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APPLICATION NOTE

Impact of Process Loading on Optimization and Scale-Up of TFF Microfiltration

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Introduction

Tangential flow filtration (TFF) microfiltration has been used as one of the choices for clarification of mammalian cell or microbial cell culture in the biopharmaceutical industry. Unlike the ultrafiltration process for protein concentration and the diafiltration application where the feed solution is relatively clean (free of colloids or larger particles after the clarification/purification process), the microfiltration process needs to handle a rather high-fouling feed stream such as cells, cell debris, colloids, *etc*. In a previously published article[1], we discussed that a TFF microfiltration step is limited by a maximum throughput or capacity obtainable under a given set of operating conditions. Some distinct microfiltration characteristics, such as critical permeate flux, permeate flux control, and maximum throughput were explained in that article.

The microfiltration process endpoint with permeate flux control is often determined by the decrease of extracellular product or contaminant sieving (increase of rejection), or increase of transmembrane pressure (TMP) to the maximum process limit (with flux held constant). Kubota *et al.*[2] have also demonstrated higher protein recovery using permeate flux control compared to the standard TFF TMP control, with clear correlation of cake mass resistance related to membrane sieving, both empirically and theoretically. Typically, the permeate flux controlled method is applied to minimize membrane fouling. In the microfiltration process, fouling is assumed to occur first by pore blockage, with a cake formed over the blocked areas of the membrane. However, even with permeate flux control, the pores in the microfiltration membrane are slowly plugged by small colloidal particles when more feed is processed through the membrane. These solid loading or volumetric loading/throughput (capacity) limits pose constraints in the microfiltration process. There are no general guidelines yet for volumetric or solid loading to microfiltration processes applied across the biopharmaceutical industry. We conducted studies to quantitatively examine the TFF microfiltration process loading limits on the cell and cell lysate clarification (concentration) and the operating parameters that affect the process end points, comparing the plugging models at constant flow.[3] This article describes the experiments and discusses the results and future considerations for microfiltration loading challenges. Plugging models for constant flux filtration were investigated with membrane resistance (or transmembrane pressure) profiles. The experimental data analysis corresponded well with the combined cake/complete pore plugging models.

Materials and Methods

This study investigates the solid and volume loading on microfiltration process endpoints using feed solutions of whole cell yeast and yeast lysate. Microfiltration was conducted using the 0.1 m^2 Pellicon® 2 0.1 µm polyvinylidene difluoride (PVDF) membrane filter with suspended screen (V-screen) at a feed flux of 5 L/m^2/min (300 LMH). Critical flux for both whole cell yeast and yeast lysate clarification was determined by using the TFF microfiltration process protocol explained previously.[1]

Dry baker's yeast (*Saccharomyces cerevisiae*) was rehydrated and cultivated in a glucose solution at 37°C for four hours. The yeast solution was centrifuged at 3700 rpm for ten minutes using a Heraeus® Multifuge® 3 S-R centrifuge (Thermo Scientific) and the supernatant was discarded. Wet cell pellets were washed down by using 50 mM of phosphate buffered solution (PBS) and collected in centrifuge tubes. Yeast cell pellets were frozen at –20°C in order to prepare for experiments with the desired percentage packed cell volume (% PCV). For lysate solution preparation, the frozen cell pellets were thawed and lysed by using a Sonics® Vibra-Cell™ sonicator (with 1" diameter high-gain probe) at 60% amplitude (of 20,000 Hz) for a total of 14 minutes. Critical

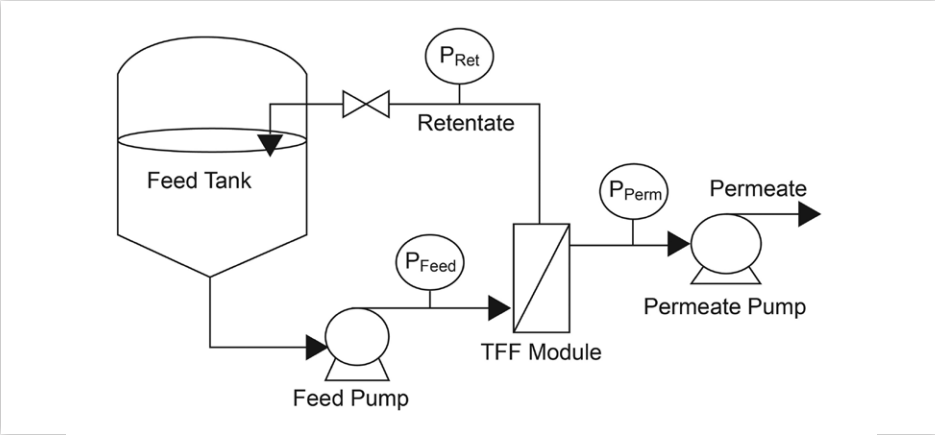

FIGURE 1. Experimental setup for microfiltration experiment with permeate flux control.

flux was determined prior to microfiltration clarification experiments. A secondary pump was set up to control the permeate flux (Figure 1). Permeate flux is expressed as the permeate flow rate per square meter of filter area (LMH = $Lm^{-2}h^{-1}$).

With the permeate pump off (Watson-Marlow 323S, four-roller 323 DW pump head with 3.1 mm ID Tygon LFL Masterflex® 06429-16 tubing), the feed pump (Watson-Marlow 520U, pump head 520REM, 6.4 mm ID silicone platinum-cured Masterflex 976410-24 tubing) was ramped up to a cross flow rate of 5 L/m^2/min. The permeate pump was then slowly ramped to 5 LMH and TMP was recorded every five minutes for 15 minute intervals. The permeate flux was incrementally increased until

$$\text{the } \frac{TMP_{final}}{TMP_{initial}} \text{ was} > 1.5 - 2.0$$

where initial and final referred to the beginning and end of the 15 minute intervals.

