CHO CELL CULTURE

Clarification of a mAb-producing cell culture

NOTE
Product names and filter sheet grades may have changed since the application note was created.

Author: Bettina Ledergerber

FILTROX AG, Moosmühlestrasse 6, 9001 St.Gallen, Switzerland

E-Mail: applications@filtrox.com

Date: November 2021

Key terms: CHO, monoclonal antibodies, mammalian cells, cell culture, depth filtration, single-use, alluvial filtration, pre-coat, filter aid, purification, process optimization, diatomaceous earth

Content

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

Abstract

Depth filtration is one of the most efficient and economical types of filtration and is often used in the biotechnology industry as the first step after culturing of cells to produce a respective protein. The purification of a monoclonal antibody (mAb) produced by CHO (Chinese Hamster Ovary) cells will therefore be optimized in filtration experiments. In this application note, the challenges and possible solutions for the purification of mAbs from a mammalian cell suspension after cultivation are shown.

Up to now, the clarification of a respective CHO cell culture after cultivation has been carried out via three filtration steps. By adding a filter aid to the unfiltered cell culture, a filter cake is formed on top of the filter sheet, which results in an increased filtration capacity and thus, reducing the three process steps to two. The initial turbidity of over 2000 NTU is reduced to 26 NTU, which allows the subsequent sterile filtration via a 0.2 µm filter membrane. After this, further purification steps in the downstream process (DSP) can be carried out.

1 The Challenge

The clarification process of monoclonal antibodies can be realized in different ways. The cell harvest step is either the last step in the Upstream Process (USP) or the first step in the Downstream Process (DSP). Thus, the term Midstream Process (MSP) is used in this application note. After the successful removal of cells and cell fragments after cultivation, further purification steps of the DSP follow.

By applying the alluvial filtration technology (also known as cake filtration), larger particles like cells, cell fragments but also smaller impurities (e. g. host cell DNA (HCD) or host cell protein (HCP)) can be removed in an efficient way. An appropriate amount of filter aid is added to the unfiltered suspension (so-called body-feed), which builds up a filter cake on the filter sheet together with the solid particles, like cells or cell fragments, during filtration. This can significantly increase the filtration capacity compared to filtration without the addition of filter aid (see figure 1).

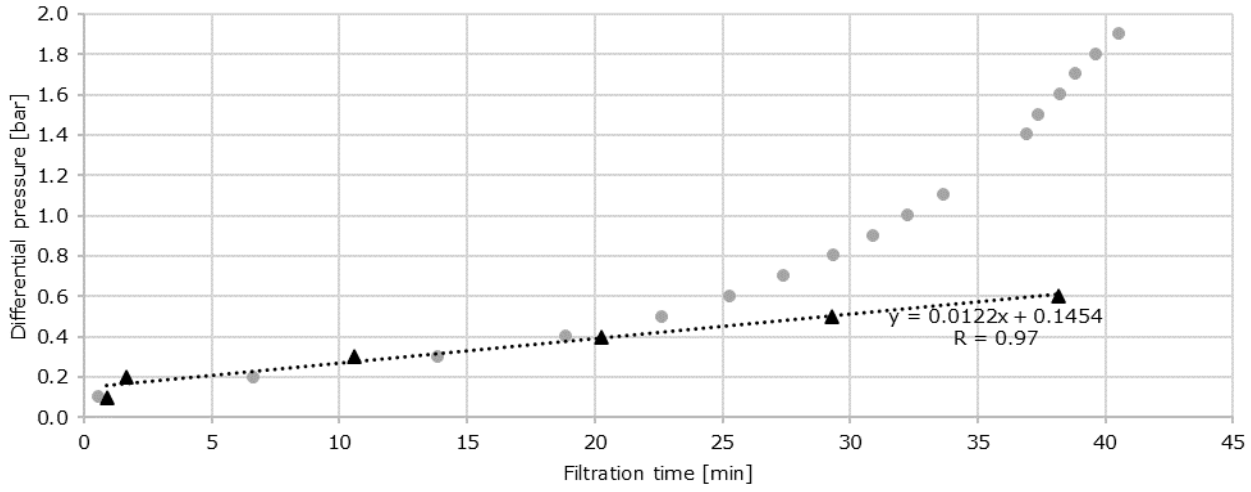

Figure 1: Comparison of standard depth filtration with alluvial filtration. Without the addition of filter aid, a rapid and exponential increase in differential pressure is monitored (●). By adding filter aids, a linear increase in pressure can be seen, which significantly increases the filtration capacity (▲).

