

# Efficiency increase for single-use cell removal (midstream) using filter aid

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## INTRODUCTION

Continuous process optimization is a key factor in the biotech industry. With higher and higher particle loads (>10<sup>8</sup> cells/ml), standard technologies for cell removal (midstream processing) – e.g. centrifugation, separation, membrane- and depth filtration – find their limits. The goal of the following study was to focus on making the midstream process more economical and investigate the efficiency increase for the cleaning of fermentation broths through alluvial filtration (see box) and its ease of scalability. This technology leads to a maximum product yield and highest economic efficiency.

### Alluvial filtration

Alluvial filtration is a type of depth filtration and a well-established, economical method in pharmaceutical industries (e.g. plasma fractionation). Instead of using an immobilized depth filter medium, filter aid (e.g. diatomaceous earth, perlite) is used to constantly build a filter cake during filtration. The filter cake with its resistance acts then as the actual filter medium. Alluvial filtration, therefore, leads to a higher filter capacity<sup>2</sup> – especially with compressible particles, e.g. microbial or mammalian cells.

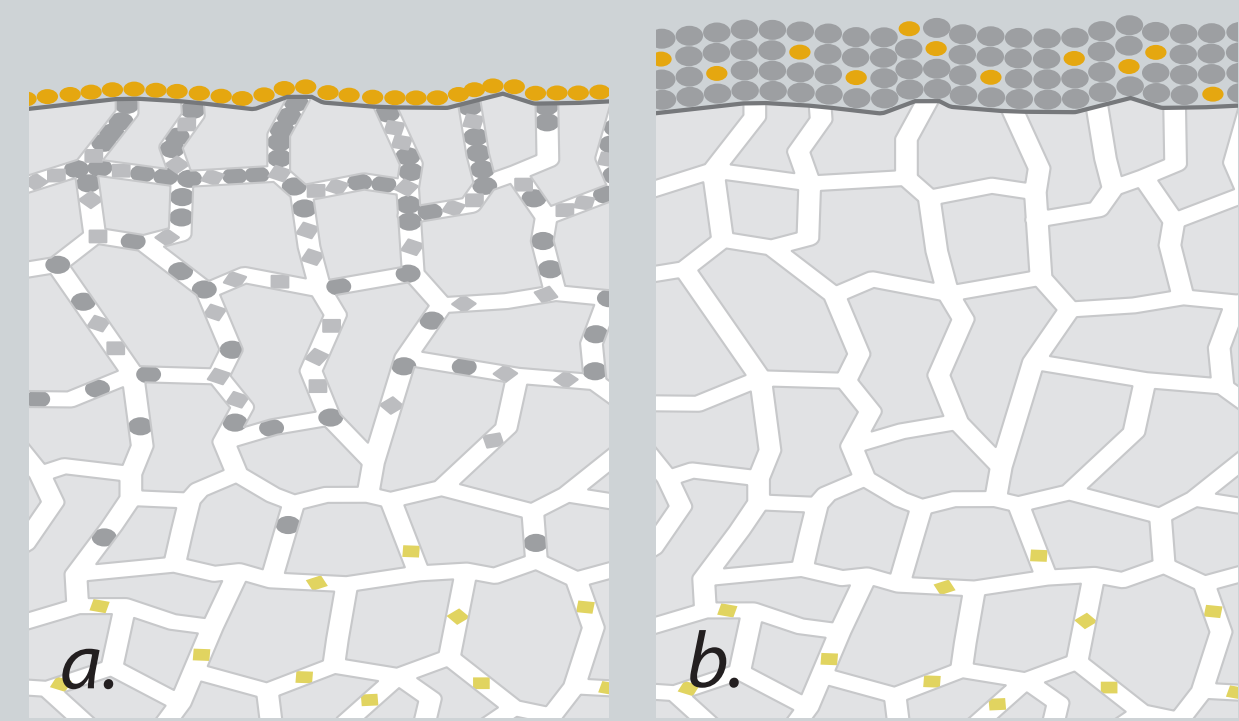


Figure 1: Principle of standard depth filtration (a.) vs. alluvial filtration (b.)