Subsequent whole cell yeast and lysate clarification (concentration) experiments were performed at 50% of critical flux with the permeate directed to a collection container (Figure 1) using a feed solution of different % PCV and starting volume. Pressure and flow rates were recorded manually according to sampling intervals. The microfiltration process endpoint was predefined as the maximum TMP limit of 5 psi or when a sudden and rapid increase of TMP was observed. The final concentration was calculated by volume reduction ratio and verified by using a GENESYS™ 10 Bio (Thermo Scientific) spectrophotometer at OD_{600} and measuring % PCV before and after microfiltration. After each clarification experiment, the membrane was cleaned by using 300 ppm of NaOCl for 30 minutes. Cleaning efficiency was assessed by measuring normalized water permeability (NWP) with acceptance criteria to be within ± 90% of original NWP for every cycle. The first set of loading experiments was performed using 2 L, 6 L, 10 L, and 20 L of 2% yeast lysate solution with a 10× volumetric concentration. The increasing volumetric loading was done to determine the maximum loading of the microfiltration membrane in a progressive manner. Volumetric loading is defined as the amount of fluid that has passed through the test filter, expressed as liters per square meter of filter area. The loading experiment was repeated by using a yeast lysate solution of 6% PCV to achieve higher solid loading (% PCV) with a lesser processed volume. For whole cell yeast experiments, low solid (2% PCV) containing high volume (>20 L) and high solid (18% PCV) with low volume (2 L) experiments were carried out.

Results

Yeast Lysate

The critical flux of 2% PCV yeast lysate in 50 mM PBS was found to be 41 LMH, with a TMP ratio of 1.58 reached at 45 LMH (Table 1). The complete profile of the critical flux

TABLE 1. TMP ratio of critical flux (2% PCV yeast lysate).

Permeate Flux (LMH)	TMP Initial (psi)	TMP Final (psi)	TMP Ratio	Critical Flux Reached
37	2.1	2.5	1.20	NO
41	3.3	4.6	1.37	NO
45	**4.7**	**7.4**	**1.58**	**YES**

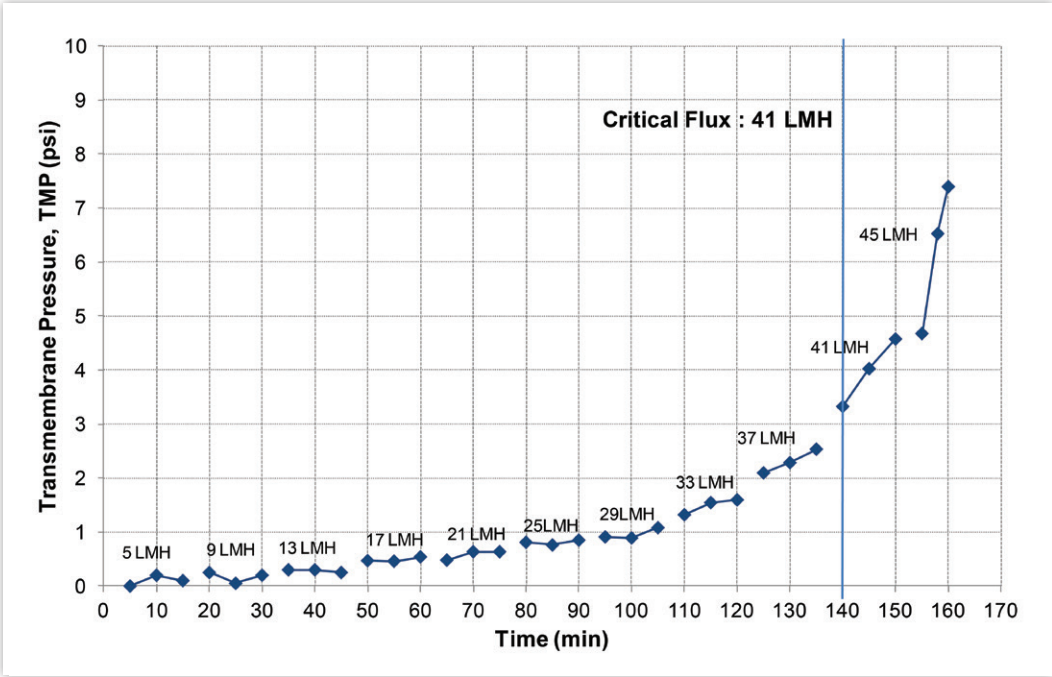

FIGURE 2. Critical flux experiment results with yeast lysate (2% PCV) solution.

experiment is presented in Figure 2.

The TMP profile of the 2% PCV lysate solution showed a similar linear shape for all the 2 L, 6 L, 10 L, and 20 L volumetric loading experiments with a 10× volume concentration, demonstrating consistency in membrane performance and experimental design (Figures 3 [below] and 4 [next page]). Maximum volumetric loading was reached when the TMP started to increase rapidly. As shown in Figure 3, the maximum volumetric loading for 2% PCV lysate solution was 187 L/m^2. This phenomenon is better illustrated in Figure 4 with the TMP increment observed only after 180 L/m^2 when the % PCV was

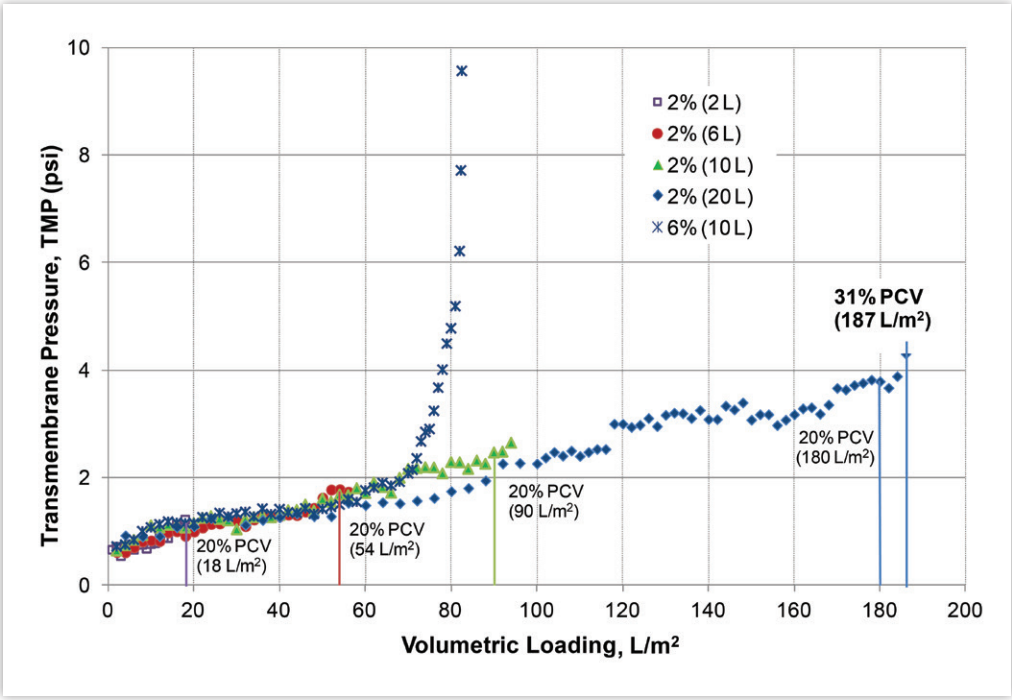

FIGURE 3. TMP vs. volumetric loading for various volumes of 2% and 6% PCV yeast lysate.