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

In the presence Application Note, the optimization of the filtration process of a mAb-contained CHO cell culture is shown in detail. So far, the suspension is filtered in three steps, before a sterile filtration using a 0.2 µm membrane is applicable (see figure 2 below). To ensure the sterile filtration step, a flocculant has to be added prior to each filtration. In order to reduce the number of process steps in the cell harvest process, filtration tests with FILTROX depth filter sheets should be performed in the laboratory scale. The aim is to determine the most suitable filter aid and its ideal quantity and, in addition, to achieve turbidity values below 30 NTU to ensure subsequent sterile filtration. Diatomaceous earth (DE), also called kieselguhr, is usually used as a filter aid. Depending on the properties of the particles to be removed, a different DE type may be more or less suitable. For the clarification of a cell suspension containing mAbs, the highly purified pharmaceutical kieselguhr grades Celpure® (Imerys, France) is used. However, there are four different Celpure® types available and it is necessary to find out which one suite best for the particular product to be filtered and in what quantities.

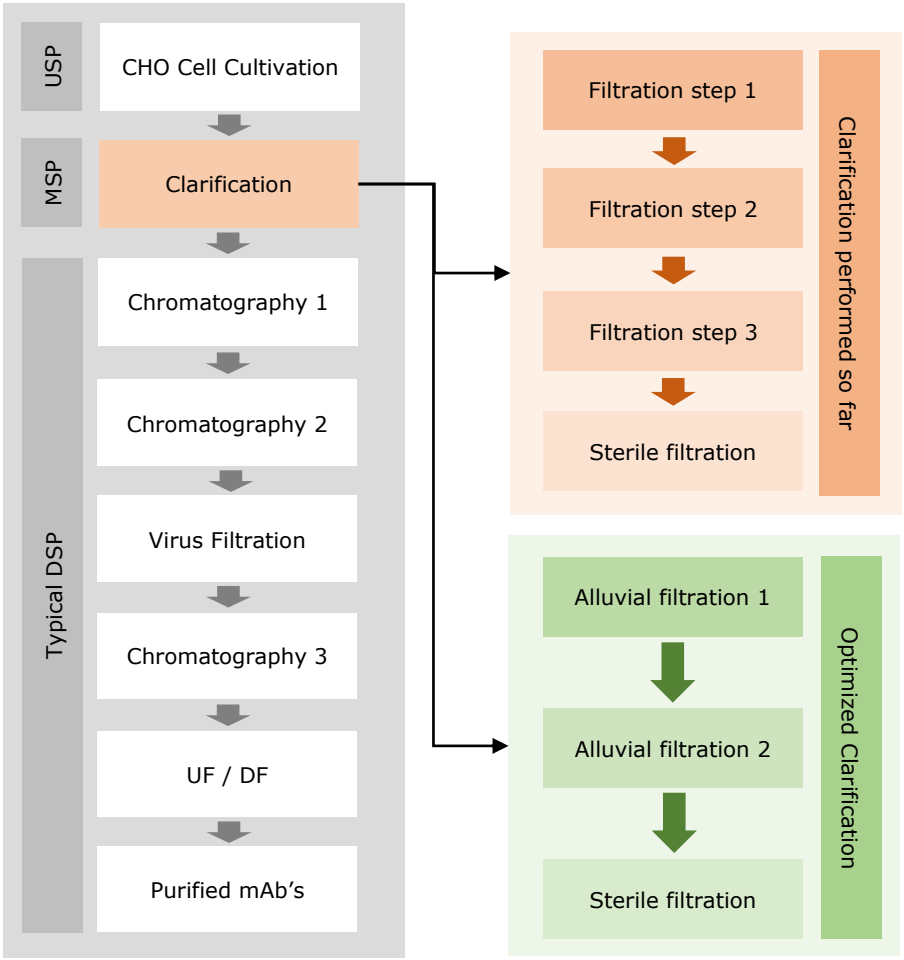

Figure 2: Clarification and Purification of a mAb-producing CHO cell culture. So far, three standard filtration steps were applied to ensure subsequent sterile filtration with a 0.2 µm membrane. This process can be optimized by using FILTROX depth filter sheets and by using the alluvial filtration technology. This reduces the number of purification steps.

