Single-use workflow for recovery of a domain antibody from *E. coli* culture feed in an automated manner

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This application note demonstrates the performance of the automated ÄKTA™ readyflux single-use filtration system in microfiltration applications. Here, a domain antibody (dAb) expressed in *E. coli* was recovered from fermentation broth. The dAb-containing sample was clarified (2.5-times concentration) and further washed with three wash volumes, with a recovery of approximately 90% of product in the permeate.

Introduction

Following the success of monoclonal antibodies (mAbs), antibody fragments (e.g., Fab, scFv, and dAb) are an increasingly important class of protein-based therapeutics. With their small size, antibody fragments possess many advantageous properties, such as easier tissue penetration, suitable for a range of diagnostic and therapeutic applications. For this experiment, a dAb was expressed in the periplasm of *E. coli* and released into the cultivation medium through heat treatment.

Hollow fiber filters offer effective upstream filtration for harvest of proteins expressed in bacteria. Hollow fiber filters are well suited for use with high solid or viscous feeds, as the crossflow sweeping the membrane prevents clogging of the pores. After clarification, the permeate containing the target molecule can be collected and further purified in downstream processes for removal of remaining impurities. Hollow fiber filters are available in both reuse and single-use cartridges.

The ÄKTA readyflux is a tangential flow filtration (TFF) system intended for both microfiltration and ultrafiltration applications. The system can be used in upstream harvesting as well in downstream processes, ranging from pilot to small commercial manufacturing scales. For flexibility and ease-of-use, ÄKTA readyflux is designed with a single-use flow path and can be used with both hollow fiber filter cartridges and filter cassettes.

This work aims to show how ÄKTA readyflux can be used in automated microfiltration applications to meet the industry demand for increased flexibility and efficiency in operations, while minimizing failure and batch changeover time. Automated TFF equipment are operated based on current conditions of the process and, therefore, can save time and increase process consistency. Automation provides sophisticated control for TFF operations, where two interdependent control modes are included for the recirculation and permeate side, respectively. The harvest of dAb (approx. M_r 15 000) from *E. coli* culture feed was used as model process. Hence, for clarification and wash, the system was equipped with a M_r 750 000 nominal molecular weight cut-off (NMWC) hollow fiber filter. Hollow fiber filter cartridges are especially suited for processes where the process stream needs to be contained for health and safety reasons. In addition to reducing the cross-contamination risk between runs, the disposable flow path eliminates several of the cleaning steps required for filtration using reuse systems. When using single-use assemblies, all process components that have been in contact with the process material, including the filter cartridge, can be conveniently disposed after use without the need for open handling of the product.

To achieve good process economy, acceptance criteria for the process were set to a dAb recovery of more than 85% and for all procedures (incl. pre-and post-filtration operations) to be completed within one working day.

Materials and methods

Sample preparation

A dAb-expressing *E. coli* strain was cultured to an OD of 110. Product content was 1.4 mg/mL at time of harvest, when dAb was released from the periplasm into the fermentation broth by heat treatment at 48°C for 3 h. Prior to filtration, the sample was stirred at 100 rpm for 1 h in room temperature before transferred to a 2D bag using a transfer pump.

Microfiltration process

ÄKTA readyflux was installed with a Flow Kit plus TC flow path and a hollow fiber filter cartridge with M_r 750 000 NMWC. A 2D disposable bag was connected to the system for use as a recirculation reservoir. Sample weight at start was 8.5 kg. The sample was concentrated 2.5-fold. When the bag weight reached 3.4 kg, constant retentate volume (CRV) was set to 3.4 kg and wash was initiated. The system was run at a shear rate (feed flow) of 8000 s^{-1}. The outlet was directed to a graduated beaker to allow measurement of volume over the process time.

The dAb-containing sample was thereafter washed with 3 sample volumes (10.2 L) of PBS. At every 3.4 L of permeate volume during wash, the container was changed and product was collected separately to enable analysis of each wash volume. Wash was conducted until an exchange factor of 3 was reached, after which the pump was stopped and sample was collected for analyses.