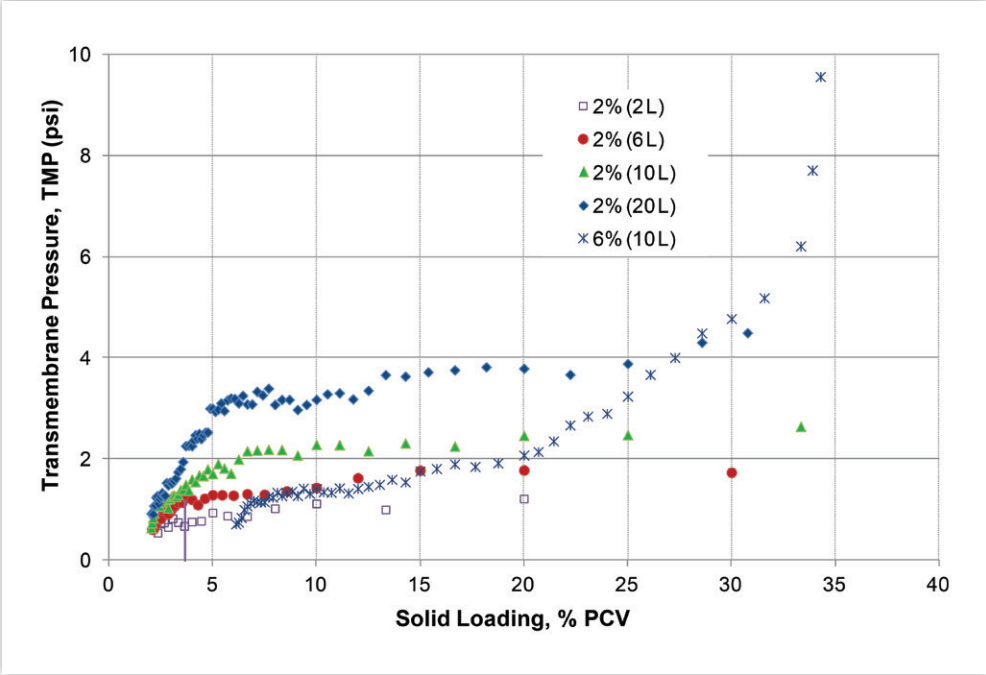

FIGURE 4. TMP vs. solid loading for various volumes of 2% and 6% PCV yeast lysate.

progressively increased from 20% to 30%. The loading experiments using 6% PCV lysate solution also showed TMP increments after reaching 20% PCV (Figure 4), with the TMP increased exponentially to 5 psi after a solid loading of 30% PCV. The maximum volumetric loading for 6% PCV lysate was found to be 80 L/m^2 (Figure 3).

Whole Cell Yeast

Unlike our observations in the yeast lysate feed, for our 2% PCV whole cell yeast critical flux experiment, a rapid TMP increment was not observed even when the permeate conversion was >50% of feed flux (Figure 5). Similar phenomena were also observed when the experiments

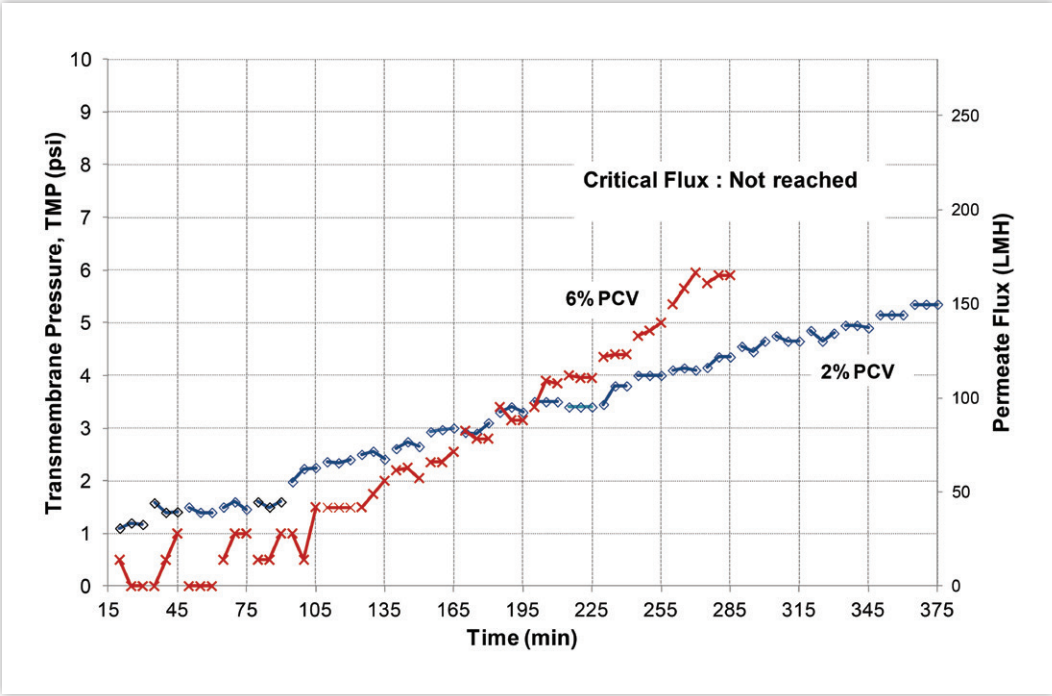

FIGURE 5. Critical flux experiment results with whole cell yeast (2% and 6% PCV) solution.

were run using higher starting feed concentration (*i.e.*, 6% PCV of whole cell yeast solution [Figure 5]).

