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# Secondary Membranes for Flux Optimization in Membrane Filtration of Biologic Suspensions

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## Abstract

We employ in situ deposited secondary membranes of yeast (SMYs) to optimize permeate flux during microfiltration and ultrafiltration of protein solutions. The deposited secondary membrane was periodically removed by backflushing, and a new cake layer was deposited at the start of the next cycle. The effects of backflushing time, backflushing strength, wall shear rate, and amount of secondary membrane deposited on the permeate flux were examined. Secondary membranes were found to increase the permeate flux in microfiltration by severalfold. Protein transmission was also enhanced owing to the presence of the secondary membrane, and the amount of protein recovered was more than twice that obtained during filtration of protein-only solutions under otherwise identical conditions. In ultrafiltration, the flux enhancement owing to the secondary membrane was only 50% or less. In addition, the flux for ultrafiltration was relatively insensitive to changes in the concentration of yeast used during deposition of SMY and to the backflushing strength used to periodically remove the secondary membrane.

**Index Entries:** Secondary membrane; backflushing; microfiltration; ultrafiltration; direct visual observation; fouling.

## Introduction

Membrane filtration, with gentle conditions, no phase change, and low energy expenditure, has great potential for separations in biotechnology and other fields. However, fouling has hindered the adoption of membrane technology for separations *(1)*. Membrane fouling during filtration of biotechnological fluids with cells, proteins, cell debris, colloidal particulate matter, and dissolved solutes is quite complex *(2)*. Fouling in this case can be broadly divided into two types: external fouling and internal fouling.

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External fouling is caused by the formation of a cake layer of cells or other materials on the membrane surface, leading to a reduction in permeate flux (defined as the volume of permeate produced per time and membrane area). Internal fouling is caused mainly by proteins and particles smaller than membrane pores. Proteins and protein aggregates can adsorb or deposit at the pore entrance or inside the pores and cause pore blockage or narrowing, leading to increased hydraulic resistance *(2)*.

Fouling can often be minimized by using backflushing, which involves periodic reversal of flow for a few seconds after several minutes of forward filtration. Redkar and Davis *(3)* achieved a 30-fold increase in permeate flux using more rapid backpulsing conditions of 0.5 s of backpulse after every 5 s of forward filtration. Backflushing or backpulsing has also been applied to ultrafiltration to improve separation performance. Rodgers and Sparks *(4)* observed that the permeate flux could be increased as much as two orders in magnitude in crossflow ultrafiltration of protein solutions when transmembrane pressure pulsing was applied. Although some studies show that backflushing is not always effective in increasing the permeate flux for crossflow ultrafiltration *(5)*, other studies have demonstrated flux improvement and optimization, using backflushing or backpulsing for microfiltration *(6–9)* and ultrafiltration *(10–12)*.

Backflushing and backpulsing are least effective in tackling irreversible or internal fouling, such as when proteins adhere to the membrane surface or inside the membrane pores. Kuberkar and Davis *(13)* carried out backflushing during microfiltration of mixtures of yeast and bovine serum albumin (BSA). They observed that backflushing is only partially effective in removing the internal foulants. The flux decreased with increased internal fouling and finally appeared to reach a nearly steady value as low as 5% of the clean membrane flux. However, the permeate flux for filtration of the protein-cell mixture with backflushing showed a marked improvement over that of a protein-only solution. This finding is related to the concept of dynamic secondary membranes. The yeast cake layer formed simultaneously with membrane separation during filtration of yeast-BSA acts as a selective barrier, screening the primary membrane from fouling by protein aggregates, while allowing individual protein molecules to pass through the cake layer and primary membrane. Protein aggregates are notorious for depositing at the entrance of and inside the membrane pores and acting as nuclei for further protein deposition *(14,15)* leading to pore narrowing or blockage. This type of fouling is, to a large extent, irreversible and is the primary cause of lower fluxes and lower protein transmission.

Marcinkowsky et al. *(16)* were the first to use dynamic secondary membranes in reverse osmosis for rejection of salts. Güell et al. *(17)* later investigated protein transmission and permeate fluxes in microfiltration of protein mixtures using yeast to form a predeposited secondary membrane, and they observed higher flux and protein transmission in the presence of the secondary layer. Kuberkar and Davis *(18)* also observed higher flux and transmission of BSA in the presence of a cake layer of yeast,

showing that the separation is improved by using the secondary membrane. At low yeast concentrations (<1g/L), BSA transmission was 50–100% and a high BSA recovery was achieved; at higher yeast concentrations, the transmission was higher but recovery was lower owing to low fluxes. Even with a secondary membrane, the flux gradually decreased owing to slow fouling of the secondary membrane. Periodic removal of the secondary membrane can temporarily recover the flux, but then irreversible fouling of the primary membrane occurs due to its temporary exposure to the feed solution after each time the secondary membrane is removed *(13)*.

In the current work, we employed a modified approach, with predeposition of a secondary membrane of yeast (SMY) before starting the filtration of protein. Backflushing was employed periodically to remove the deposited secondary membrane to recover the flux, and a new secondary membrane was deposited subsequently with the start of each new cycle, prior to restarting the filtration of protein. Microfiltration experiments were performed with yeast as the secondary membrane and BSA-only solutions and yeast-BSA mixtures as the feed. Ultrafiltration experiments were performed with yeast as the secondary membrane deposition medium and cellulase enzyme solutions, used in the conversion of biomass into ethanol, as the feed. In this article, we also present direct visual observation images *(19)* of the formation of the secondary membrane and its subsequent removal.