## Midstream, the (missing) link between up- and downstream

The removal of cells and cell debris are the process steps between upstream processing (fermentation) and downstream processing (product purification). These intermediate process steps are being referred to as midstream processing (fig. 2). Midstream processing is often done by a combination of several operation units<sup>1</sup>. A highly efficient method is alluvial filtration (filtration with filter aid) and can be done with FILTRODISC™ BIO SD. Midstream processing is amongst the most important steps in biotech processes (fig. 1). Nowadays, cell culture systems are the method of choice to produce therapeutics and diagnostics. For this purpose, the use of mammalian cells is predominant, but also bacteria, yeast and insect cells are being used. Involved in the process design for the right cell removal system are questions about: process efficiency, process robustness, economic feasibility, as well as legal aspects. Challenges for process efficiency are higher and higher cell titers, amount of cell debris, scalability, robustness and flexibility in terms of process changes and future process adaptations and process optimizations. The industry asks for more efficient and economic methods.

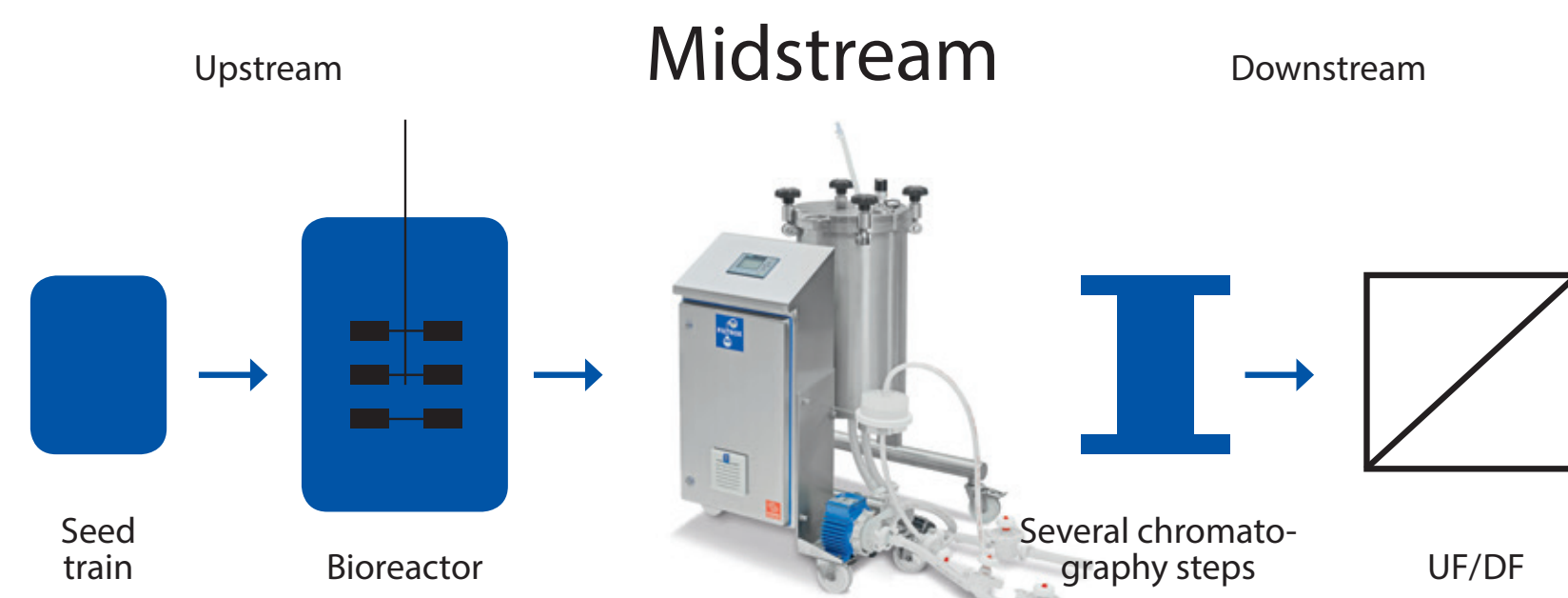


Figure 2: Biotech processing steps: upstream (fermentation), midstream (cell removal) and downstream (product purification)

## Experimental set-up

Filtration experiments for the removal of cell debris of *E.coli* were done to compare filtration efficiency between standard depth filtration and alluvial filtration. The experimental set-up is shown in figure 3. The filter capsule used was FILTRODISC™ BIO SD 2" CH 103P and the filter aid was Celpure® C65. The cell broth (with or without mixed-in filter aid) was constantly stirred by a magnetic stirrer to avoid sedimentation. A peristaltic pump was used to pump the unfiltered liquid through the filter at a steady flow rate of 330 l/m<sup>2</sup>\*h. When reaching a differential pressure of 2 bar, the filter could be considered as clogged and the filtration was stopped. The filtered volume was determined by gravimetric analysis.

