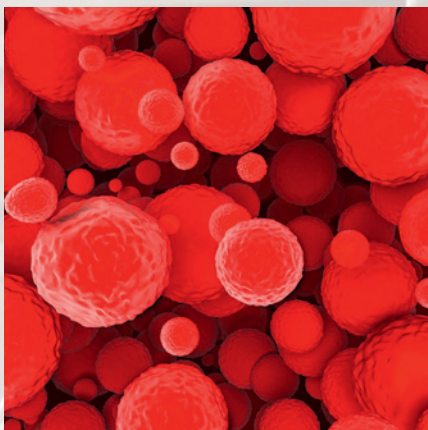




SWISS QUALITY.
SINCE 1938.



FILTRODISC™ BIO SD

Single-use clarification in a new dimension
Disposable high-performance microfiltration system
Flexible scalability from lab to process

DEPTH FILTRATION FOR VALUABLE LIQUIDS. SINCE 1938.

Introduction

Clarification of fermentation broths is one of the most important and unattended steps in biotech processes.

Cell culture processes have become the most popular systems for producing therapeutics and diagnostics in the biotech world. These processes consist of bacterial, yeast, insects or mammalian cells.

Optimized fermentation processing has led to dramatically increased cell densities over the recent decade.

The first purification step after fermentation is the cell removal. The goal of the selected method is to remove the cells and cell debris as well as to reach the maximum product yield in compliance with the existing regulatory environment.

Standard technologies (centrifugation, separators, membrane and depth filtration) can no longer handle the high particle loads ($>10^8$ cells/ml) in an economical way.

Centrifugation and separation, an application in which mechanical stress is applied to cells, increases turbidity through fine particles which have to be removed further downstream during separation and purification.

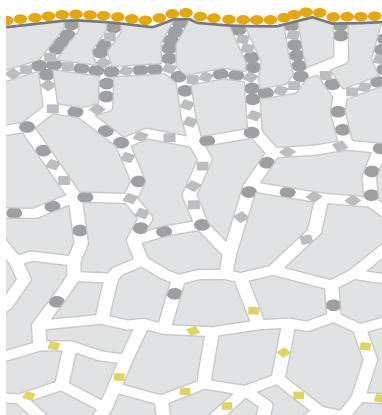
Membranes are very cost-intensive and it is not practical to scale them up. The required footprint for standard depth filtration increases with the particle load. A depth filter with a higher capacity per area would solve these issues.

Alluvial filtration, e.g. diatomaceous earth (DE) filtration, is a well-established method in pharmaceutical industries (plasma fractionation). Until recently, however, it has not been used for single-use cell separation as it was unavailable as a scalable and disposable solution with all validation requirements. FILTRODISC™ BIO SD is the first depth filter using the advantages of alluvial technology in a disposable format. It is also useful for other applications such as activated carbon removal or similar processes with demanding cleaning requirements.

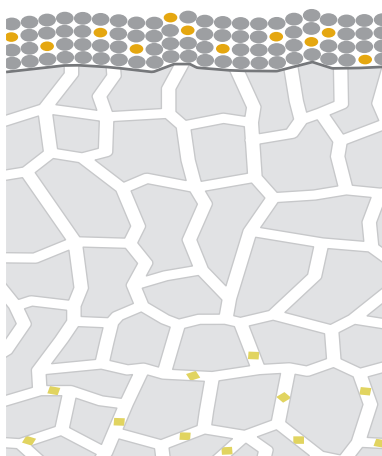
Mammalian cell culture is the most popular method for producing therapeutic proteins or monoclonal antibodies. The product is usually secreted into the cell culture medium during the fermentation. Generally, these processes start in research with several ml, scaled up to development with ~1 – ~25 l and to production with up to several thousand liters. Separating the cell mass is the first step in the purification lines followed by several other steps as filtration and chromatography.

