Clarification of *Pichia pastoris* using cross flow microfiltration

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Abstract

Pichia cell cultures with different biomass were studied under different process conditions at different scales. Results show that permeate flux rates were 25 lmh and 15 lmh for cell cultures containing 20% and 45% wet cell weight (WCW), respectively. Results also show that larger lumen diameter fibers may be beneficial for achieving higher permeate flux and less filter clogging.

Introduction

Pichia pastoris (*P. pastoris*) has been increasingly recognized as a choice of expression systems for therapeutic protein production in biotechnology and biopharmaceutical industries. However, the primary product recovery from yeast cell cultures usually requires an effective separations method, and the high yeast cell density has made such a separation difficult. In addition, *P. pastoris* is sensitive to shear force, and its broth is also easy to foam. Centrifugation may be used for such separations; however, additional filtration steps may still be needed before subsequent column loading. Conventional dead-ended filtration may also be used; however, its filtration capacity is usually very low. Cross flow filtration appears to be a better fit for such applications, but filter clogging may occur easily if a "screen channel" filter is used. Cross flow filtration with open channel filters may be less problematic, demonstrates good filtrate quality, and shows high membrane filtration capacity (filtrate volume/membrane surface area). Hollow fiber cartridges are such open channel filters.

When using open channel filters for yeast cell clarification, microfiltration membranes are usually the choice for high product passage. Due to the high fluxes of most microfiltration membranes, premature membrane fouling can be a challenge. When one half of the required delta *P* (the difference between feed and retentate pressures—e.g., 5 psig in Figure 1) is higher than the desired transmembrane pressure (TMP—e.g., 0.5 psig), permeate pressure must be applied (e.g., 2 psig in Figure 1). A traditional way to apply permeate pressure to regulate permeate flux involves using a permeate flow control pump, as shown in Figure 1.

Experiments were conducted using GE Healthcare hollow fiber cartridges on different *Pichia* cell cultures. Process conditions, permeate flux, membrane filtration capacity, process scale up, and the effects of filter geometry were examined in this study.

Experiments and results

Primary study on process conditions

Initial study was conducted with a 20% wet cell weight, *P. pastoris* cell culture using a 0.2 µm, 1-mm lumen diameter, 30-cm fiber length hollow fiber cartridge (CFP-2-E-5A) from GE Healthcare, which contains a total of 0.12 m^2 membrane surface area. Cartridge clean water flux data are shown in Table 1.

Cell clarification was conducted in two phases for studying the primary process conditions. A total of 23 liter (l) cell broth was first concentrated to 14 l (9 l of filtrate) in 180 minutes. The cross flow rate was 6.8 l/min, and the permeate flux was controlled at 50 ml/min (Table 2). The cartridge was then flushed with buffer and water before being stored in NaOH overnight. The remaining 14 l sample was stored at 4ºC overnight, and processed the next day using the same cartridge. A lower cross flow rate of 4.8 l/min. was used, and permeate flux was not controlled in phase II. In phase II, 1.9 l of filtrate was first collected in 40 minutes and the process was then continued for another 72 minutes to collect 1.8 l more filtrate before finishing (Table 2). The cartridge filtration capacity was about 75 l/m^2 altogether. The cartridge was buffer/water flushed in between phase I and phase II.

Table 1. Cartridge clean water flux at 25ºC

TMP (psig)	Cross flow (l/min)	Clean water flux (ml/min)	Clean water flux (lmh/psig TMP)
2.5	4.0	350	70
10	7.2	1100	55

Table 2. Results of sample yeast cell culture process conditions

Phase	Temperature (ºC)	Shear rate (1/sec)	Beginning TMP (psig)	End TMP (psig)	Average flux (lmh)
I	29.5	~6800	5	7	25.0
II-a	10.0	~4800	5	7	23.7
I-b	16.8	~4800	7	-	13.1

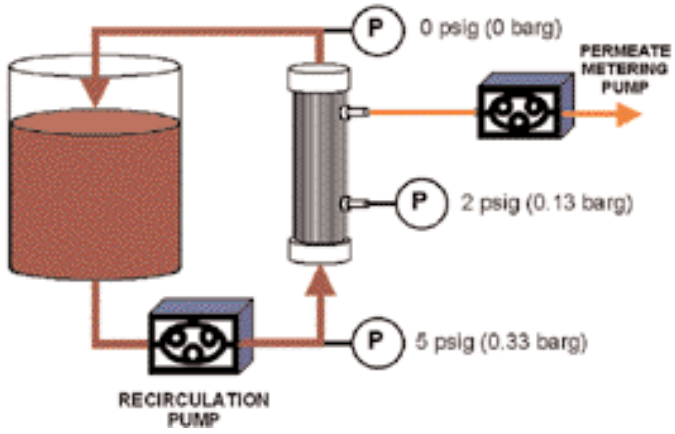

Figure 1. Cross flow filtration system with permeate flow control

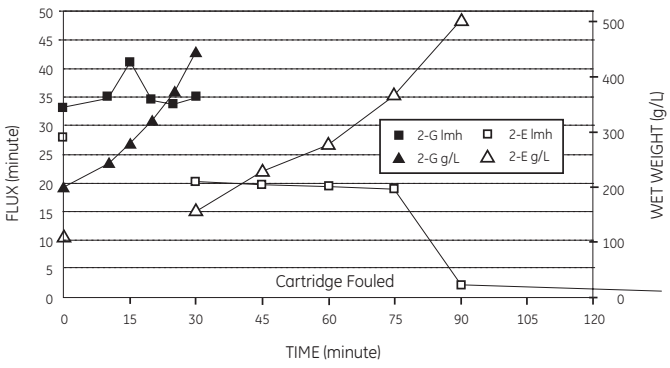

Figure 2. Results of *Pichia* cell clarification using different lumen diameter fiber cartridges

Study on a scale up-process

A scale-up study was conducted on a 280 l *Pichia* fermentation broth with 45% (WCW) cell density. Two large-scale filters (CFP-2-E-45A, GE Healthcare) were used in parallel, and each cartridge contained 2.5 m^2 of 0.2 µm membrane with 1-mm lumen diameter and 30-cm fiber length.