## Materials and Methods

### *Secondary Membrane Experiments*

The membrane filtration experiments were carried out using the setup shown in Fig. 1. The primary feed reservoir, the backflush reservoir, and the secondary feed reservoir (model DIV. WT 304, 1 gal; Alloy Products, Waukesha, WI) used for depositing the secondary layer of yeast were pressurized using high-pressure nitrogen. In the microfiltration experiments, the feed was pumped using a peristaltic pump (model no. XX 800 000 000; Millipore, Bedford, MA) and passed over a flat-sheet membrane module fabricated previously *(19)*. The membrane had an effective area of 5.5 $cm^2$. The module had a single channel 2.35 cm long, 2.35 cm wide, and 0.4 mm high. Permeate was collected and its mass is measured using an electronic balance (PG 5002; Mettler Toledo, Columbus, OH) interfaced to a personal computer using a serial port. The retentate from the feed was returned to the feed reservoir, and the retentate from the secondary feed was returned to the secondary feed reservoir. Three-way solenoid valves (model no. 453239F 3/2; Burkert, Irvine, CA) interfaced to the computer were used to switch between primary feed and secondary feed (valves A and B) and between forward filtration and reverse filtration (valve C). The valves were controlled using a QuickBasic program via a data acquisition card (DT 2805; Data Translation, Marlboro, MA). The amount of fluid lost during reverse

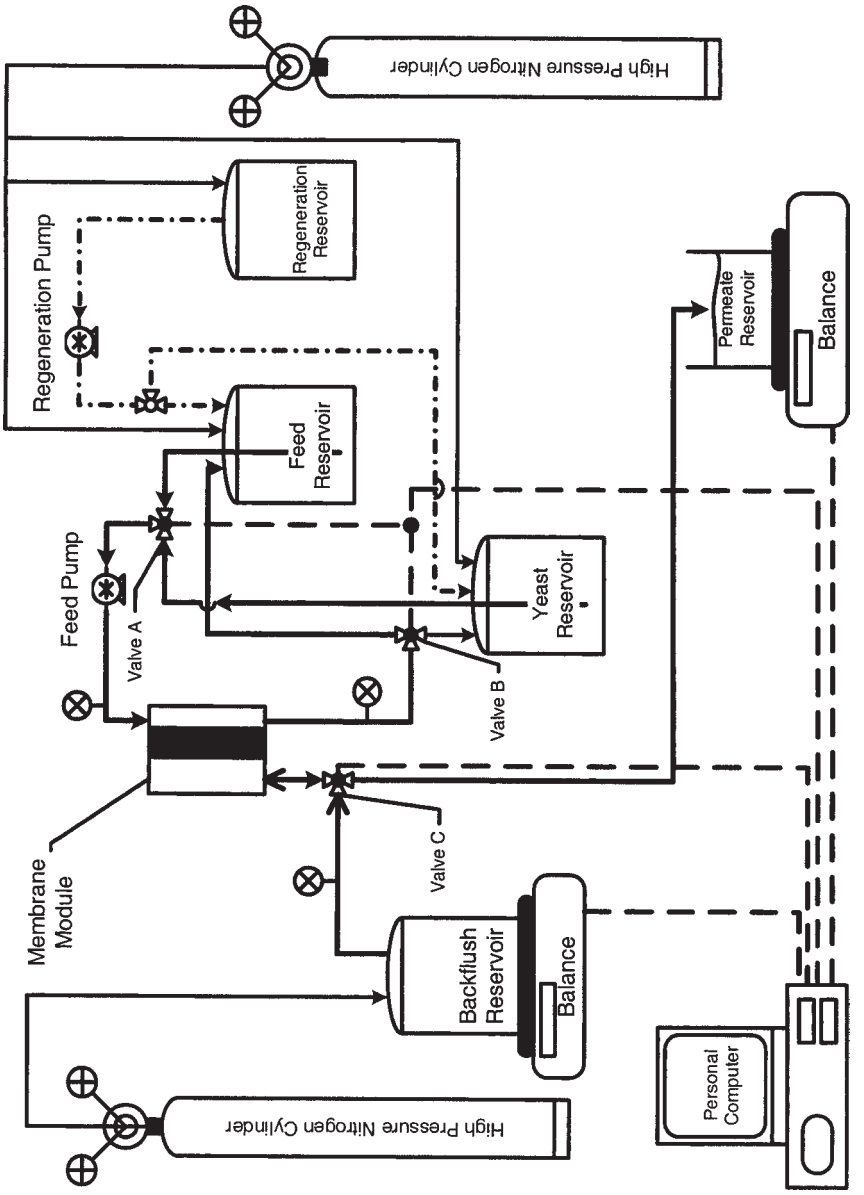


Fig. 1. Schematic of experimental setup for membrane filtration.

filtration or backflushing was measured using another balance (PM-11; Mettler Toledo, Columbus, OH), also interfaced to the computer.

Flat-sheet cellulose-acetate membranes (Acetate plus, 0.22 µ, plain, supported, catalog no. A02SP04700, batch no. 166421; GE Osmonics, Minnetonka, MN) were used in the microfiltration experiments. The membranes were hydrophilic and symmetric and had an approximate thickness of 85 µ. Each membrane was used once and then discarded. Washed yeast (*Saccharomyces cerevisiae*, commercially available Fleischmann's Active Dry Yeast) was used in preparing the yeast-only suspensions for depositing the secondary membrane. BSA (heat-shocked fractionate, fraction V powder, catalog no. A7906; Sigma, St. Louis, MO) was used for the protein-only feed suspensions. Mixtures of yeast and BSA were used as feed in a few experiments. Commercial dry yeast contains solutes and cell debris, which are removed during the process of washing. The weight of dry washed yeast remaining is found to be 68% of the weight of dry unwashed yeast. Washed yeast was prepared by adding the appropriate amount of yeast to 250 mL of deionized water and then centrifuging (Beckman GPR Centrifuge) at 1500*g* for 10 min, the supernatant was discarded, and the pellet was resuspended. This procedure was repeated three times. Feed consisted of BSA solutions (2.0–4.0 g/L) and yeast-BSA mixtures (0.68–1.34 g/L of washed yeast, 2.0–4.0 g/L of BSA).