A summary of the process is given in Table 1.

Table 1. Process summary

Start feed volume	8.5 L
Load	30 L/m^2
Concentration factor	2.5
Wash	3 sample volumes
Feed flow rate	8 L/min
Flux	30 L/m^2/h
Shear rate	8000 s^{-1}
Filter area	0.28 m^2

Analysis of dAb concentration

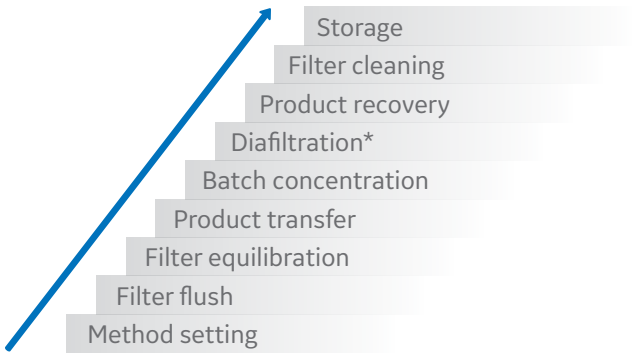
After completed run, samples were collected at various stages according to Table 2. The dAb concentration was determined on a HiTrap™ Protein L column in bind-elute mode. The resulting peaks were integrated and compared to a standard.

Table 2. Sample collection

Sample stage	Sample volume
Initial protein sample	10 mL
Initial concentration (after 2.5×): permeate from concentration	10 mL
Wash 1	10 mL
Wash 2	10 mL
Wash 3	10 mL

Method creation

Preprogrammed phases were used to create the automated method in the ***Method Editor*** of the UNICORN™ software. Choosing ***Create new method, Empty method*** was selected. From the phase library, individual phases were drag-and-dropped to the method outline, starting from method settings:



* Wash step

In ***Phase properties*** under each phase selected, process parameters were entered. The created method was named and saved. To run the process, the method was accessed from ***Method navigator*** under ***System control***.

Results

Figures 1 to 3 show data exported from the ***Evaluation*** module of the UNICORN software. Total dAb recovery of from the UF/DF process was found to be ≥ 89% (Table 3).

The system was operated using its automation features, allowing automated end-point control and data logging to liberate time for other tasks in the lab. Alarms were used to alert of any warnings that needed user attention. Methods were easily created using the ***Method editor.*** Evaluation of results was conducted using the ***Evaluation*** module of the UNICORN software.

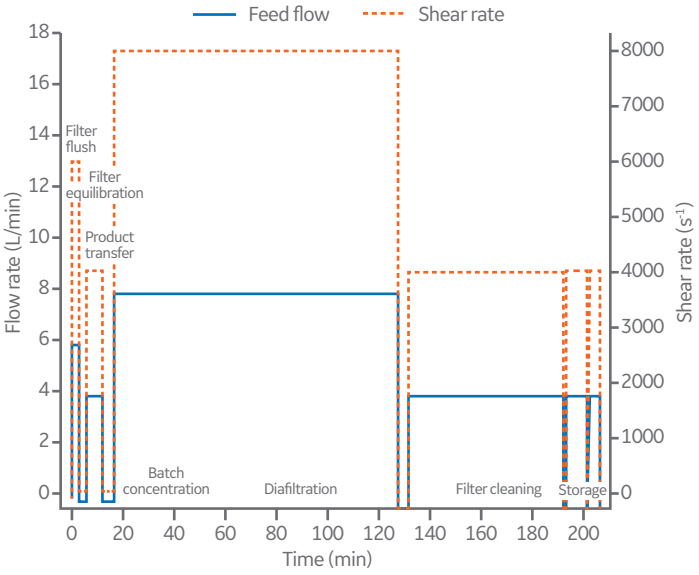


Fig 1. Shear and feed flow stability during the process. During microfiltration of the *E. coli* dAb, the shear and feed flow were maintained at set values throughout the process without fluctuation.