The decision regarding the right clarification/purification system involves questions with regard to process performance, economics and existing regulatory requirements. Process performance challenges include higher and higher cell titers, cell debris content, scalability and flexibility for process changes and future processes, higher product yields and constant high quality product flow for further downstream purification. Economic challenges include consumables costs, capital investment and maintenance costs. Regulatory challenges include adaptation of processes to the changing requirements of the regulatory bodies, with regard to cleaning regulations, cross-contamination and extractables & leachables.

Difference between standard depth and alluvial filtration



Standard depth filtration



Alluvial filtration

Principle / Applications

Principle

A liquid with a cell density of 10^8 often represents a clarification challenge. The turbid state of a cell broth is not only caused by cells but also the feed, and other degraded ingredients have an effect on the turbidity.

The performance of the system depends on the amount of cells, bacteria, yeast or cell debris in the solution. Together with the filter aid it builds a filter cake in the filter. The dosage of filter aid depends on the concentration of cells, bacteria, yeast or cell debris. To define the dosage of filter aid

the FILTRODISC™ BIO SD 2" capsule kit (picture 1) is used. This kit contains 10 capsules and 4 different types of filter aid. The volume of the resulting filter cake is determined following filtration. This cake volume is directly proportional to the batch size.

The FILTRODISC™ BIO SD system provides a linear scale-up from lab to production (picture 2), as well as for non-single-use applications.



Picture 1: FILTRODISC™ BIO SD 2" kit



Picture 2: FILTRODISC™ BIO SD 16" double for up to 1000 l

Applications

FILTRODISC™ BIO SD comprises a filtration system which removes particles (e.g. cells, bacteria, yeast), impurities (e.g. HCP, DNA) and other turbid matter from process liquids such as fermentation broth.

This system is completely disposable; meaning all material that comes in contact with the product is meant for single-use and can be disposed of after the filtration is completed. This "disposability" or "single-use" reduces cross-contamination risks, cleaning validation efforts and reduces the downtime during filter changes, making this method the ideal choice for contract manufacturers (CMO) and other operations with a high frequency of product change. All sizes can be ordered gamma sterilized (upon request).

The system is scalable from lab to industrial-size filtration (see product range). The transfer from the method development stage to large-scale processes can be easily achieved due to linear scalability.

The pharma grade filter aid (e.g. diatomaceous earth, CELPURE® an Advanced Minerals product) is used for filtration. CELPURE® can be used without any restrictions in the biopharmaceutical industry due to its high purity (96–99 % SiO₂) and very low contaminants. It is certified according to USP-NF requirements (Purified Siliceous Earth. USP35-NF30 2012, 1948). A validation guide is available.

FILTROX can provide these in single-use transfer bags, which can easily be connected directly to the single-use bioreactor or mixing device. The fermentation broth is mixed with filter aid in a bioreactor or mixing bag and then pumped via the filter unit. The filter aid and cells form a filter cake, thereby increasing the filtration time and preventing premature blocking of the filter media. After the filtration is completed, the remaining liquid from the bag can be pumped via a FILTRODISC™ BIO SD 5" capsule to recover the valuable liquid (figure 1). After the filtration is finished, both, the module and the capsule can be disposed of.

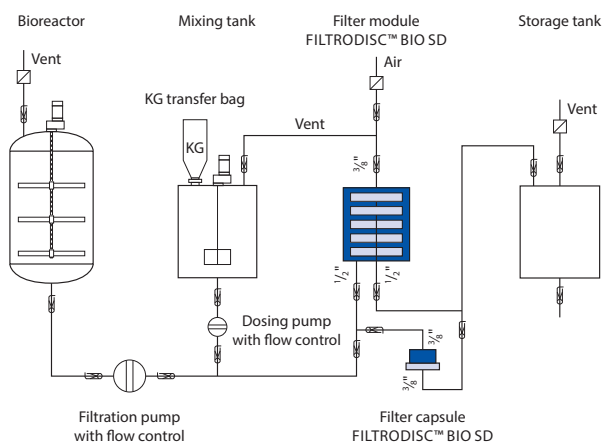


Figure 1: typical filtration scheme

Performance and advantages

Why is the use of filter aid necessary? Standard depth filters can no longer handle the higher and higher cell titers ($>10^8$ cells/ml) in fermentation broths. By using a filter aid such as diatomaceous earth (e.g. CELPURE®) the filtration volume per filter area can be increased up to 4-fold (see figure 2). The filter aid is suspended in the fermentation broth or in buffer/water and then washed onto the FILTRODISC™ BIO SD modules to form a filter cake. When doing this, the filter sheet (e.g. PURAFIX® CH 09P) in the modules, themselves function not as filters, but merely as a support for the filter cake, which is where the actual slurry removal takes place.