Since 2% and 6% PCV as starting concentrations of feed (yeast cells) did not result in an identification of the critical flux point, an experiment was conducted using further higher solid content (20% PCV) as the starting feed concentration of yeast cells. The results are presented in Figure 6 and Table 2. It is evident that the TMP became unstable after a flux of 77 LMH and later increased consistently, reaching 13 psi.

Considering 77 LMH as the observed critical flux point, subsequent experiments were conducted at 35 LMH (50% of critical flux). The loading experiments (Figures 7 [right] and 8 [next page]) indicate that the loading limit for low % PCV of whole cell yeast solution at an operating flux of 35 LMH was about 55% PCV, which translates to a volumetric loading of about 200 L/m^2.

To investigate the potential limitation of solid loading independent of volume processed, high % PCV was used as starting feed solution. The TMP versus loading profile with 18% PCV whole cell yeast challenge is illustrated

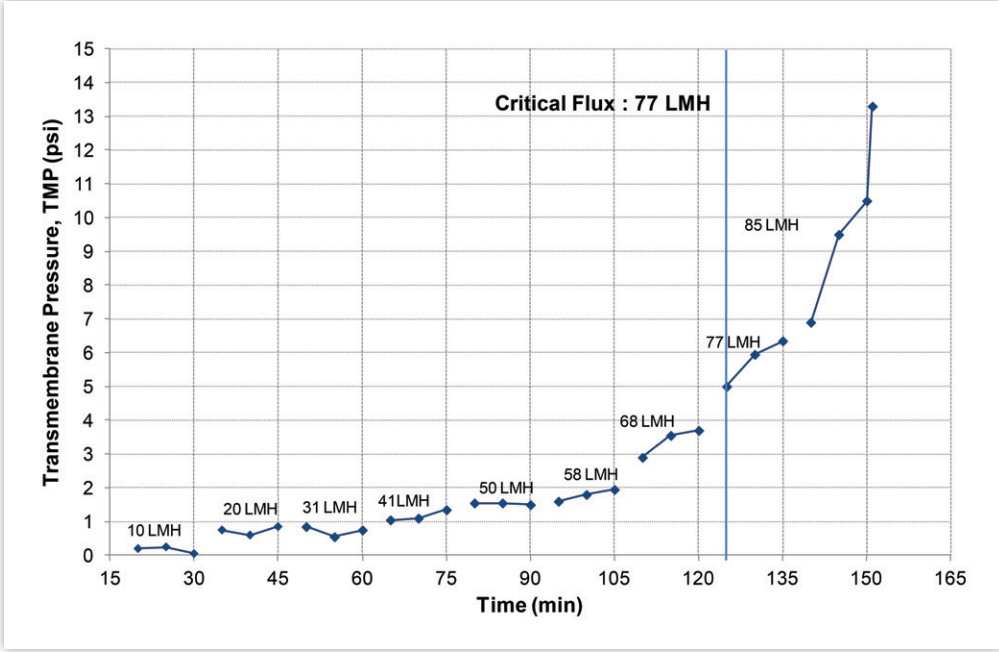

FIGURE 6. Critical flux experiment results with whole cell yeast (20% PCV).

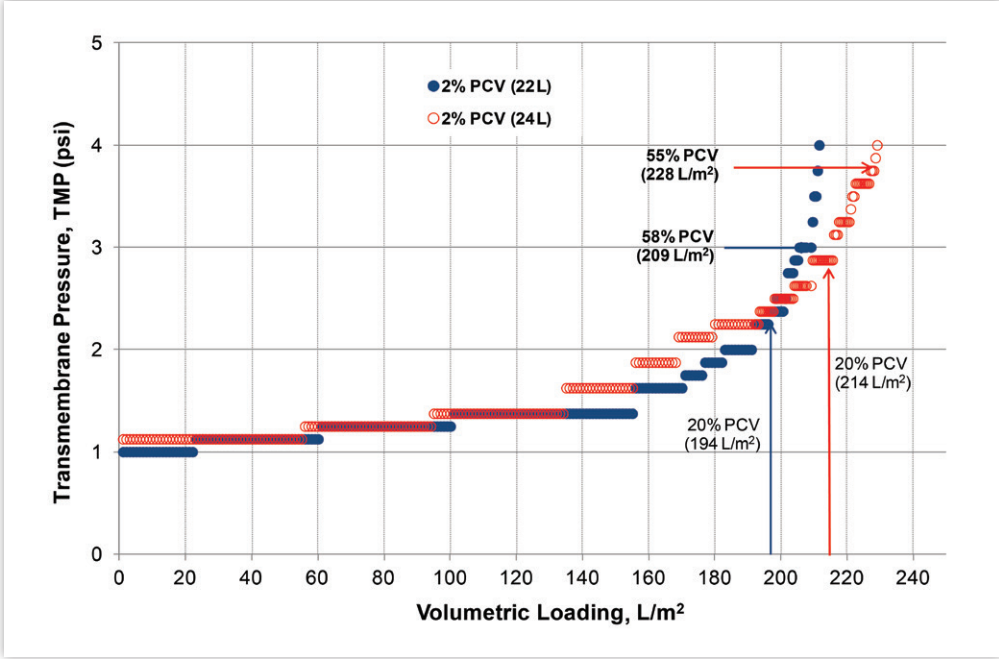

FIGURE 7. TMP vs. volumetric loading for low % PCV whole cell yeast.

TABLE 2. TMP ratio of critical flux experiment using 20% PCV of whole cell yeast.