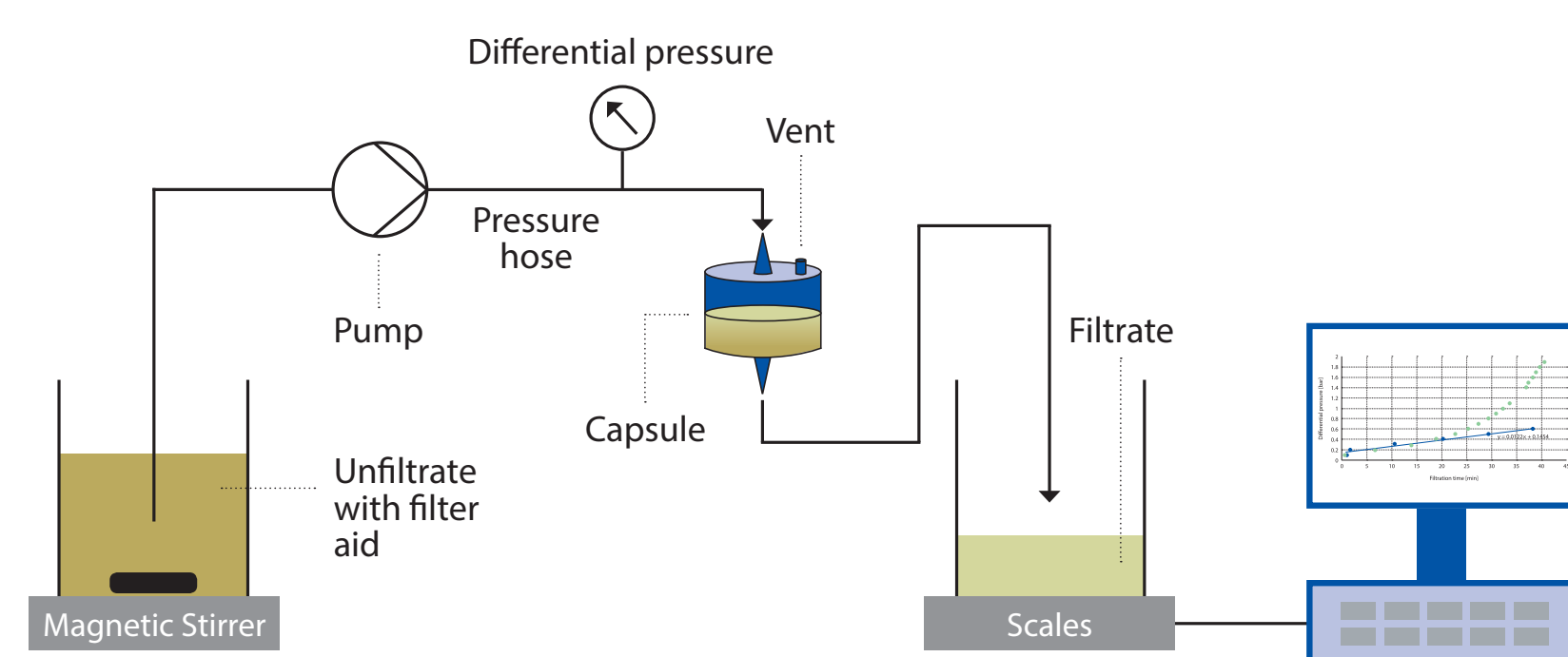


Figure 3: Experimental set-up

## Comparison between standard depth filtration and alluvial filtration

Figure 4 shows the increase of differential pressure throughout the filtration with and without the addition of filter aid. It shows that without the addition of filter aid, the filter clogged already after 41 minutes filtration time. Whereas, when filter aid was added, the differential pressure was only at 0.6 bar after about the same filtration time. Considering the linear increase of the differential pressure of an optimal alluvial filtration, the clogging differential pressure of 2 bar would have been reached after 152 minutes filtration time. Therefore, with alluvial filtration, the filtration time – and therefore the filtered volume per square meter of filter area – can be increased up to 4-fold compared to standard depth filtration.