The secondary feed used for deposition of the secondary membrane consisted entirely of yeast suspensions with concentrations ranging from 1.34 to 4.0 g/L. Yeast concentrations are reported as dry weight after washing. An average transmembrane pressure (TMP) of 7.5 psi was maintained during forward as well as reverse filtration (1 psi = 6894.7 Pa). A recirculation flow rate of 5.2–6.2 mL/s was used, with corresponding wall shear rate of 2100–2500 $s^{-1}$. The clean membrane steady flux was found to be $J_o = 3000 \pm 200$ L/($m^2$·h) at a forward transmembrane pressure of $P_f$ = 7.5 psi, and it was found to vary almost linearly with TMP. This flux value is reported as average ±1 SD for 25–30 repeats. The filtration cycle started with deposition of the SMY for $t_{sf}$ = 5–25 s, followed by forward filtration for 275–300 s, and finally removing the SMY by backflushing with a reverse TMP of $P_b$ = 7.5 psi for $t_b$ = 0.1–10 s. The cycle was then repeated. The experiments were carried out at room temperature (22°C). Protein transmission was measured by analyzing samples of permeate for the total protein content using a Coomassie® Plus Protein Assay (no. 23236; Pierce, Rockford, IL,) at 595 nm on a Perkin-Elmer Lambda 40 UV/VIS spectrometer. The Coomassie Plus Protein Assay uses a Coomassie dye-protein-binding colorimetric method based on modification of the Bradford method for total protein quantization.

Ultrafiltration experiments were carried using the same setup, except the flat-sheet membrane module had a filtration area 38 mm long, 29 mm wide, and 1.6 mm high, with a surface area of 11 $cm^2$. The permeate side had 11 grooved channels that supported the membrane, leaving an effective filtration area of 6.4 $cm^2$. However, the total membrane area including the supports was used in calculating the fluxes. The feed consisted of cellulase enzyme solution (5.0 g/L of supplied cellulase, unless noted otherwise).

The cellulase enzyme was a commercial preparation made specifically for the National Renewable Energy Laboratory by Iogen (Ottawa, Ontario, Canada). It is supplied as a liquid solution, reported to contain 205 g/L of total soluble protein, of which 158 g/L is high molecular weight proteins retained by a 30,000-Dalton mol wt cutoff ultrafiltration membrane *(20)*.

The secondary feed used for deposition of the secondary membrane consisted entirely of yeast suspensions with concentrations ranging from 1.34 to 6.8 g/L. Ultrafiltration experiments were carried out using a flat-sheet, asymmetric polysulfone membrane (lot no. 0589702311M11 3355-04P; Millipore) with a 30,000-Dalton mol wt molecular weight cutoff. The membranes were used once and then discarded after each experiment. The feed was circulated at 5.0-16.2 mL/s, corresponding to a wall shear rate of 100–1300 $s^{-1}$. The steady flux of deionized water for clean membrane was found to be $J_o$ = 375 ± 40 L/($m^2$·h), independent of the wall shear rate. Ultrafiltration experiments employed a filtration cycle similarity to that of the microfiltration experiments, with a forward filtration time of $t_f$ = 300 s and an SMY deposition time of $t_{sf}$ = 5–30 s. Average TMPs of $P_f$ = 15–30 psi and $P_b$ = 5–15 psi were maintained during forward and reverse filtration, respectively, with a fixed backflushing time of $t_b$ = 2 to 3 s.

### *Direct Visual Observation*

Fouling of the cellulose-acetate microfiltration membranes was observed using a Nikon Labophot microscope in a direct visual observation setup described elsewhere *(19)*. The membrane was observed from above using a glass cover slip for the top of the channel. The images were captured with a color digital camera (JVC TK-1270; Victor, Yokohama, Japan) connected to a PowerMac G4 containing an LG-3 framegrabber card (Scion, Frederick, MD). The real-time images were displayed on the screen of a PowerMac monitor using NIH Image (US National Institutes of Health, http://rsb.info.nih.gov/nih-image/). Cellulose-acetate membranes were viewed at a magnification of ×350 using a Nikon E-10 ×10 objective. Scanning electron microscopy (SEM) images were also taken to observe the surface of fouled membrane more precisely.

## Results and Discussion

### *Direct Visual Observation of Membrane Fouling*

Figure 2A depicts pictures of the microfiltration membrane surface for various stages of deposition of yeast. The concentration of yeast in the secondary feed was 2.0 g/L, with the primary feed containing 2.0 g/L of BSA. A complete cycle consists of $t_{sf}$ = 15 s of SMY, followed by $t_f$ = 275 s of forward filtration, and ending with $t_b$ = 10 s of reverse filtration, all at a TMP of $P_f = P_b$ = 7.5 psi. The pictures become progressively darker with increasing yeast deposition during the first cycle. After 9 to 10 s of yeast deposition, the images of the membrane surface are completely dark, an indication that multiple layers of yeast were deposited over the membrane surface.