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

2 Material and methods

2.1 Set-up

The schematic set-up is shown in figure 3. The corresponding FILTROX PURAFIX® depth filter sheet was inserted into a FILTRODISC™ BIO SD 2" capsule (FILTROX AG), which consists of two individual parts. A cake can be built up in the upper part of the BIO SD 2" capsule (see figure 3b). After clamping the FILTRODISC™ capsule into the synthetic filter holder (FILTROX AG, see figure 3c) and attached to a laboratory stand, the inlet was connected to the pressure gauge (TRI-MATRIX AG) by a Silicone tubing #17 (Shenchen Precision Pump Co. Ltd.). A peristaltic pump (Baoding Shenchen Precision Pump Co. Ltd. with the pump head YZ1515x) was used to convey the CHO cell culture. The pressure gauge is necessary as a maximum differential pressure of 2.5 bar should not be exceeded. Since the used peristaltic pump cannot exceed a maximum pressure of 2 bar, the tests were stopped prematurely in each case. The turbidity of the unfiltered and the filtered samples were measured with a turbidity meter (Thermo Scientific™). To ensure the subsequent sterile filtration step, a sterile filter membrane (Millipore Express SHC 0.2 µm with 3.5 cm² filter area) was used.

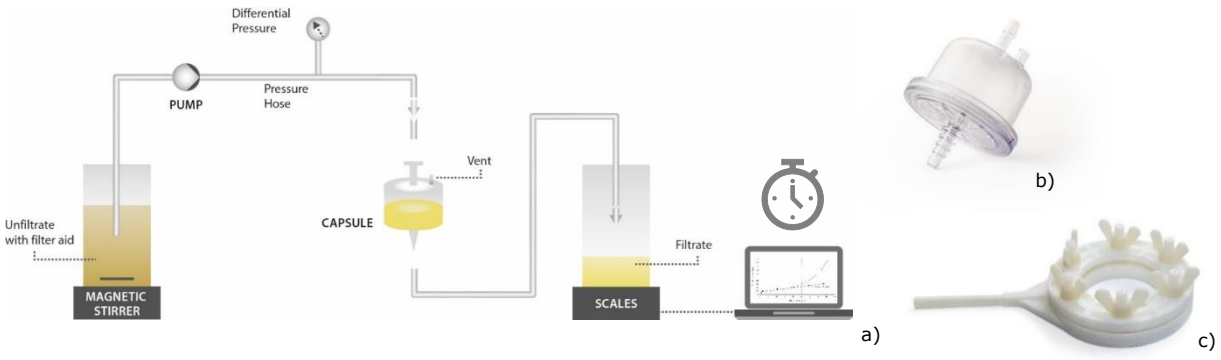

Figure 3: Schematic overview of the test set up (a). Following the filtration using a PURAFIX® depth filter sheet inserted into a FILTRODISC™ BIO SD 2" filter capsule (b) and synthetic filter holder (c), the filtrate is collected in a measuring cylinder in order to record the time-based curves of the filtration flux and the differential pressure. As an alternative to the volume by using a measuring cylinder, a scale can be used.

2.2 Rinsing and Precoat

To wash out any loose components from the depth filter sheet (e. g. cellulose particles) the sheets were rinsed with 50 L/m² of PBS buffer prior to each filtration test. Since the filter sheet area on a lab scale is 0.0021 m², this corresponds to a rinsing volume of 100 mL.

With high particle loads, it can happen that a very rapid pressure increase occurs within a few seconds. To prevent this, a so-called precoat can additionally be applied. Since pre-rinsing of the filter sheet is recommended anyway, an appropriate amount of the filter aid can be added directly to the PBS buffer.

2.3 Alluvial Filtration

Eleven filtration trials were performed in the laboratory scale. As not all experiments could be carried out on the same day, the cell density and the vitality of the cells were measured on both days. The filtration experiments 1 – 4 were carried out on the first day with 12.7 x 10^6 cells/mL and a viability of 82.3 %, the remaining filtration experiments with

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

a cell density of 11.5 x 10^6 cells/mL and with an 80 % viability. The unfiltered CHO cell culture exhibited a turbidity value of more than 2000 NTU. As it is listed in table 1, the following parameters were varied in each filtration experiment:

- Filter sheet type
- Type and amount of filter aid (for the pre-coat)
- Type and amount of filter aid (for the body-feed)

Table 1: Overview of all filtration trials.

