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Model based adaptive control of a continuous capture process for monoclonal antibodies production

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ABSTRACT

A two-column capture process for continuous processing of cell-culture supernatant is presented. Similar to other multicolumn processes, this process uses sequential countercurrent loading of the target compound in order maximize resin utilization and productivity for a given product yield. The process was designed using a novel mechanistic model for affinity capture, which takes both specific adsorption as well as transport through the resin beads into account. Simulations as well as experimental results for the capture of an IgG antibody are discussed. The model was able to predict the process performance in terms of yield, productivity and capacity utilization. Compared to continuous capture with two columns operated batch wise in parallel, a 2.5-fold higher capacity utilization was obtained for the same productivity and yield. This results in an equal improvement in product concentration and reduction of buffer consumption. The developed model was used not only for the process design and optimization but also for its online control. In particular, the unit operating conditions are changed in order to maintain high product yield while optimizing the process performance in terms of capacity utilization and buffer consumption also in the presence of changing upstream conditions and resin aging.

1. Introduction

In the last years the concept of continuous processing is broadly discussed in the production of therapeutic proteins. In general the expected benefits include a higher productivity, resulting in reduced inventory and smaller footprint [1,2] as well as a more uniform product quality.

In addition, the integration of several continuous processes avoids non-productive unit operations like storage and filtration. Thereby the facility footprint is further reduced [3,4].

Within the concept of continuous downstream processing, the capture step is the first one and its correct operation affects the entire subsequent purification train. The general idea is to load one column beyond its dynamic binding capacity (*DBC*) and capture the unbound flowthrough on subsequent columns. Afterwards the columns are shifted countercurrent to the feed direction and the saturated column is eluted and regenerated. Several authors have shown that, comparing such operations to batch-wise capture, a higher productivity with better resin usage is obtained [3,5–8]. It is worth noting that a process with high capacity utilization makes better use of the often expensive affinity resin, resulting in a higher amount of processed product per cycle and hence less buffer consumption per gram of product. In cases where the lifetime of the column is given by a certain number of cycles due to the harsh conditions of the cleaning-in-place step, more product can be processed with the same resin amount. Additionally, the product stream will be at a higher concentration, from which further filtration or purification steps will benefit.

In batch-wise capture steps higher resin utilization while maintaining a given yield can only be achieved by decreasing the feed flowrate, hence lowering the productivity. This trade-off of batch chromatography can be overcome by multicolumn countercurrent chromatography [8]. Such concepts have been previously implemented in processes with 4 columns [9,10], 3 columns [11,12] or two columns [7,13], with the capture of monoclonal antibodies by Protein A affinity resin as the reference application. However the benefits are also observed in the case of other (affinity) capture processes [3,14]. While all these processes elute the purified product in a non-continuous way, there are differences in varying or keeping a constant feed flow rate as well as in implementing the wash steps. Of course the complexity of the equipment increases with the number of columns.

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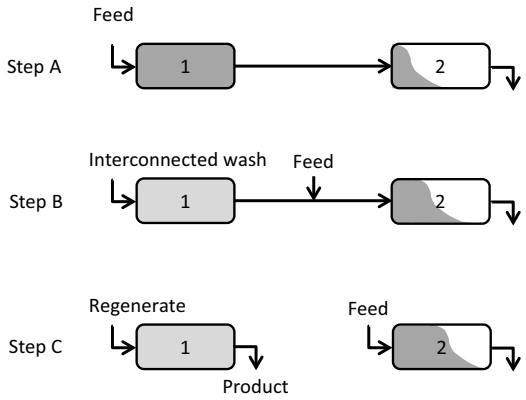

Fig 1. Schematic representation of a continuous capture process with two columns. Each cycle consists of three consecutive steps. During the first two the two columns are interconnected while they are operated batch-wise in the third step. Since during each step cell culture supernatant (feed) is loaded onto the columns, a continuous feed flow is ensured. After the end of step C the two columns switch position and the same steps are repeated to complete the cycle.

In general process design, e.g. choosing the right column size and process parameters, can either be done using empirical approaches [3,7,11] or process simulation [8,12]. In this work the process is simulated using a lumped mass-transfer model coupled with a heterogeneous binding model. Using such a model, process operating conditions for the continuous capture process were determined and then verified experimentally. In agreement with the model predictions it was found that the process was able to capture an IgG monoclonal antibody at high yield with increased amount processed per cycle and capacity utilization compared to the corresponding batch process.