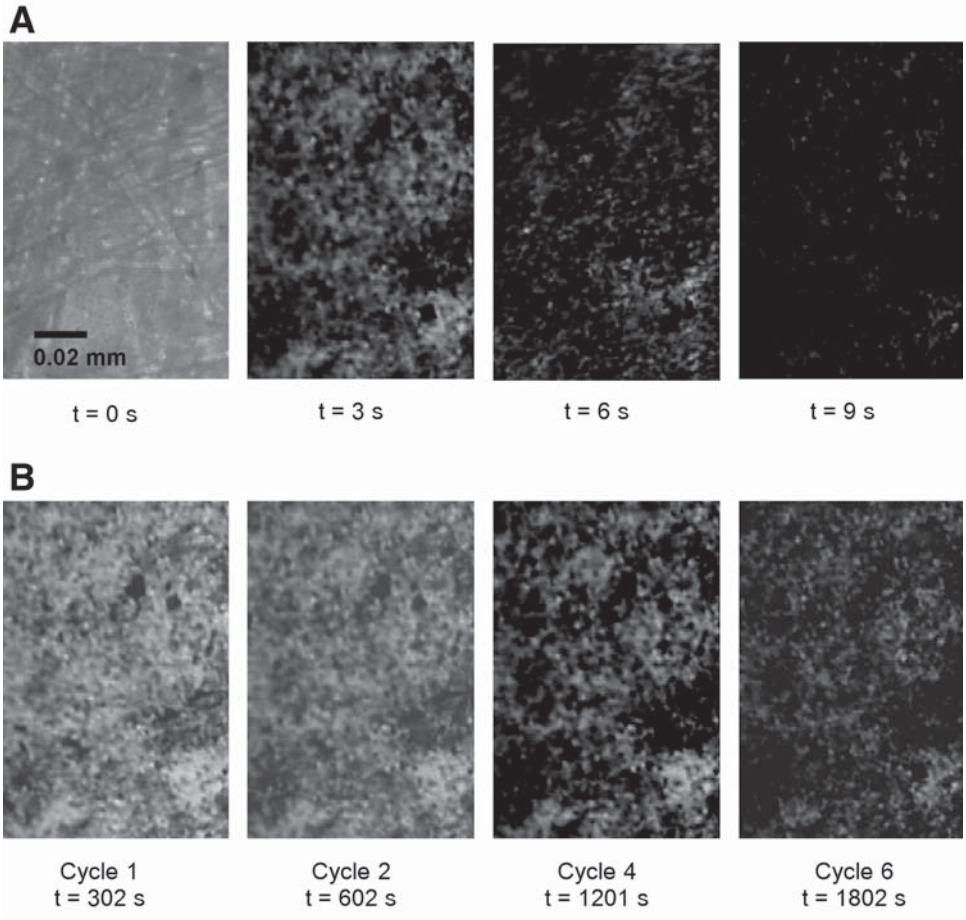


Fig. 2. Direct visual observation pictures of surface of membrane **(A)** during first cycle of deposition of SMY, and **(B)** at end of several cycles of yeast-BSA microfiltration with a secondary membrane, with the pictures taken just after backflushing portion at end of indicated cycle.

Images of the membrane surface taken just after completion of backflushing for several cycles are shown in Fig. 2B. It is apparent that backflushing is partially effective in removing the deposited SMY. The images of the membrane become progressively darker as the number of cycles increases. Therefore, backflushing becomes less and less effective over time, owing to irreversible fouling of the membrane. Apparently, the BSA in the primary feed gradually foul the primary membrane and caused yeast cells to adhere to the membrane.

Figure 3A is an SEM image of a CA microfiltration membrane with a nominal pore size of 0.22 m, fouled by BSA. The image is taken after 3100 s of filtration of 2.0 g/L of BSA at a TMP of 7.5 psi without an SMY. The image is characterized by heavy fouling of the membrane owing to deposition of BSA, leading to pore narrowing and blockage seen in the image.