Trial No.	Product	Filter sheet	Pore size [µm]	Precoat	Body Feed
-	Unfiltered Cell Culture	N/A	N/A	N/A	N/A
1	Unfiltered Cell Culture	PURAFIX® CH 9P	30-10	N/A	30 g/L, C300
2	Unfiltered Cell Culture	PURAFIX® CH 9P	30-10	N/A	30 g/L, C100
3	Unfiltered Cell Culture	PURAFIX® CH 9P	30-10	N/A	30 g/L C300 + C1000**
4	Unfiltered Cell Culture	PURAFIX® CH 9P	30-10	N/A	50 g/L, C300
5	Unfiltered Cell Culture	PURAFIX® CH 9P	30-10	3 g, C300	30 g/L, C300
6	Unfiltered Cell Culture	PURAFIX® CH 9P + CH 31HP*	30-10 / 12-5.0	3 g, C300	30 g/L, C300
7	Unfiltered Cell Culture	PURAFIX® CH 101HP	1.5-0.6	3 g, C300	30 g/L, C300
8.1	Unfiltered Cell Culture	PURAFIX® CH ST 140P	0.4-0.2	3 g, C300	30 g/L, C300
8.2	Filtrate 1.1	PURAFIX® CH ST 140P	0.4-0.2	3 g, C65	30 g/L C65
9.1	Unfiltered Cell Culture	PURAFIX® CH 101HP	1.5-0.6	3 g, C300	30 g/L C300 + C65**
9.2	Filtrate 2.1	PURAFIX® CH ST 150P	0.2-0.04	N/A	N/A

* double layer (both sheets in one capsule).

** mixture of both Celpure® grades (1:1).

Filtration Tests No. 1 – 7: For the first filtration experiment the filter aid Celpure® C300 was chosen to start with. 30 g/L was used for the body feed. For comparison reasons of the trials 1 to 3, the grade of filter aid was changed only. Therefore, the coarse filter sheet PURAFIX® CH 9P was used for all three trials. Within trial 4, the PURAFIX® CH 9P filter sheet was used again, but a higher amount (50 g/L) of the DE Celpure® C300 was added to the cell culture. For the first four trials, no precoat was created.

Prior to the tests 5 to 7, a precoat with 3 g Celpure® C300 was generated. Afterwards, 30 g/L of the Celpure® C300 was added as the body feed. To reduce the turbidity values further, the finer filter sheet grades PURAFIX® CH 31HP and CH 101HP were applied in trial 6 and 7.

Filtration Tests No. 8 and 9: To achieve turbidity values below 30 NTU, the filtrates generated in the experiments 8.1 and 9.1 were filtered in a second step, no. 8.2 and 9.2 respectively. The sterile filter sheet PURAFIX® CH ST 140P was chosen for the trials 8.1 and 8.2. To remove bigger particles (e. g. whole cells) the Celpure® grade C300 was added in the first step. To remove smaller cell fragments and further turbid matters, the slightly finer DE grade Celpure® C65 was used in the second step.

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

A mixture of two Celpure® grades in equal amounts was used in trial 9.1. The filter sheet PURAFIX® CH 101HP is more coarse-pored than the sheet used in the test 8, but it is thicker, which means that more particles can be retained within the filter sheet. The subsequent step 9.2 was performed as a sheet filtration, which means that no filter aid was added. The filter sheet PURAFIX® CH ST 150P was used, which represents the finest filter layer of the FILTROX PURAFIX® series.

To assess the filtration performance of all filtration tests, the filtration flux, differential pressure (DP), and turbidity values were recorded over time.

3 Results

3.1 Filtration Tests No. 1 – 7

Various combinations of filter sheets, amount of filter aid and type of filter aid were applied, as it was shown in table 1. Based on the results (in terms of filtration capacity and turbidity values achieved) of the first seven filtration trials, a two-stage filtration seems to be necessary.