Permeate Flux (LMH)	TMP Initial (psi)	TMP Final (psi)	TMP Ratio	Critical Flux Reached
68	2.9	3.7	1.28	NO
77	5.0	6.4	1.28	NO
85	**6.9**	**13.0**	**1.89**	**YES**

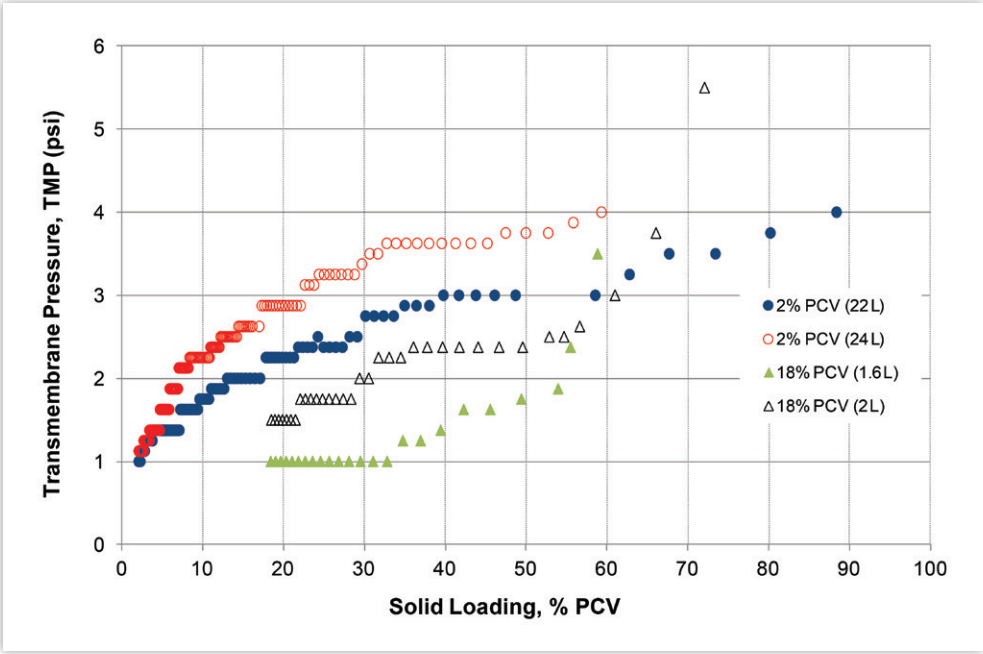

FIGURE 8. TMP vs. solid loading for whole cell yeast.

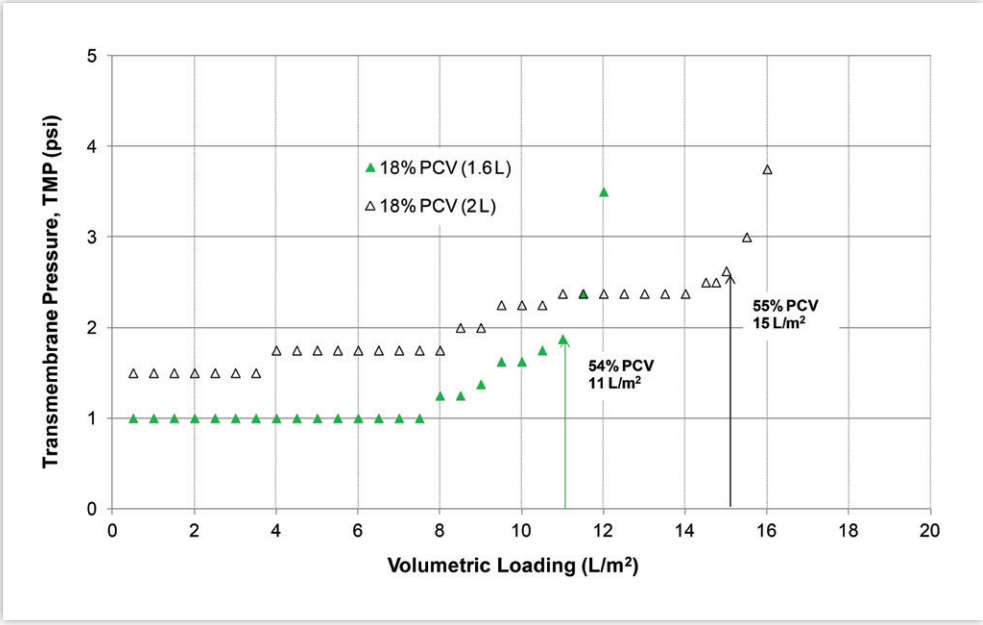

FIGURE 9. TMP vs. volumetric loading for high % PCV whole cell yeast.

in Figures 8 and 9. It is not surprising to note that only minimal volumetric loading (11–15 L/m^2) was achieved with such high starting solid content in the feed stream (Figure 9). TMP only began to increase when the solid loading increased to more than 55% (Figure 8). This again corroborated that the maximum solid loading for whole cell microfiltration is about 55%, which is significantly higher than yeast lysate solution. The overall loading results are presented in Table 3.

Discussion

Loading Limits

Prior to the experiments, it was assumed that 20% PCV was the maximum solid loading capacity for plate and frame-type microfiltration devices. This assumption was based on some historical internal data (not shown here). In contrast to historical data, the 2% lysate (representing small particles) volumetric loading challenge experiment resulted in TMP instability only when volumetric loading exceeded 180 L/m^2 (30% PCV). The volumetric loading was reduced to 80 L/m^2 when 6% PCV was used as the starting feed solution. It is clear that % PCV is the limiting factor for stable microfiltration operation. Experiments with whole

TABLE 3. Solid and volumetric loading for whole cell yeast and lysate solution.

Feed Solution	Permeate Flux (LMH)	Max. Volumetric Loading (L/m^2)	Max. Solid Loading (% PCV)
2% lysate	20	180	30
6% lysate	20	80	30
2% whole cell	35	200	55
18% whole cell	35	11–15	55

cell solutions (representing larger plugging particles) resulted in maximum achievable solid loading of 55% PCV (almost double the solid loading of yeast lysate) or volumetric loading of 200 L/m^2. These differences in achievable final % PCV could be due to differences in feed characteristics, as colloidal particles tend to increase in lysate solution rather than whole cell yeast solution. This has been described by the inertial migration model.[4] From the loading experiments, it is shown that the final solid concentration (% PCV) dominates in the maximum design capacity of microfiltration filter sizing at the selected constant permeate flux.