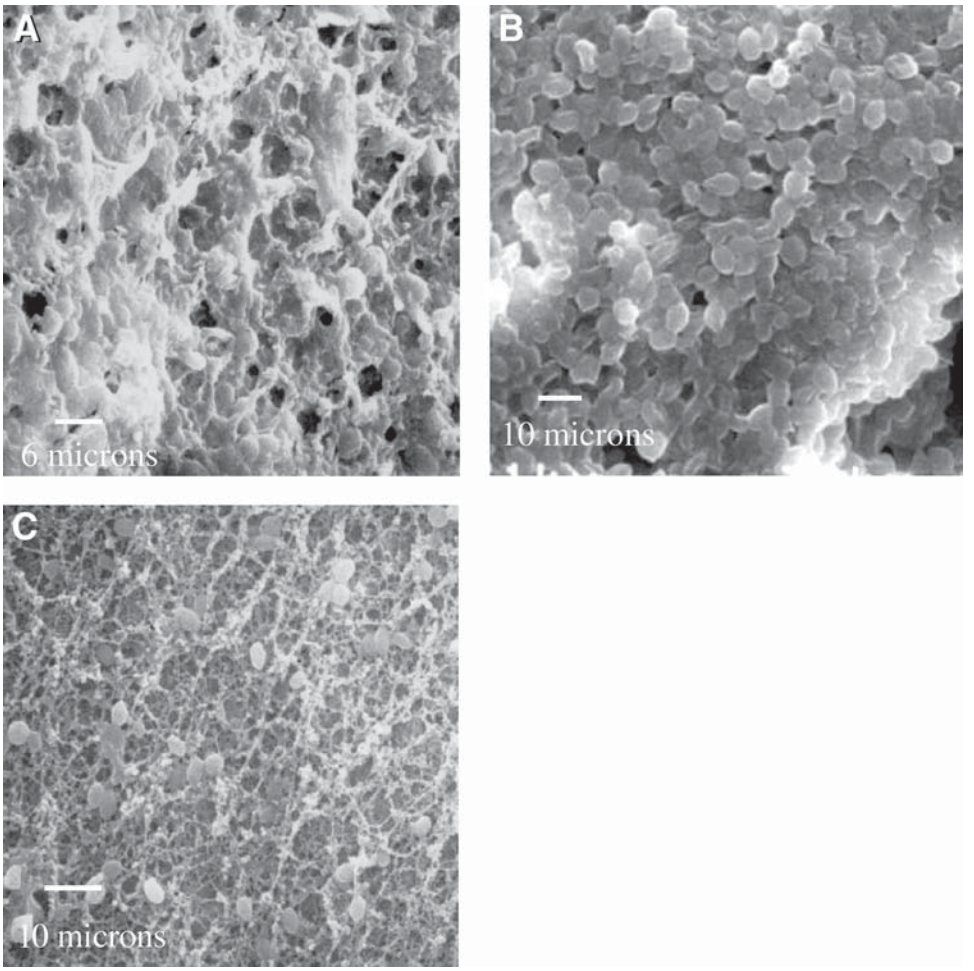


Fig. 3. SEM micrograph of a CA microfiltration membrane fouled **(A)** after filtration of BSA only for 3100 s, **(B)** after completion of SMY deposition portion of cycle 11, and **(C)** after completion of backflushing portion of cycle 11. The primary feed contained 2.0 g/L of BSA, and the secondary feed contained 1.34 g/L of yeast. The cycle conditions were $t_f$ = 300 s, $t_{sf}$ = 15 s, and $t_b$ = 3, with an average TMP of 7.5 psi maintained during forward as well as reverse filtration.

Figure 3B is a representative SEM image of another CA microfiltration membrane taken immediately after the SMY deposition portion of cycle 11, for an SMY experiment with 2.0 g/L of BSA in the primary feed, 1.34 g/L of yeast in the secondary feed, and $P_f = P_b$ = 7.5 psi. In this image, the primary membrane is completely covered by multiple layers of yeast. These layers will shield the primary membrane from internal fouling by BSA. Figure 3C is a representative SEM image of another CA microfiltration membrane taken immediately after the backflushing portion of cycle 11. Here, the membrane is relatively clean with most of the SMY removed, but there is evidence of internal fouling owing to BSA in a few places.

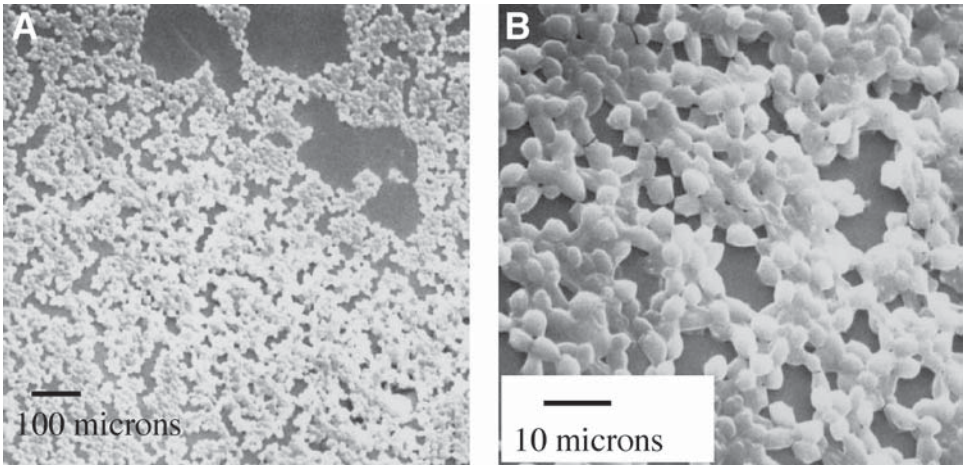


Fig. 4. SEM images of a SMY deposited over a polysulfone ultrafiltration membrane at **(A)** low magnification (↔200) and **(B)** high magnification (↔700). The image is captured during the protein filtration portion of cycle 10. The primary feed contained 5 g/L of cellulase, and the secondary feed contained 5.36 g/L of yeast. The cycle conditions were $t_f = 300$ s, $t_{sf} = 30$ s, and $t_b = 2$, with an average TMP of 15 psi maintained during forward as well as reverse filtration and a wall shear rate of 100 $s^{-1}$.

Figures 4A and 4B are scanning electron micrographs of a an ultrafilitration polysulfone membrane with a 30,000-Dalton mol wt cutoff at low and high magnification, respectively. The image was taken during the protein-filtration portion of cycle 10 (~3100 s), for an SMY experiment with 5.0 g/L of cellulase in the primary feed, 5.36 of g/L yeast in the secondary feed, and $P_f = P_b = 15$ psi. The majority of the membrane is covered by an SMY, but in a few places the SMY is absent or has been eroded. Note that the SMY is mostly monolayered, compared with the multilayered SMY seen during microfilitration. This difference is apparently due to the lower flux in ultrafiltration.