Pichia was first concentrated from 280 l to 130 l in about 112 minutes with a constant permeate flow about 13.9 lmh. TMP was increased from 2 psig to 5 psig, and delta *P* was increased from 5 psig to 20 psig during the process due to the increased cell concentration. To increase product yield, the remaining 130 l of the concentrated sample was diafiltered with 200 l of water for 155 minutes with an average flux rate about 15.4 lmh. The same procedure was repeated for 20 more batches.

Study on filter geometry

Two GE Healthcare 0.2 µm, 60-cm fiber length hollow fiber cartridges with different lumen diameter fibers were studied for Pichia cell clarification. CFP-2-E-6A contains 0.28 m^2 of 1-mm lumen diameter fibers, and CFP-2-G-6A contains 0.24 m^2 of 1.75-mm lumen diameter fibers. *Pichia* was first diluted with an equal volume of buffer before filtration started. All processes were temperature controlled between 4ºC and 8ºC using a heat exchanger. The cartridge containing 1.75 mm lumen diameter fibers exhibited a sample flux between 33 and 40 lmh at 15 psig of TMP, with a starting cell concentration of 19% (WCW) and a final cell concentration of 45% (WCW) (Fig 2). The cartridge containing 1 mm fibers yielded a sample flux between 20 and 28 lmh at the same TMP. The cell starting concentration was 10% (WCW), and the cartridge was fouled when the cell concentration reached 50% (Fig 2).

Summary and discussion

Pichia cell cultures with different cell densities (20% to 45% WCW) were able to be concentrated and diafiltrated at different scales using GE Healthcare hollow fiber cartridges. Average sample flux of 25 lmh at 20% (WCW) cell density was achieved using permeate flow control; and the average flux was above 15 lmh for a cell culture with even higher cell density (45% WCW) without pre-sample dilution. Membrane filtration capacity was found to be more than 70 l/m^2 (including the diafiltration volume) even for the sample with high cell concentration (Fig 3).

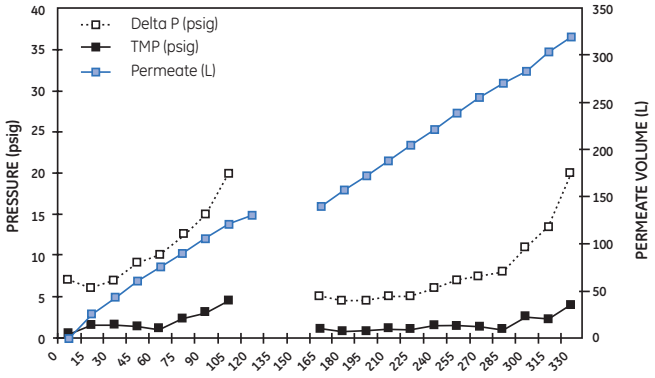

Figure 3. Results of a large scale *Pichia* cell clarification study

Membrane pore size selection

Even though only 0.2 µm hollow fiber cartridges were used in these studies, membrane pore size selection should be made based on the sizes of the target protein. If the target protein is small, ultrafiltration membranes (nominal molecular weight cutoff [NMWC] 750 kD or less) may be considered since no permeate flow control is necessary for ultrafiltration processes. However, if the target protein passage is not acceptable, microfiltration membranes (0.1 µm or larger) should be used.

Fiber lumen diameter and length selection

Due to the high viscosity of most yeast cell cultures, larger lumen diameter hollow fibers (1 mm or larger) should be used. Test results (Fig 2) show that higher sample flux and less membrane fouling was achieved with a 1.75-mm fiber. For the same membrane surface area, a longer flow path will require less cross flow for the same shear rate than the shorte cartridges, but they will also require a greater delta *P*. With high viscosity samples, the delta *P* increase can be dramatic; therefore, for viscous samples a short path length may be recommended for ease of operation.

Permeate flow control

For most *Pichia* cell clarification processes using microfiltration membranes, the required delta *P* for the desired shear rates often leads to an undesired high TMP, resulting in premature membrane fouling (greatly reducing membrane filtration capacity). Therefore, permeate flow control is typically recommended for reducing permeate flux in order to achieve a stable process (Fig 1). Permeate flow can either be controlled by a pump or a valve. The use of a pump for permeate flow control is usually preferred for manually controlled cross flow systems.

Pre-dilution and optimization of diafiltration

To achieve high product yield, yeast cell clarification processes usually include a wash (diafiltration) step. This step can be optimized for flux or buffer consumption. Although a diafiltered concentrated sample will reduce buffer consumption, the high yeast cell concentration may dramatically reduce permeate flux, greatly extending the process time, or even stopping the process because of membrane fouling. For solutions with high biomass, pre-dilution or diafiltration before reaching final concentration may be beneficial.

In summary, high yeast cell concentration samples can be clarified with hollow fiber cartridges. Yeast cell clarification with hollow fiber cartridges has shown high performance, linear scalability, high filtrate quality, and easy operation.

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