3.2 Filtration Tests No. 8 and 9

An overview of all results of the experiments 8 and 9 is given in table 2. The minimum specific filtration flux of 300 L/m²×h (10.5 mL/min) was observed in all trials.

Table 2: Results of filtration trials No. 8 and 9.

Trial No.	Product	Time [min]	Specific flux [L/m²×h]	Max. DP [bar]	Volume [mL]	Cake height [cm]	Turbidity [NTU]
-	Unfiltered Cell Culture	N/A	N/A	N/A	N/A	N/A	≥ 2000
8.1	Unfiltered Cell Culture	38	300	1.8	390	3.2	49.55 ± 2.55
8.2	Filtrate 1.1	34	310	0.55	370	3.2	26.50 ± 6.03
9.1	Unfiltered Cell Culture	40	300	2.0	420	3.2	45.17 ± 5.18
9.2	Filtrate 2.1	26	280	1.4	265	0	35.35 ± 8.65

Filtration Test 8: In trial 8.1, a linear pressure increase was measured from minute 12. After 38 minutes, the filtration was stopped due to the increasing differential pressure (DP) up to 1.8 bar. The generated filter cake is shown in figure 4. The entire filtrate obtained in this test was filtered in the second step (8.2), where the same filter sheet (PURAFIX® CH ST 140P) but a different type of kieselguhr was used. Here, a stable DP of approximately 0.5 bar was measured over time. Turbidity values of 49.55 ± 2.55 NTU (n=4) for trial 8.1 and 26.50 ± 6.03 NTU (n=4) for trial 8.2 respectively, were obtained. The time-based curves of the filtration flux, the differential pressure and the turbidity is shown in figure 5.

Figure 4: Filter cake generated in trial 8.1. The cells have settled between the DE particles, allowing the liquid to flow through the cake.

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

In addition to the visual comparison, the generated filtrate in trial 8.2 was filtered again using a sterile filter membrane (Millipore Express SHC 0.2 µm with 3.5 cm² filter area). No further turbidity reduction was achieved, but the entire filtrate of 370 mL could be filtered. A final pressure of 1.4 bar was measured.

Filtration Test 9: A larger volume could be filtered in trial 9.1 compared to the previous filtration test 8.1. A turbidity value of 45.17 ± 5.18 NTU (n=4) was measured. As it can be seen in figure 6, the differential pressure increased up to 1.4 bar and decreased after 24 minutes to 0.7 bar. However, due to the turbidity value of 35.35 ± 8.65 NTU (n=2), the filtration was stopped after 26 minutes, and the generated filtrate was not further purified by sterile filtration.

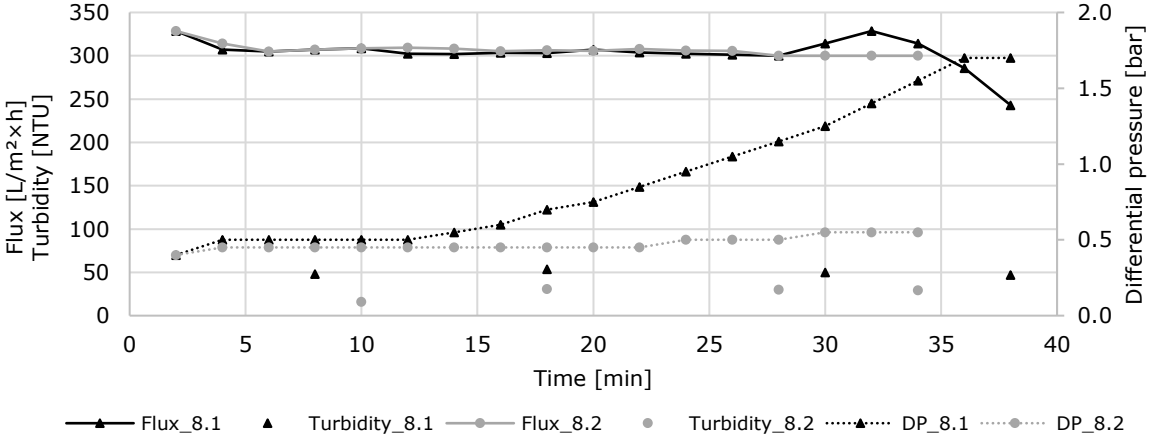

Figure 5: Time-based curves of the filtration flux, DP and turbidity of the trials 8.1 and 8.2. Within the first trial, a linear pressure increase was measured from minute 15. A stable differential pressure of approximately 0.45 bar was observed during the second filtration. The initial turbidity of 2000 NTU was reduced to 50 NTU (8.1) and 26.5 NTU (8.2) respectively.