### *Microfiltration Experiments with Secondary Membranes and Backflushing*

The goal of microfiltration of protein solutions is to remove particulate matter and protein aggregates while passing individual protein molecules through the membrane. Figure 5 shows a comparison of the permeate flux, *J*, vs time obtained during microfiltration in various configurations, including microfiltration of a 2.0 g/L BSA solution with backflushing and SMY deposition, with backflushing but without predeposited SMY, and without backflushing or SMY deposition. Also shown are results for microfiltration of a mixture of 1.34 g/L of yeast and 2.0 g/L of BSA with and without backflushing. A steep decline in permeate flux was observed during microfiltration of the yeast-protein mixture without backflushing,

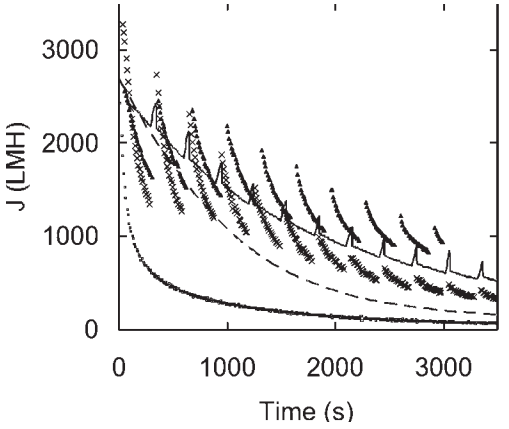


Fig. 5. Permeate flux during microfiltration of mixture of 2.0 g/L of BSA and 1.34 g/L of yeast with deposition of SMY and with backflushing (▲), without SMY but with backflushing (×), and without deposition of SMY and without backflushing (□); permeate flux for filtration of BSA without deposition of SMY and with backflushing (——), and without SMY and without backflushing (– – –). The feed for the SMY contained 0.68 g/L of yeast. The cycle conditions were $t_f$ = 280 s, $t_{sf}$ = 10 s, and $t_b$ = 10 s, with $P_f = P_b$ = 7.5 psi. LMH = L(m²·h).

and it appeared to reach a nearly steady value of 60 L/(m²·h) or only 2% of the clean membrane flux. A slightly higher flux was obtained during filtration of the BSA-only solution, but a rapid decline in permeate flux was again observed and reached a nearly steady value of 90 L/(m²·h) after 5000 s. The permeate fluxes obtained during filtration of the BSA-only solution and the yeast-BSA mixture accompanied by periodic backflushing showed improvement; however, the fluxes continued to decline steadily. The best performance was obtained during filtration of the BSA-solution with periodic deposition and removal of the secondary membrane of yeast. Apparently, the secondary membrane was effective in partially shielding the primary membrane from irreversible fouling by protein aggregates and monomers. After 3000 s, a fivefold enhancement in permeate flux was obtained owing to use of secondary membranes and backflushing over BSA-only filtration without backflushing. However, when a secondary membrane was not redeposited after each backflush, the primary membrane was exposed to fouling by BSA in the feed, and a lower flux enhancement was obtained.

Average permeate fluxes (normalized by the clean membrane water flux, $J_o$) for yeast-BSA mixtures are plotted vs time in Fig. 6 for different concentrations of yeast in the primary and secondary feed reservoirs. The average flux, $\langle J \rangle$, was calculated by dividing the amount of net permeate collected by the time required to complete one cycle of yeast deposition, feed filtration, and backflushing ($t_{sf} + t_f + t_b$); the net permeate collected is

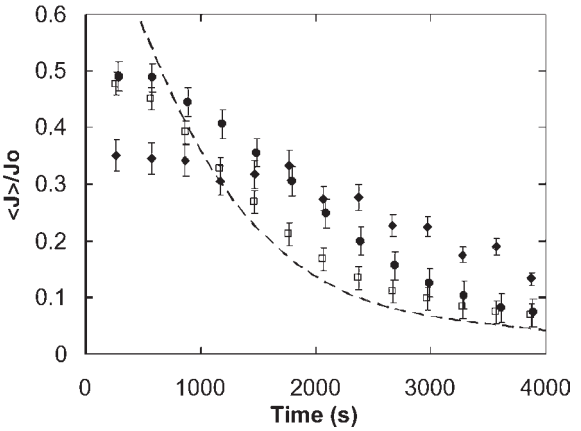


Fig. 6. Normalized microfiltration permeate flux vs time for different concentrations of yeast in secondary feed reservoir: (◆) 1.36 g/L; (●) 0.68 g/L; (□) 0.34 g/L. The cycle conditions were $t_f = 280$ s, $t_{sf} = 10$ s, and $t_b = 10$ s, with $P_f = P_b = 7.5$ psi. The primary feed contained a mixture of yeast (with the same concentration of yeast as in the secondary feed) and 2.0 g/L of BSA. The dashed line represents the normalized permeate flux during filtration of protein alone without deposition of SMY and without backflushing. Error bars represent ± SD for two to three repeats.

the difference between the mass of permeate collected during filtration of primary feed and the mass lost during backflushing. The permeate flux for the highest yeast concentration (1.36 g/L) was low initially, owing to the resistance of the thicker secondary membrane formed, but the flux tended to remain steady for longer times. By contrast, with lower yeast concentrations (0.34 and 0.68 g/L), the flux decreased more rapidly. After only 1800 s of filtration, the permeate fluxes for the lower yeast concentrations fell below that obtained for the higher yeast concentration. With a higher yeast concentration in the secondary feed reservoir, the secondary membrane of yeast formed was thicker and did a better job of shielding the primary membrane from protein foulants. By comparison, the flux for the forward filtration of BSA only started at a higher level, owing to a lack of yeast cake with added resistance, but it dropped below those with SMY after about 1000 s, as a result of protein fouling of the unprotected primary membrane.