Advantages

1. *Extension of filtration volume per area:* Due to the formation of the filter cake the filtration volume per filter area can be extended greatly, in some cases up to 4-fold (figure 2).
2. *Removal of impurities:* Removes cells and impurities (e.g. HCP) in one step, e.g. up to 30–40 % HCP from the fermentation broth.
3. *Flexibility*
 - a. Based on the results of the trials with the 2" capsules the number of lenses and therefore the space for filter cake can be adapted. Even a complete module with 16 lenses can be built into the bag
 - b. Connections: All common connector types can be integrated into the system.
 - c. All available FILTROX filter sheets can be built into the system, therefore, different retention rates are possible; a two-step filtration in one step is possible.
 - d. Activated carbon sheets can also be built in.
4. *Very small dead volumes.*
5. *Widely linearly scalable* from lab (2" capsules) to production (16" double module).
6. *Well known and accepted technology* (no validation risks).
7. *Elimination of cleaning validation* and therefore, reduction of cross-contamination risks.
8. *Reduction of downtime during filter change.*
9. *Reduction of overall production costs.*
10. *No need to change pH values or add flocculants.*

"It's like building your own depth filter."
(Comment from a famous US biotech company)

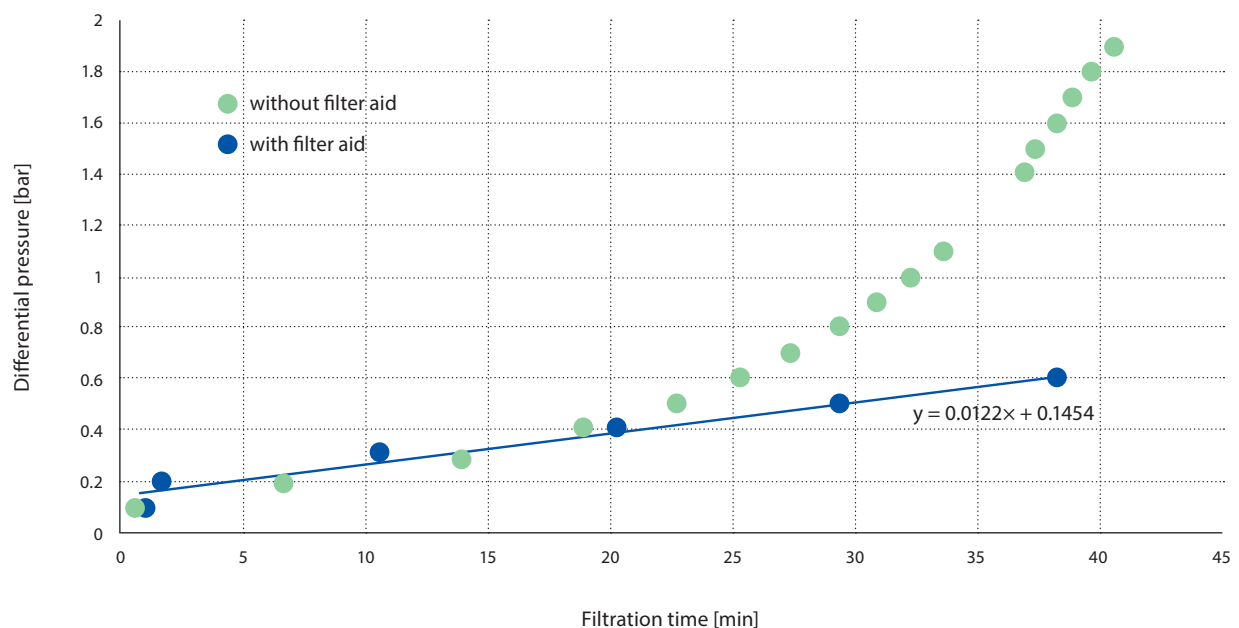


Figure 2: Increase of differential pressure during filtration without and with the addition of filter aid

Scalability

The system is scalable from lab to industrial-size filtration. The transfer from the method development stage into large-scale processes can be easily achieved due to linear scalability. The most important parameter is not the filter area but the space required to build the filter cake.

