the dynamic gassing out method [4].

Results

Initial experiments were carried out with CHO cells. Based on the initial results, the perfusion process was adapted for HEK293 cells.

CHO perfusion process

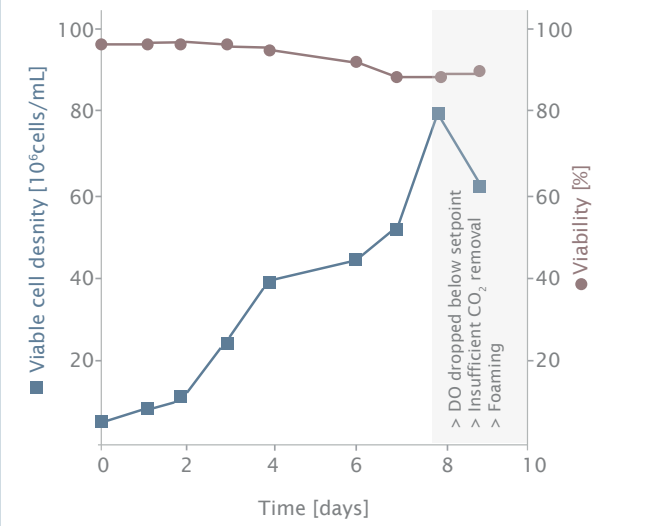

Fig. 4: CHO cell bioprocess in perfusion mode. Viable cell density and viability.

IgG-expressing CHO cells were cultivated in a DASbox Mini Bioreactor System equipped for perfusion as described in the Material and Methods section. The bioreactors were equipped with one marine-type impeller mounted at the bottom of the impeller shaft. The bioreactor was inoculated with 5×10^6 cells/mL and cultivated in batch mode for one day, before switching to perfusion mode. Within 8 days, the cell density increased to 80×10^6 cells/mL with a viability of around 90 % (Figure 4). Throughout the process, the IgG sieving coefficient was between 91 and 99 %.

When the cell density exceeded 80×10^6 cells the process control deteriorated. CO_2 was insufficiently removed from the liquid phase. The DO demand of the high-density culture could not be fulfilled, causing the DO to drop below the setpoint. A high stirring speed was used and a high gas flow rate of up to 0.07 VVM was sparged into the bioreactor in the attempt to control the DO at setpoint, which caused excessive foaming. The viability stayed high, indicating, that the CHO cells tolerated the suboptimal process conditions (Figure 4).

HEK293 perfusion process

In a first step, the experimental setup described above for CHO cell cultivation was tested for the cultivation of HEK293 cells producing rhEPO. In contrast to CHO cells, these cells did not tolerate the unfavorable process conditions, which occurred at high cell densities and which are described

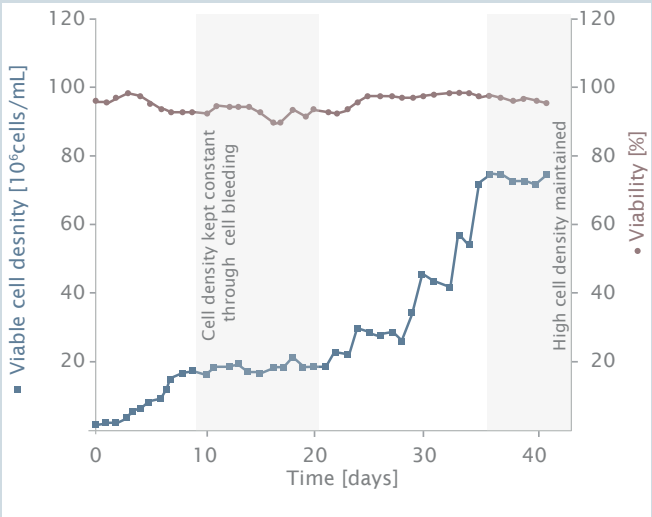

Fig. 5: HEK293 cell bioprocess in perfusion mode. Viable cell density and viability.

above. Therefore, the oxygen transfer to the bioreactor needed to be optimized.

To do so, the four impeller setups described in the Material and Method section were tested. Preliminary tests in batch processes indicated that all four setups are suitable for the cultivation of HEK293 cells.

Measuring the $\kappa_L a$ values showed, that the impeller configuration with one Rushton-type impeller placed at the top and one marine-type impeller placed at the bottom of the impeller shaft led to the highest oxygen transfer to the bioreactor. Therefore, this arrangement was chosen for the following experiments. The required $\kappa_L a$ can be estimated from the equation

$$X = \kappa_L a \times (C^*O_2 - [O_2]) / qO_2 \quad [5]$$

where X is the target cell density, C^*O_2 the saturation oxygen concentration, $[O_2]$ the oxygen concentration in the liquid and qO_2 the specific oxygen consumption rate. For example, for $X = 10^8$ cells/mL, $C^*O_2 = 1$ mM, $CO_2 = 0.1$ mM (at 50% air saturation) and $qO_2 = 5.77$ pmol/cell/day for CHO [6], the required $\kappa_L a$ is approximately 27 h^{-1}. This requirement was met by the optimized impeller configuration.

The process parameters are summarized in Table 1. The bioreactors were inoculated with 1×10^6 cells per mL. Around day 8 the cell density reached 20×10^6 cells/mL with viability of approximately 90 %. The cell density was maintained at this value for 14 days by applying cell bleeding. Subsequently, the cell density was stepwise increased by interrupting the bleed function up to 80×10^6 cells/mL with a viability around 95 %. The process was kept stable at these values for one week (Figure 5). Therefore, by optimizing oxygen transfer, a cell density comparable to the previous runs with CHO cells was reached.

The concentration of rhEPO was quantified at various time points and the volumetric productivity was calculated. It increased linearly with cell density, from approximately 129 mg/L/day at cell densities of 15 to 25 $\times 10^6$ cells/mL to approximately 392 mg/L/day at densities of 70 to 80 $\times 10^6$ cells/mL.

Conclusions

Using the DASbox Mini Bioreactor System, ATF perfusion cultivation of CHO cells and HEK293 cells in a working volume as low as 230 mL was established. Cell densities of up to 80 x 10^6 cells/mL were maintained at a viability > 90%, showing that high cell densities could be achieved at small scale, even though the bioreactor was slightly undersized for the ATF system used.

The described perfusion setup was suitable for process optimization at small scale. This application note describes only a part of the process optimization, namely oxygen transfer to the bioreactor. When optimizing a perfusion process, additional parameters need to be considered. Several experiments were conducted to analyze, how temperature, feed composition, and perfusion rate influenced process productivity and cell metabolism [3]. These findings demonstrate the potential of the presented small scale perfusion bioprocess system for screening process parameters at small scale in parallel in a time- and cost-saving manner.

Literature

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Ordering information

Description	Order no.
DASbox® Mini Bioreactor System, for cell culture applications, max. 5 sL/h gassing, 4-fold system	76DX04CC
DASbox® Vessel, 60 – 250 mL, overhead drive, marine-type impeller	DS0250ODSS
Rushton-Type Impeller, 6-blade, stainless steel, O.D. 30 mm, I.D. 5 mm	78107304
Pitched-Blade Impeller, 3-blade, 30° pitch, stainless steel, O.D. 30 mm, I.D. 5 mm, marine impeller	78107325

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