The normalized average flux is plotted in Fig. 7 vs the duration of backflushing at the end of cycles 2, 3, and 4 for filtration of a BSA solution with an SMY. It is seen that the recovered permeate values decreased with each cycle, owing to gradual irreversible fouling. More significantly, the average flux obtained for each cycle is a maximum at a backflushing time of about 1.0 s. For lower backflushing durations, the removal of cake from the primary membrane was incomplete, and thus the flux declined owing to external foulant buildup. For higher backflushing times, there was sig-

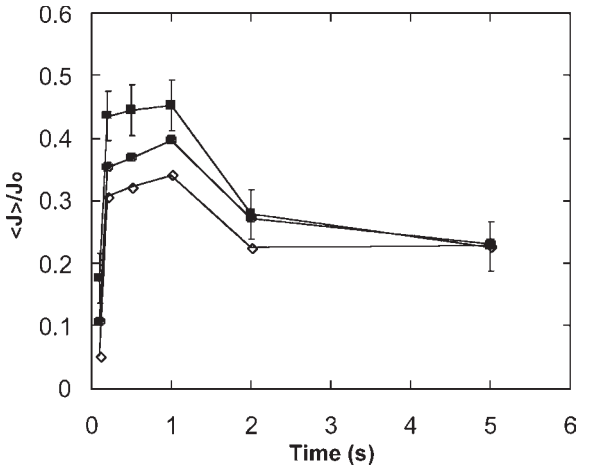


Fig. 7. Normalized recovered flux plotted after 600 s (■, cycle 2), 900 s (●, cycle 3), and 1200 s (◇, cycle 4) of microfiltration with deposition of SMY. The SMY was deposited for $t_{sf}$ = 25 s, followed by forward feed filtration of 2.0 g/L of BSA for $t_f$ = 275 s and then $t_b$ = 0.1, 0.2, 0.5, 1.0, 2.0, or 5.0 s of backflushing. The points are joined by straight lines for clarity. The error bars represent ± SD for three repeats.

nificant loss of permeate through the membrane in the reverse direction without additional cleaning, which reduced the net flux from the maximum values.

Protein transmission data (Fig. 8) indicate nearly 100% transmission of protein initially, but the fraction of protein transmitted decreased rapidly for forward filtration of a BSA-only solution and appeared to reach a steady value of only 35% transmission after 10,000 s. When a secondary membrane of yeast was deposited at the beginning of each cycle, and then filtration of a BSA solution was carried out, the protein transmission values remained at nearly 100% for about 4000 s and subsequently decreased gradually to about 60% after 18,000 s of filtration. With SMY, the amount of protein recovered in the permeate is more than two times that recovered after filtration of the BSA-only solution after 18,000 s.

### *Ultrafiltration Experiments with Secondary Membranes and Backflushing*

The goal of ultrafiltration, in contrast to microfiltration, is to retain protein molecules by the membrane while passing smaller solutes through the membrane with the permeate. Ultrafiltration experiments were performed with polysulfone membranes (30,000-Dalton mol wt cutoff). Figure 9 shows a comparison of the permeate flux vs time obtained during ultrafiltration of cellulase in the presence and absence of SMY that was periodically removed by backflushing and then replaced with a new SMY.

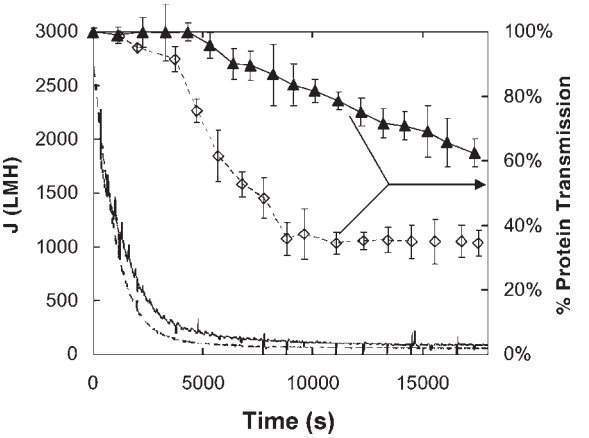


Fig. 8. Protein transmission during microfiltration of 2.0 g/L BSA only (◇) and 2.0 g/L of BSA in presence of SMY and backflushing (▲), and permeate flux vs time for microfiltration of BSA only (– – –) and for microfiltration of BSA with deposition of SMY and backflushing (——). The cycle conditions were $t_f$ = 300 s, $t_{sf}$ = 15 s, and $t_b$ = 2 s, with $P_f$ = $P_b$ = 7.5 psi, and the yeast concentration in the secondary feed was 1.34 g/L. The error bars represents ± SD for two to three repeats. LMH = L(m²·h).

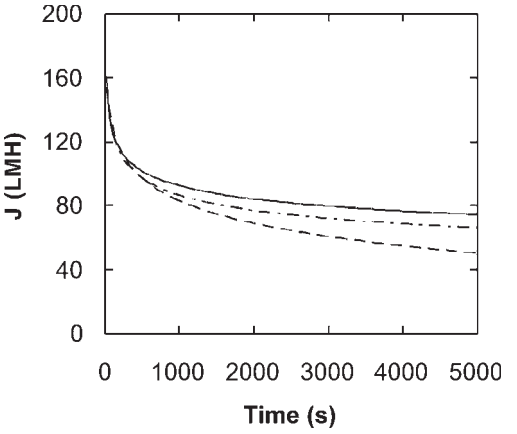


Fig. 9. Permeate flux vs time during ultrafiltration of 5.0 g/L of cellulase. The solid line represents SMY and backflushing with $P_f$ = 30 psi, $P_b$ = 15 psi, $t_f$ = 300 s, $t_{sf}$ = 5 s, and $t_b$ = 2 s; the line with short and long dashes represents SMY and backflushing under the same conditions but with $t_{sf}$ = 10 s. The yeast concentration in the secondary feed was 4.0 g/L. The dashed line is the permeate flux obtained without deposition of a secondary membrane or backflushing. A wall shear rate of 1300 $s^{-1}$ was used. LMH = L(m²·h).