The filter cake height reached in the 2" capsule multiplied by the filter area results in the filter cake volume per fil-

tered volume of liquid e.g. 1 l. Again multiplied by the volume of the production batch results in the cake volume for production scale. This volume can be used to select the right production size filter.

Depending on the cell density and the amount of filter aid added to the solution, the filterability and volume may vary.

Filter module range

2" capsule

Batch range [liter]	Up to 2 l
Cake volume [liter]	0.06
Filter area m ²	0.002
Application	Proof of technology, evaluation of DE grade



5" capsule

Batch range [liter]	Up to 10 l
Cake volume [liter]	0.38
Filter area m ²	0.0127
Application	Evaluation of 2" results, small pilot scale



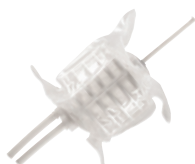
12" short module

Batch range [liter]	Up to 100 l
Cake volume [liter]	3.5
Filter area m ²	0.33 (up to 0.675)
Application	Pilot scale



12" single module

Batch range [liter]	Up to 200 l
Cake volume [liter]	9.9
Filter area m ²	0.55 (up to 1.8)
Application	Bio process scale



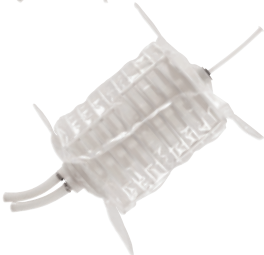
12" double module

Batch range [liter]	Up to 500 l
Cake volume [liter]	19.8
Filter area m ²	1.1 (up to 3.6)
Application	Bio process scale



16" double module

Batch range [liter]	Up to 1000 l
Cake volume [liter]	40.7
Filter area m ²	2.26 (up to 7.2)
Application	Bio process scale



Since all applications are unique, the tables above are intended as a preliminary guide only. Please contact the FILTROX Academy for customized advice regarding your specific application (example calculated with 20 g/l DE). For

CHO cells with a high viability, for example, DE amount of around 10 g/l will be sufficient. A good starting point for the evaluation trials is a DE to cell mass (g/l) ratio of 1:2.

Support systems

For the use of FILTRODISC™ BIO SD modules, reusable stainless steel shells are necessary to allow homogeneous filling of the bags, and as protection in the event of pressure shocks. The support system (DISCSTAR™ BIO SD) for larger modules can be pivoted for easy discharge of the used bags. All systems (except version 12K) will be delivered with a

separate trolley for bag discharge. A pressure gauge is installed on the top of the support system for full pressure control without product contact.

For the 12" double support system, a stainless steel inlet can be ordered, so that the system can also be used for 12" short and 12" single modules.



Picture 3: DISCSTAR™ BIO SD 12" short support system



Picture 4: DISCSTAR™ BIO SD 12" double support system

The DISCSTAR™ BIO SD skids are complete systems combining a single-use pump incl. control panel, automated valves, hose assemblies and support system for 12" or 16" modules plus a holder for a 5" capsule or cartridge. Figure 3 shows the scheme of single BIO SD skid and picture 5 the actual system. The software can be easily integrated into the existing software environment.

Picture 6 shows a completely automated double 16" skid for production scale up to 2000 l. The control software is able to control the speed of the pump via the flow rate and collect the data from the digital manometer. Controlled single-use valves e.g. GEMÜ can be installed upon request. The whole skid is mounted on wheels.