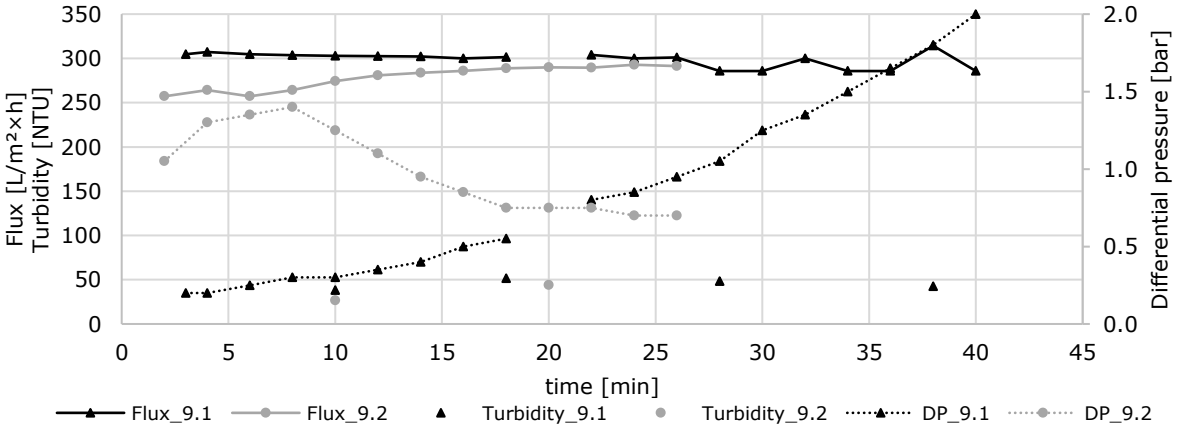

Figure 6: Time-based curves of the filtration flux, DP and turbidity of the trials 9.1 and 9.2. The pressure increase of the first trial is comparable to the one of trial 8.1. A slightly pressure increase after a few minutes after the start of the filtration was measured within trial 9.2. However, it decreased again and remain stable at approximately 0.7 bar. The initial turbidity of 2000 NTU was reduced to 45 (9.1) and 35 (9.2) NTU respectively. As turbidity values of less than 30 NTU should be achieved, the trial 9.2 was stopped after 26 minutes.

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

4 Discussion

The filtration experiments 1 – 7 were necessary in order to determine the best suited Kieselguhr (grade and quantity) and PURAFIX® filter sheet for this particular CHO cell culture. Based on the results of these trials, the parameters (filter sheet and DE types and quantities) for trials 8 and 9 were derived. The generated filtrate from trial 8 exhibited a turbidity of 49.55 ± 2.55 NTU, which indicates that mainly whole cells and larger cell fragments were removed. By using the slightly finer filter aid Celpure® C65, smaller cell fragments and further turbid matters were removed in the second step 8.2.

The turbidity of over 2000 NTU measured in the unfiltered suspension was reduced to turbidity values of approx. 26 NTU by applying a two-stage alluvial filtration. Thus, turbidity values below the required value of 30 NTU were achieved and the entire filtrate could be filtered using a 0.2 µm filter membrane.

In a next step, additional filtration trials with other combinations of Celpure® (grades and quantities) with FILTROX depth filter sheets can be carried out for further optimization, if requested. After evaluating the suitable combinations for the corresponding process, a scale-up for a specific batch size can be calculated. For this purpose, different sized capsules and modules from the FILTROX FILTRODISC™ BIO SD series are available (see figure 7). The following calculations [1] and [2] are used to determine the minimal cake volume and filter area which is necessary for the respective batch size in the pilot or production scale:

$$A_{prod.}[m^2] = \frac{V_{prod.}[L] \times A_{test}[m^2]}{V_{test}[L]} \qquad [1]$$

$$c_{prod.}[m^3] = \frac{V_{prod.}[L] \times c_{test}[m^3]}{V_{test}[L]} \qquad [2]$$