For the conditions in Fig. 9, improvements of 35–50% in the permeate flux were observed when a secondary membrane was used, owing to a reduction in the protein fouling of the primary membrane. Little or no flux recovery was observed with each backpulse, as might be expected from the relatively low resistance of the yeast layer and the irreversible nature of the protein fouling. The flux continuously declined with time owing to irreversible fouling, though the rate of decline was reduced by the SMY.

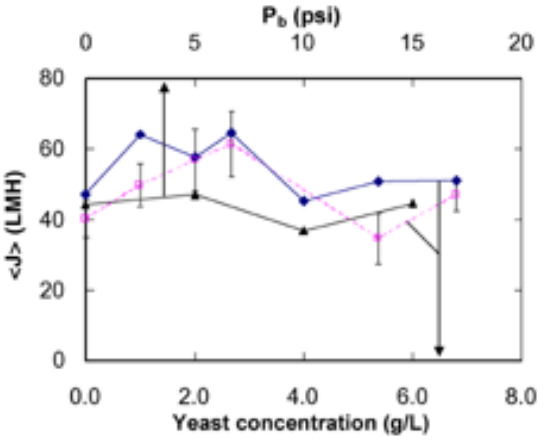


Fig. 10. Average permeate flux after 6000 s of ultrafiltration of 5.0 g/L of cellulase for different concentrations of yeast in secondary feed for wall shear rates of 400 $s^{-1}$ (—◆—) and 100 $s^{-1}$ (– –□– –), with $P_f = P_b = 7.5$ psi, $t_f = 300$ s, $t_b = 3$ s, and $t_{sf} = 30$ s. Also shown is the average permeate flux after 6000 s of ultrafiltration of 5.0 g/L of cellulase for varying reverse TMP (—▲—), with $P_f = 30$ psi, $t_f = 300$ s, $t_b = 2$ s, $t_{sf} = 5$ s, a wall shear rate of 1300 $s^{-1}$, and a concentration of yeast in the secondary feed of 4.0 g/L. The error bars represent ± SD for two to three repeats. LMH L($m^2$·h).

In Fig. 10, the average permeate flux after 6000 s of ultrafiltration is plotted vs the concentration of yeast in the secondary feed, for two shear rates of 100 and 400 $s^{-1}$. Without any secondary membrane or backflushing, the average permeate fluxes are 48 ± 3 and 40 ± 6 L/($m^2$·h) at the high and low shear rates, respectively. When an SMY was employed, the average permeate flux increased by about 20%, to maxima of 65 and 61 L/($m^2$·h) for high and low shear rates, respectively. These maxima occur at an intermediate yeast concentration in the secondary feed of about 2.7 g/L. At even higher yeast concentrations, there was no longer an improvement in the average flux. Apparently, the increased resistance of the thicker SMY at higher yeast concentrations offset the reduced protein deposition on the primary membrane.

Also depicted in Fig. 10 is the variation in the average permeate flux after 6000 s of ultrafiltration vs the reverse TMP. The average permeate flux do not appear to vary significantly with the reverse TMP in the range investigated (0–15 psi). This finding may reflect the trade-off between better removal of the yeast layer and protein foulants at higher reverse TMP and less loss of the permeate in the reverse direction across the membrane at lower reverse TMP. The lower reverse TMP is therefore recommended, because it is less likely to cause membrane damage. A comparison of the average permeate flux obtained with SMY and backflushing and that obtained without SMY or backflushing (Fig. 9) indicates that the improvement flux with SMY for ultrafiltration is small, if any. Previously, Knutsen and Davis *(21)* demonstrated an improve-

ment in performance of cellulase ultrafiltration by the addition of lignocellulosic solids, but this was attributed to cellulase binding to the solids (which were removed by sedimentation prior to ultrafiltration) rather than the secondary membrane effect. They also demonstrated that there is essentially no transmission of cellulase enzyme through the 30,000-Dalton ultrafiltration membrane, as desired.

## Conclusion

An increase in overall permeate flux was observed when microfiltration of protein solutions was carried out in the presence of secondary membranes. This increase was observed in microfiltration of both protein-only and yeast-protein mixtures. The backflushing time and the concentration of yeast used during deposition of the secondary membrane are the principal parameters governing the permeate flux for microfiltration. A higher yeast concentration led to a lower flux at shorter times, owing to increased resistance, but higher flux at long times, owing to increased protection of the primary membrane. There is an optimum backflushing duration for the removal of the secondary membrane, owing to a trade-off between cleaning the membrane and losing permeate during reverse filtration. The protein transmission during filtration of protein solutions with secondary membranes is higher than that obtained during filtration of protein solutions alone, leading to improved protein recovery. The use of secondary membranes may also increase the permeate flux in ultrafiltration, but the improvement is much smaller than in microfiltration. In microfiltration, protein aggregates were the primary cause of the fouling of the primary membrane, and these aggregates are effectively removed by the secondary membrane. In ultrafiltration, individual protein molecules were the primary cause of fouling of the primary membrane, and these individual protein molecules were less effectively removed by the secondary membrane. Thus, the primary application of the current work is microfiltration of biologic suspensions, in which the combination of increased permeate flux and increased protein transmission with the use of secondary membranes leads to high protein recovery.

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