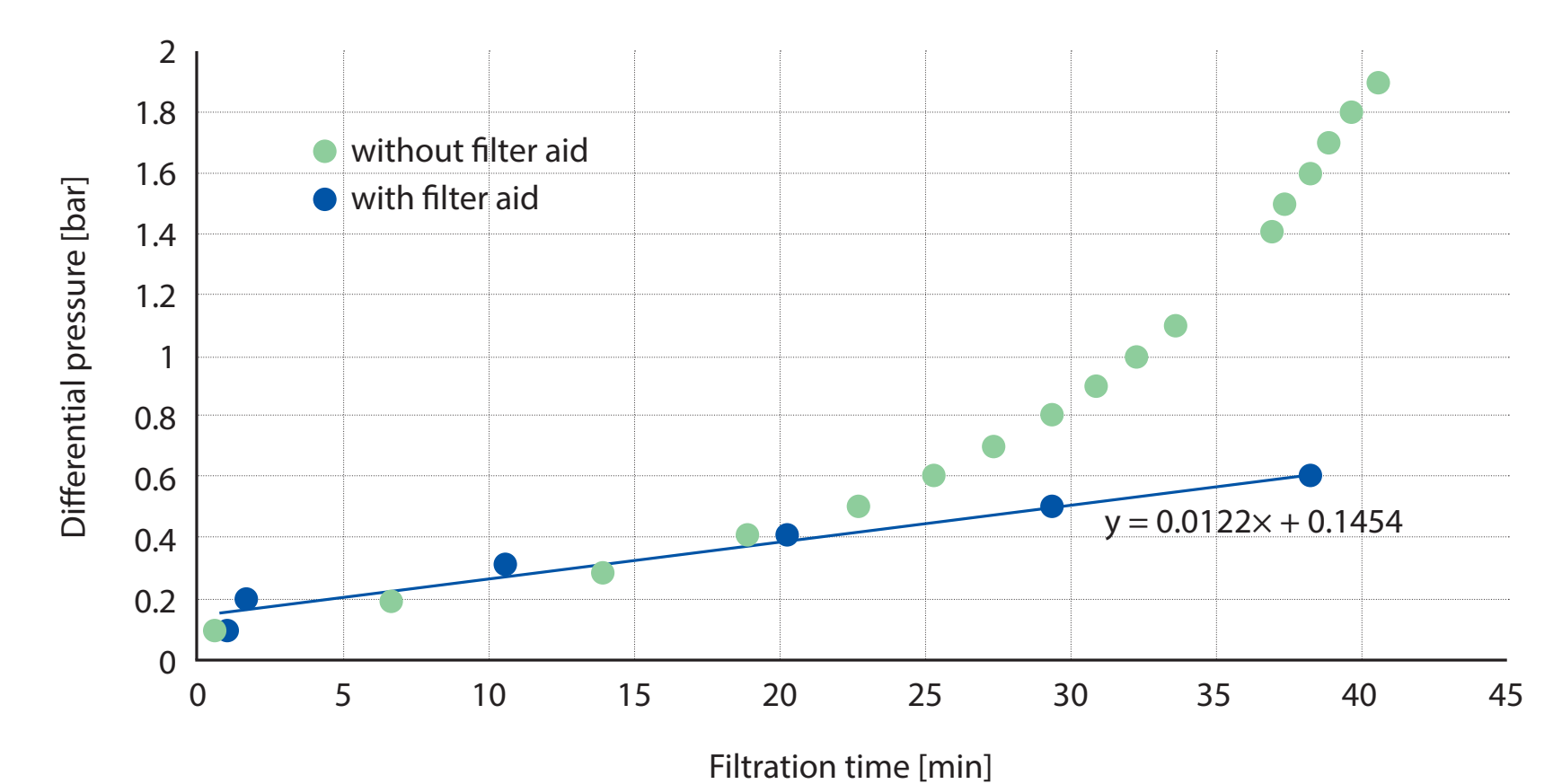


Figure 4: Increase of differential pressure during filtration without and with the addition of filter aid

## Scale-up and optimization

Scale-up from lab to production scale with FILTRODISC™ BIO SD is simple due to its linearity (fig. 5). Therefore, filtration optimization and scale-up are easily feasible. The cake volume per liter of filtered liquid, determined during lab trials, is directly proportional to the cake volume needed for the production scale filter modules:

$$C_p = \frac{V_p \times C_L}{V_L}$$

$$C = h \times A$$

C: cake volume [m<sup>3</sup>]  
V: filtered volume [L]  
L: lab scale  
P: production scale  
h: cake height [m]  
A: filter area [m<sup>2</sup>]