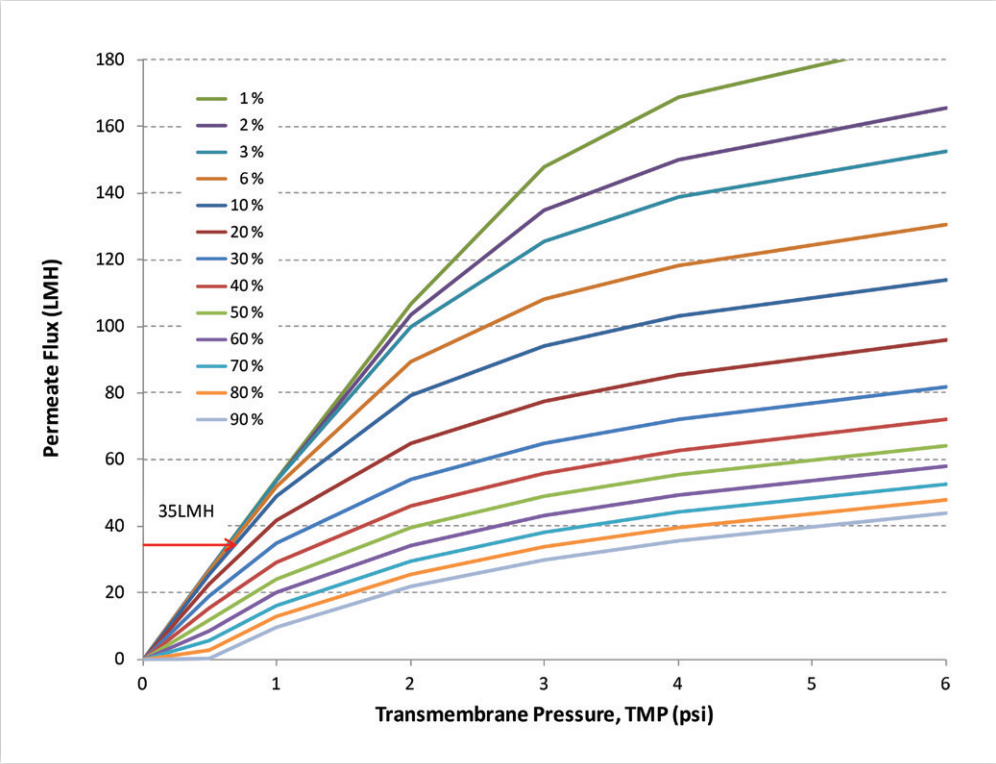

FIGURE 10. Predicted TMP vs. permeate flux (LMH) at various whole cell yeast concentrations. $R_m = 0.0185$ psi/LMH, $n = 2$, $a = 0.6$, $k = 37$ LMH

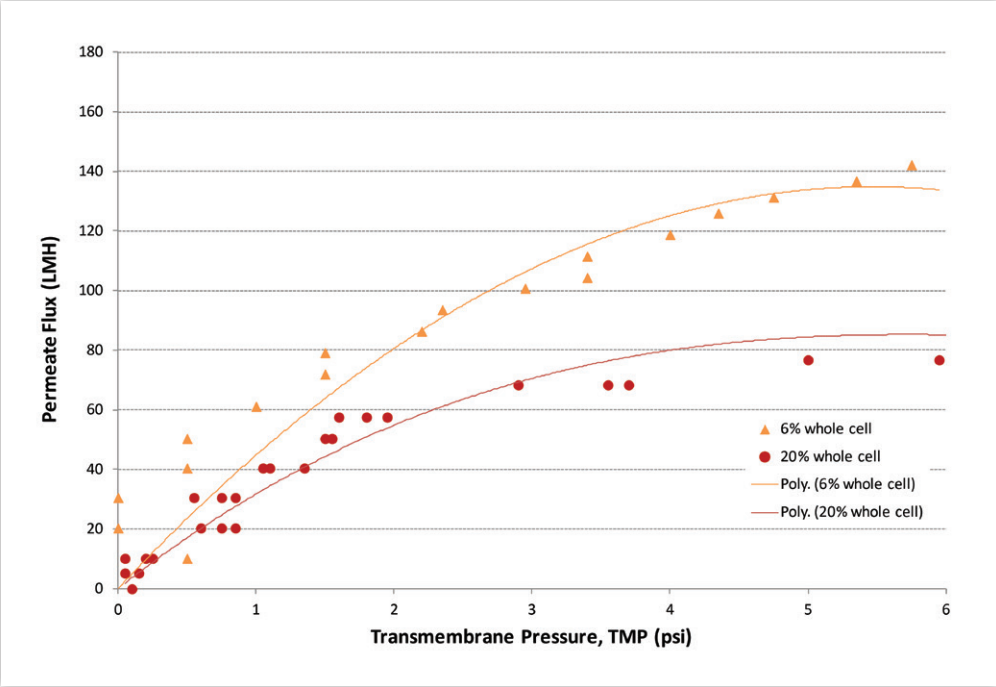

FIGURE 11. Measured TMP vs. permeate flux at 6% and 20% PCV.

Bulk Concentration Impact on TMP Curve

The impact of different initial bulk concentrations (C_b or % PCV) on a TMP profile was further investigated. Similar to the TMP excursion curve performed on ultrafiltration experiments, the flux (J) versus initial TMP data (ΔP, measured at five minutes) of whole cell yeast was fitted using the osmotic pressure model equation[5]:

$$J = \frac{\Delta P - aC_b^n \, exp\,(nJ/k)}{R_m}$$

In this equation, R_m is the hydraulic resistance of the membrane calculated by the linear region of the TMP curve of 6% PCV whole cell yeast; k is the mass transfer coefficient, and a and n are the osmotic pressure model parameters, respectively. The predicted TMP excursion curve (Figure 10) and actual TMP excursion curve (Figure 11) showed that permeate flux increases with TMP linearly up to a point and levels off. As evident from Figures 10 and 11, there is an optimum flux (critical flux) at the transition point of each curve that represents different concentrations.

When critical flux experiments were carried out, we backed out 50% from the

critical flux point to set a safe operating flux for concentration. But the final achievable concentration was not considered at that time point. To better understand the impact of operating flux selection on the final achievable concentration, we reviewed the actual concentration experiment results performed with whole cell yeast. Whole cell yeast experiments were carried out at 35 LMH at constant permeate flux (half of critical flux at 20% PCV, Figure 6) and clearly indicate no change of TMP even if the feed material (2% PCV whole cell) is concentrated to 30%. However, when the concentration exceeded 40% PCV, the TMP began increasing exponentially (Figure 12). This analysis suggests that the final maximum achievable concentration can be increased if the operating flux is reduced to 10 LMH. Thus, it is important that critical flux experimentation is carried out with the initial bulk concentration (% PCV) and final target concentration (if known) to understand the change of the TMP profile during concentration and to design better process control.