$A_{prod.}[m^2]$	Necessary filter area in production scale (for the respective Batch size)
$A_{test}[m^2]$	Filter area used for laboratory scale tests
$V_{prod.}[L]$	Volume in production scale (Batch size)
$V_{test}[L]$	Volume filtered in laboratory tests
$c_{prod.}[m^3]$	Cake volume in production scale (for the respective Batch size)
$c_{test}[m^3]$	Measured cake volume in the laboratory scale (= height of the filter cake [m] x filter area used [m²])

CHO CELL CULTURE

Clarification of a mAb-producing cell culture

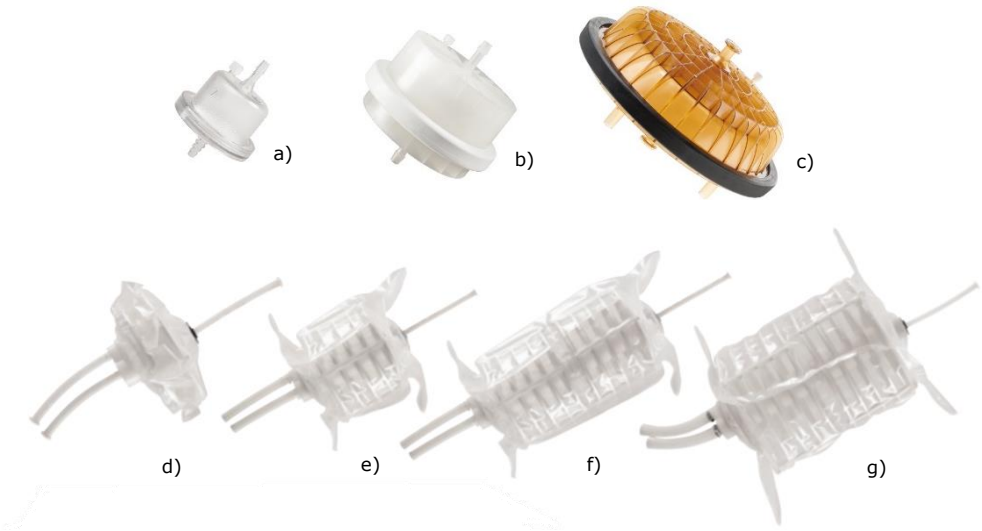

Figure 7: FILTRODISC™ BIO SD series. The two capsules BIO SD 2" (a) and 5" (b) are intended to be used for lab scale filtration experiments, whereas the BIO SD 10" capsule (c) is used for pilot scale filtrations. The modules 12K (d) and 12S (e) are slightly bigger than the 10" capsule and generally also used within the pilot scale. The biggest ones 12D (f) and 16D (g) are used for the large scale filtration and differ from each other in diameter. The modules are encased in a bag and are used for filtration in a pressure-stable stainless steel support housing.

5 Conclusion

To conclude, the laboratory scale trials showed that a reduction of the filtration steps within the midstream process can be achieved by the alluvial filtration technology (see figure 8). It can be applied immediately after the USP and is mainly used to remove whole cells and larger cell fragments. A turbidity reduction from ≥ 2000 to 26 NTU was achieved and product recovery of over 94% was measured. In order to confirm the test results obtained at laboratory scale, it is recommended to carry out pilot scale tests before starting at production scale.

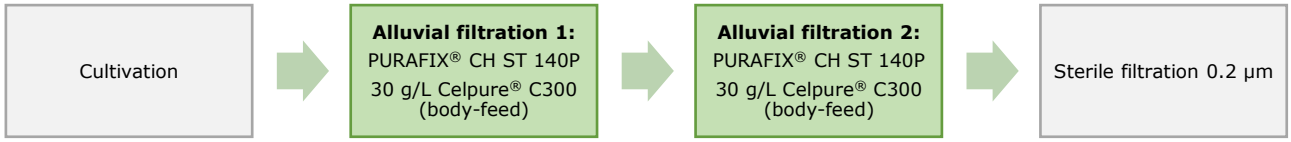

Figure 8: Optimized clarification process of a mAb-producing CHO cell culture suspension. A double-stage filtration was necessary to reduce the initial turbidity of >2000 NTU to less than 30 NTU.