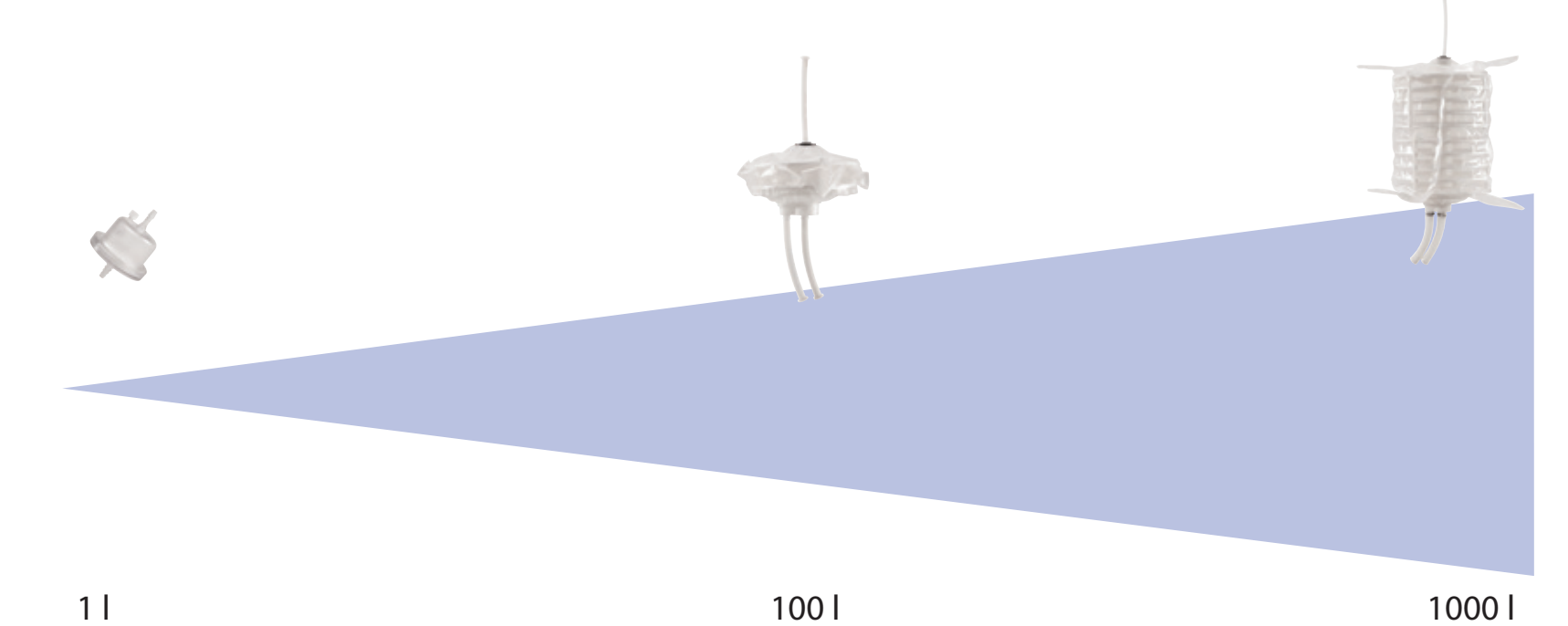


Figure 5: Scalability of FILTRODISC™ BIO SD from lab to production scale

This shows that the primary focus when working with alluvial filtration is not on the scale-up calculation of the filter area, but the required cake volume. A change of pH and the addition of flocculants to the cell broth are not necessary. In the same step as removing cells and cell debris from the fermentation broth, the FILTRODISC™ BIO SD system can also decrease impurity levels, e.g. DNA or HCP, which leads to cost reduction in the subsequent chromatography steps.

## CONCLUSION

The use of alluvial filtration (cake filtration) in midstream processing is one of the most effective, efficient, robust and easy to use methods for cell removal. FILTRODISC™ BIO SD provides a state of the art technology for this purpose.

FILTRODISC™ BIO SD is the first microfiltration system, which combines the advantages of standard depth filtration and alluvial filtration in a single-use system, resulting in new possibilities for midstream processing and subsequent downstream processing steps. Instead of a two-step cell removal system with centrifuges and depth filters, just one step is necessary to remove cells and cell debris from a fermentation broth. The centrifugation step can be eliminated.

### Literature

1. Process Scale Bioseparations for Biopharmaceutical Industry, Chapter One: Harvest of Therapeutic Protein Product; Elisabeth Russell, Alice Wang, and Anurag S. Rathore; Taylor & Friends Group, 2007
2. Dynamic Depth-Filtration: Proof of Principle; W.E. Hurst; Technical Note AMC06; Advanced Minerals