Plugging Model Analysis

The TMP profiles for both the lysate and whole cell yeast were fitted to different plugging models[3] to understand the plugging behavior. Though the two pump microfiltration was performed at a constant flow, the plugging model of TFF at constant pressure[6] was used here as reference. The analysis showed that the cake filtration model fits the data of lower loading and lower % PCV with larger particle deposition on the membrane surface, reducing the accessible filtration area but yet, permeable to fluid flow.[7] When more fluid passed through the filter (volumetric loading >100 L/m^2) the "cake growth" occurred simultaneously with the plugging of the remaining open area of the membrane, causing a more rapid increase of hydraulic resistance to flow (rise of TMP), and the plugging mechanism shifting to complete pore blocking. The combined cake-complete plugging best fit the loading experiment data and are presented in Table 4.

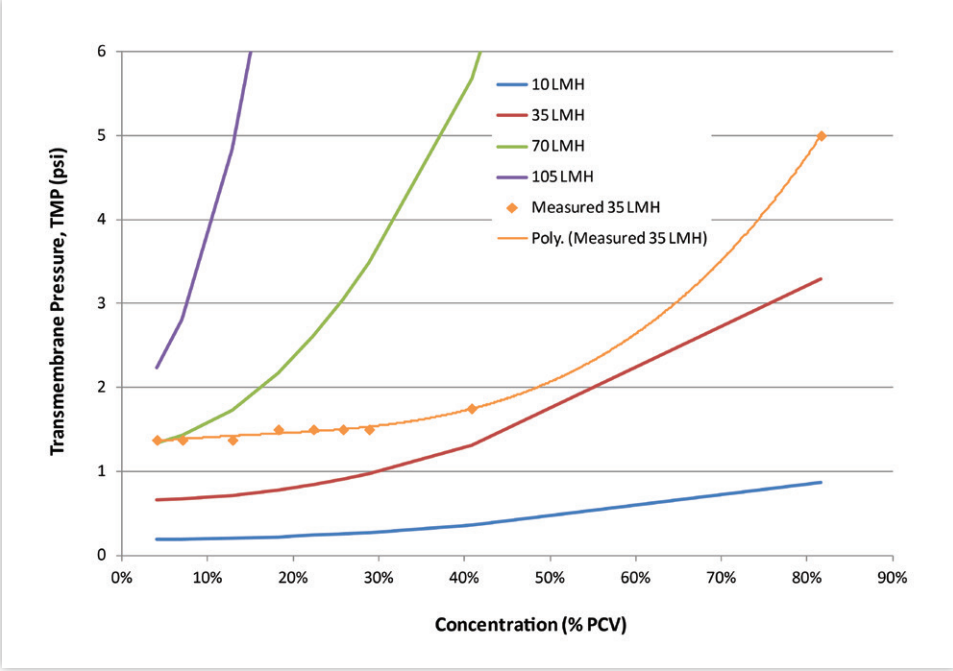

FIGURE 12. Predicted TMP of various operating fluxes compared with experiment data.

TABLE 4. Plugging model analysis.

Solid Contents	% PCV	Model	Parameter			R^2	Remarks
			P_0 [psi]	K_b [1/s]	K_c [s/m^2]		
Yeast lysate	2	Cake	0.9	—	0.320	0.940	Fitted < 100 L/m^2
Yeast lysate	2	Cake–Complete	0.9	0.026	0.330	0.961	—
Yeast lysate	6	Cake–Complete	0.9	0.240	0.041	0.990	—
Whole cell yeast	2	Cake	1.0	—	0.059	0.746	Fitted < 100 L/m^2
Whole cell yeast	2	Cake–Complete	1.0	0.140	0.016	0.750	—
Whole cell yeast	18	Cake–Complete	1.0	2.600	0.180	0.978	

Cake: $P = P_0 (1 + K_c J V)$ Cake–Complete: $P = \frac{P_0}{(1 - K_b V/J)} \left(1 - \frac{K_c J^2}{K_b} \ln (1 - K_b V/J)\right)$

Both Figures 13 and 14 show the good fit of data with the plugging model, suggesting that the microfiltration filters demonstrated similar plugging mechanisms with such feed and filter interactions.

Conclusions and Recommendations

This article highlights the dependency of loading limits on operating permeate flux in flux controlled TFF microfiltration processes. Microfiltration processes operating at low permeate flux can reach higher concentrations compared to high permeate flux. The best practice in a TFF microfiltration optimization and scale-up process is to determine the critical flux of the initial bulk and target a final concentration to avoid reaching the polarized region during the clarification (concentration) process. Permeate flux with stable TMP at the highest possible concentration is the ideal operating condition. Loading limits of microfiltration processes are expected to change with feed (particle size and type) and filter interaction. Both the lysate and whole cell yeast showed similar combined cake-complete plugging mechanisms when a V-screen 0.1 µm microfiltration filter was used. The experimental data indicated that solid loading dominates the microfiltration endpoint determination with the TMP increasing rapidly at final concentration. The recommendation is to measure solid content (% PCV) of the feed solution to better estimate the final achievable solid or volumetric loading limit of a certain microfiltration step. It is worthwhile to note that the results discussed here are only based on 0.1 µm V-screen PVDF microfiltration membrane, but a similar trend is expected in other membrane types and feed streams. There are many biotherapeutic product and vaccine processes that employ microfiltration TFF for primary or secondary clarification. It is hoped that this article adds useful insights into the design and operation of constant flux microfiltration processes and benefits potential users in the biopharmaceuticals and vaccine industries.