Next the model was used to develop an adaptive procedure which maintains yield in specification at optimal operating conditions in the presence of process disturbances, such as changes in the upstream titer and aging of the resin.

2. Process description

In the following we consider a two column capture system for the continuous processing of cell culture harvest. A two column system is particularly interesting regarding equipment footprint and operational simplicity [7]. The corresponding flow chart is shown in Fig. 1. The process is organized in three steps, where the two columns are either interconnected (step A), interconnected with an additional feed before the second column (step B) or operated in batch mode (step C). During step A, column 1 is loaded beyond its dynamic binding capacity. Any target protein in the breakthrough is adsorbed onto column 2. The load amount of column 1 at the end of this step determines the capacity utilization, as this column is washed during the subsequent interconnected state (step B). Any unbound target protein washed out of this column is mixed inline with additional feed before being loaded onto the second column. In a third step the saturated and washed column 1 is regenerated while column 2 is loaded in batch mode.

The main difference compared to previously presented process [7,13] is the additional feed of harvest inline during the interconnected wash step. This makes the unit capable to process a continuous feed flow and therefore suitable for direct and integrated capturing, for example in connection with a perfusion bioreactor. More importantly, a continuous feed facilitates the implementation of control strategies for the harvest flow in such an integrated setup.

For practical implementation, two requirements need to be considered: Firstly, the wash buffer used during the interconnected wash (step B in Fig. 1) needs to have pH and conductivity similar to that of the cell-culture supernatant, in order to avoid any precipitation in the column. This requirement is also present in the first wash step of batch chromatography. Secondly, during the interconnected wash step column 2 is loaded at a higher effective flowrate. Hence loading of this column during this step needs to be below its dynamic binding capacity, which is reached in the subsequent batch-wise loading (step C in Fig. 1).

In particular, the twin-column process considered in this work uses two different effective flow rates during loading. Ghose et al. [15] showed that a dual-flow rate approach is able to improve the process performance in the case of batch operation.

In general in all multicolumn capture processes, regeneration and loading are done in parallel and their duration needs to be synchronized. For the process in Fig. 1 this means that the two operations performed in batch mode during step C need to be synchronized. In particular, in order not to lose product in the flowthrough of column 2, the following constraint needs to be fulfilled

$$\tau_{regeneration} \leq \tau_{loadbatch} \tag{1}$$

where $\tau_{regeneration}$ is the regeneration time of column 1 in step C and $\tau_{loadbatch}$ is the time to load column 2 up to the *DBC* during step C. In case such loading takes longer than regeneration, column 2 will be idle, while otherwise loading goes beyond its *DBC*, resulting in a lower yield. Hence in an optimized process the two steps have to take equally long. The regeneration time during step C can be calculated as the sum of the duration of the wash, elution, cleaning in place (CIP) and equilibration steps, which are the same ones as in a standard batch process:

$$\tau_{regeneration} = t_{wash} + t_{elute} + t_{CIP} + t_{equilibrate} \tag{2}$$

note that t_{wash} in the above equation refers to the additional wash steps performed on a single column during step C in addition to that in the interconnected wash step B.

Since in this step the column is operated in the batch mode, the operating conditions identified experimentally in batch can be directly adopted with no need for further optimization.

On the other hand, the value of $\tau_{loadbatch}$ depends on the amount previously loaded during step A and B onto column 2, called preload, which mainly depends on the length of the interconnected load (step A). Therefore this operation parameter can be used to satisfy conditions (1) as equality and hence to obtain optimal operation performance as discussed later in more detail.

Since the *DBC* is a function of the residence time and $n_{pre-load}$ a function of the process parameters, designing a continuous capture process requires either to define the residence time in advance or measuring the breakthrough curves at different residence times. Here in contrast we follow a model based design procedure, thus minimizing the required experimental effort.

The process parameters used to quantify the process performance are the product *yield*, defined as the difference between the total load amount and the amount of product lost in the flow through divided by the total load amount, the time-based productivity representing the amount of product purified per unit time and column volume and the capacity utilization (*CU*) which is the fraction of the resin capacity saturated by the product in one cycle.

3. Process simulation

3.1. Protein adsorption

Ligands for bioaffinity separation are nowadays mainly recombinant proteins. In the case of Protein A, an oligomer of the engineered binding domain is attached to the resin [16,17]. Thereby