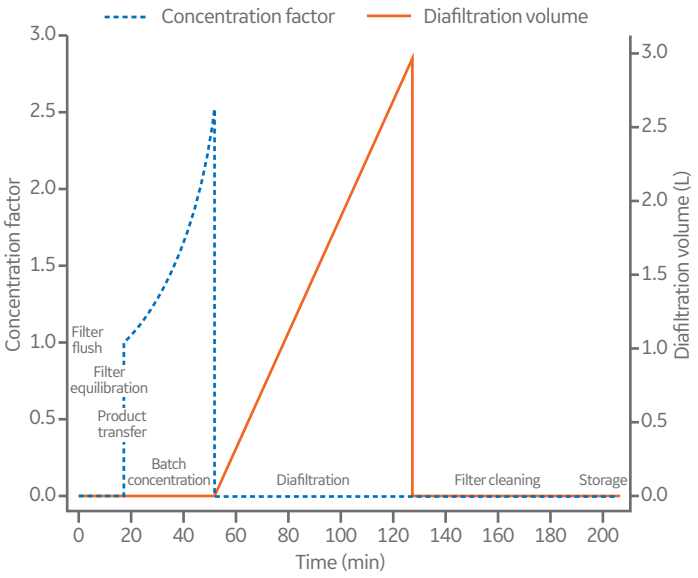


Fig 2. Accuracy of concentration and diafiltration factors. The software can calculate the exact concentration and diafiltration factors to control the process. The graph demonstrates the accuracy of the calculation implemented in the software during the process.

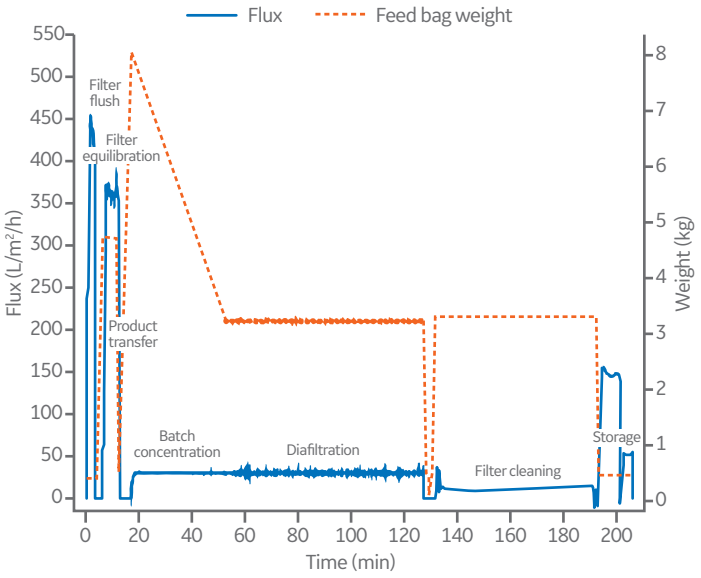


Fig 3. Stability of flux (at 30 L/m²/h) and feed bag weight at CRV during the wash step. Flux control is one of the key features for any microfiltration application. During microfiltration of the *E. coli* dAb, the system was able to maintain flux throughout the concentration and diafiltration steps with negligible fluctuations. The CRV control was highly accurate in maintaining the feed bag weight by the transfer pump during the wash step.

Table 3. Summary of results

Sample	dAb concentration (g/L)	Volume (L)	Total dAb (g)	Recovery (%)
Feed	1.423	8.5	12.1	--
Permeate from concentration	1.048	5.1	5.34	44.13
Wash 1	0.821	3.4	2.79	23.06
Wash 2	0.414	3.4	1.41	11.65
Wash 3	0.391	3.4	1.33	10.99
Overall recovery				**89.83**

Conclusions

Here, we demonstrate successful harvest of a dAb from *E. coli* culture feed using the single-use ÄKTA readyflux system. The microfiltration process (2.5-times concentration, 3-fold wash) was conducted in less than 2 h, allowing for all procedures, including pre-and post-filtration operations, to be completed within one working day. The obtained recovery was approximately 90%, which was above set acceptance criterion for the process.

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