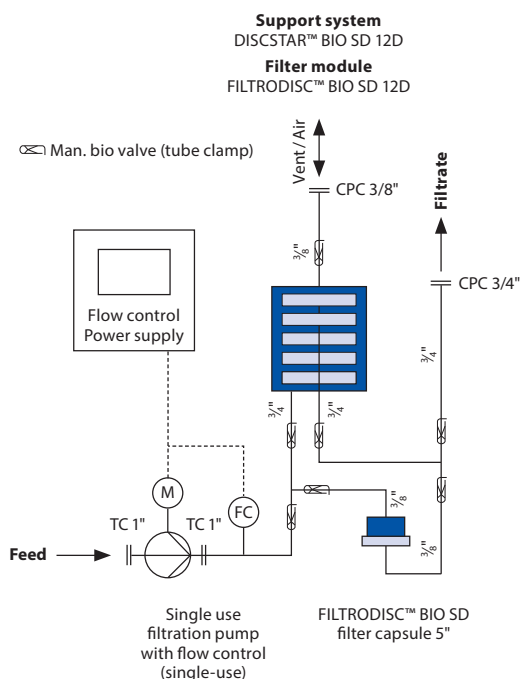


Figure 3: DISCSTAR™ BIO SD skid scheme



Picture 5: Single DISCSTAR™ BIO SD skid



Picture 6: Automated DISCSTAR™ BIO SD double 16" D skid

CPC = Colder Products
Company Connector
TC = Tri-Clamp Connector

Examples: BSD 113 2" T (BIO SD with CH 113P sheet, 2" capsule, TC connector)
 BSD 09 12K 2 VCI (BIO SD with CH 09P sheet, 12" semi-high, 2 lenses, $1/2''$ - $3/8''$ hose, CPC connector, irradiated)
 BSD 53 12D 10 XTH (BIO SD with CH 53P sheets, 12" double, 10 lenses, $3/4''$ - $3/8''$ hose, TC connector, not irradiated)

Material

PURAFIX® P (pharma grade) filter media

Purified and bleached cellulose, filter aid and polyamido-amine (< 3 %); with low ion and pyrogen content.

2" and 5" capsules

Polypropylene encapsulation.

12" and 16" modules

Backbone and bag material: Polypropylene, Polyethylene or Polyamide.

Gasket material: Silicone, others upon request.

Connections

- Hose fittings or Tri-Clamp (lab scale).
- All modules are delivered in a bag equipped with 3×25 cm pharma grade tubing (inlet, outlet, vent).

All materials are FDA approved.

Operating conditions

Max. operating temperature: 40 °C

Max. differential pressure: 2.4 bar

Max. system pressure:.....3.0 bar

Recommended rinsing volume: 50 l/m²

Sterilization: gamma or x-ray sterilized available, upon

sterilization: gamma or x-ray, sterilized available, upon request.

FILTROX quality assurance

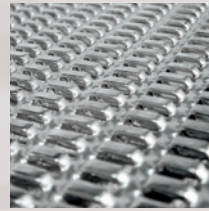
FILTROX assures the best quality control according to international standards:

- ISO 9001:2008 (Quality management)
- ISO 14001:2004 (Environmental management)
- ISO 22000:2005 (Food safety incl. HACCP)
- FDA drug master file: #16418
- EN ISO 3834-2
- DGR 97/23/EG
- China Pressure Vessel Certification
- ASME Code Section VIII Division 1

External tests of lenticular modules and filter sheets were performed and certified according to

- USP plastic class VI (BSL, Munich).
- other CFR requirements by the NAMSA.

A comprehensive validation guide can be ordered from
FILTROX.



As a global market leader in depth filtration, FILTROX offers complete solutions for filtration of high value liquids.

We are experts in development, manufacturing and supply of Swiss top quality products for a wide range of applications in pharmaceuticals, biotechnology, chemicals and cosmetics as well as in food and

beverage. Since 1938, we have been developing and manufacturing both filter media as well as filtration equipment in-house. Based on this experience, we can offer our customers a complete range of products.

FILTROX's worldwide distribution network and comprehensive technical support will help you optimize your filtration process.

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