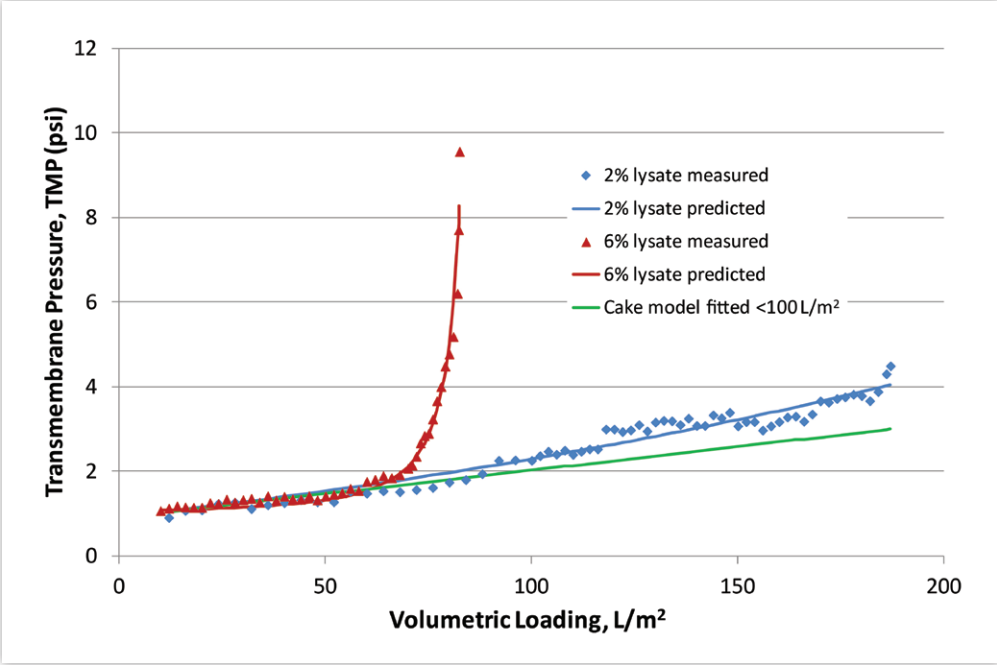

FIGURE 13. Lysate data fitted into a combined cake–complete plugging model.

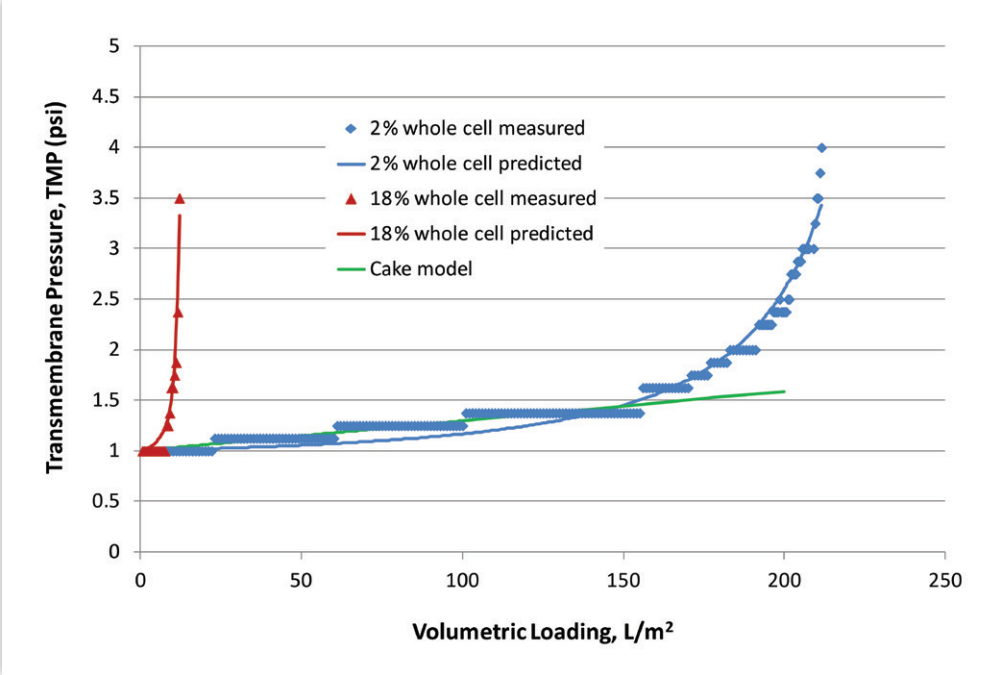

FIGURE 14. Whole cell data fitted into a combined cake–complete plugging model.

Acknowledgement

The experimental work carried out by Wang Meng Meng and Chan Ming Zhan is duly acknowledged.

References/Further Reading

[1] Raghunath B, Bin W, Pattnaik P, Janssens J. Best practices for optimization and scale-up of microfiltration TFF processes. *BioProcess J*, 2012; 11(1): 30-40. http://dx.doi.org/10.12665/J111.Raghunath.

[2] Kubota T, Kawakami Y, Ito T. *Enhancement of protein recovery with permeate flux control for open ultrafiltration membrane in tangential flow filtration*. Poster presented at YABEC (Young Asian Biochemical Engineers' Community); 2012; Japan.

[3] Lutz H, Blanchard M, Abbott I, Parampalli A, Setiabudi G, Chiruvolu V, Noguchi M. Considerations for scaling-up depth filtration of harvest cell culture fluid. *BioPharm Int*, March 2009; 22.

[4] Ho C-C, Zydney AL. Transmembrane pressure profiles during constant flux microfiltration of bovine serum albumin. *J Membrane Sci*, 2002; 209: 363-377. http://dx.doi.org/10.1016/S0376-7388(02)00282-X.

[5] Wijmans JG, Nakao S, Smolders CA. Flux limitations in ultrafiltration: osmotic pressure model and gel layer model. *J Membrane Sci*, 1984; 20: 115-124. http://dx.doi.org/10.1016/S0376-7388(00)81327-7.

[6] Razaei H, Ashtiani FZ, Fouladitajar A. Effects of operating parameters on fouling mechanism and membrane flux in cross-flow microfiltration of whey. *Desalination*, 2011; 274: 262-271. http://dx.doi.org/10.1016/j.desal.2011.02.015.

[7] Belfort G, Davis RH, Zydney AL. The behavior of suspensions and macromolecular solutions in cross-flow microfiltration. *J Membrane Sci*, 1994; 96(1-2): 1-58. http://dx.doi.org/10.1016/0376-7388(94)00119-7.

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