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Cellulase Recovery via Membrane Filtration

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Abstract

A combined sedimentation and membrane filtration process was investigated for recycling cellulase enzymes in the biomass-to-ethanol process. In the first stage, lignocellulose particles longer than approx 50 μm were removed by means of sedimentation in an inclined settler. Microfiltration was then utilized to remove the remaining suspended solids. Finally, the soluble cellulase enzymes were recovered by ultrafiltration. The permeate fluxes obtained in microfiltration and ultrafiltration were approx 400 and 80 L/(m^2·h), respectively. A preliminary economic analysis shows that the cost benefit of enzyme recycling may be as much as 18 cents/gal of ethanol produced, provided that 75% of the enzyme is recycled in active form.

Index Entries: Sedimentation; microfiltration; ultrafiltration; cellulase enzyme.

Introduction

As traditional energy resources become more depleted, the drive to develop new, nontraditional energy sources grows. One area that has been of interest for decades is the conversion of biomass to ethanol *(1–3)*. Although cellulosic biomass can be converted to ethanol in existing processes, this conversion is not currently employed on a large scale because of its expense. One way to reduce the process cost is to recycle the cellulase enzyme used to hydrolyze cellulose. The enzyme used in this process is extremely expensive, representing approx 20% of the total ethanol cost *(4)*. Because much of it remains active after hydrolysis, recycling this enzyme could considerably decrease operating costs.

One possible way to separate and recover cellulase enzyme is through the use of sedimentation followed by microfiltration or ultrafiltration. In the sedimentation step, the larger particles are removed so as not to block the tubing or membrane filter in the subsequent filtration step *(5)*.

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Particle removal may be accomplished efficiently using sedimentation vessels with inclined walls *(6–8)*. This simple and inexpensive technique involves pumping feed into an inclined vessel that has a large surface area but small spacing between the inclined surfaces. As the fluid moves up the channel, the particles settle owing to gravity onto the upward-facing surfaces and then slide down to the bottom of the settler, where they can be either removed separately or returned to the feed material. Inclined vessels provide the advantages of short settling distances and large surface areas for sedimentation *(9)*.

After sedimentation, the suspension may be further clarified using microfiltration. In this step, suspended solids >0.2 μm in diameter are removed, but the cellulase enzyme (mol wt = 60–90 kDa) passes through to the permeate side *(10)*. Finally, in the ultrafiltration step, these enzymes are retained by the membrane while the water, sugars, ethanol, and other small molecules pass through the membrane for further processing. The retained cellulase may then be reused for further hydrolysis. The proposed separation strategy may be used either after simultaneous saccharification and fermentation or between separate hydrolysis and fermentation steps. For the latter, lignocellulosic particles and cellulase enzyme would be retained in the hydrolysis reactor while inhibitory sugars pass through the membranes to the fermentation vessel.

In this article, we present the results of initial laboratory studies on sedimentation, microfiltration, and ultrafiltration experiments with lignocellulosic particles and cellulase enzyme. We also present a preliminary cost-benefit analysis.

Materials and Methods

Sedimentation

Feed was drawn through an inclined settler to remove the larger particles from the feed stream prior to microfiltration. A schematic of the inclined settler is shown in Fig. 1. A rectangular glass sedimentation channel of 30-cm length (L), 5-cm width (w), and 0.5-cm depth (b) was used as the settler. A peristaltic pump (Millipore) was used to draw fluid from the feed tank at a prescribed rate (Q_o) between 0.01 and 1.4 mL/s. Particles then settled onto the upward-facing wall of the settler and slid back into the feed tank, producing a clarified overflow. The angle of inclination from vertical, θ, was varied from 15 to 60°. Experiments were conducted using aqueous suspensions of lignocellulosic particles (ground yellow poplar) obtained from the National Renewable Energy Laboratory (NREL) after pretreatment in its pilot plant in Golden, CO. These particles contain approx 40% lignin and 60% cellulose, plus acetic acid and other residual components. The particles are oblong, with a typical length-to-diameter ratio of 2:1, and range in length from 1 to 1000 μm (with the nominal length about 100 μm). The pH of the lignocellulose feed was 3.5.

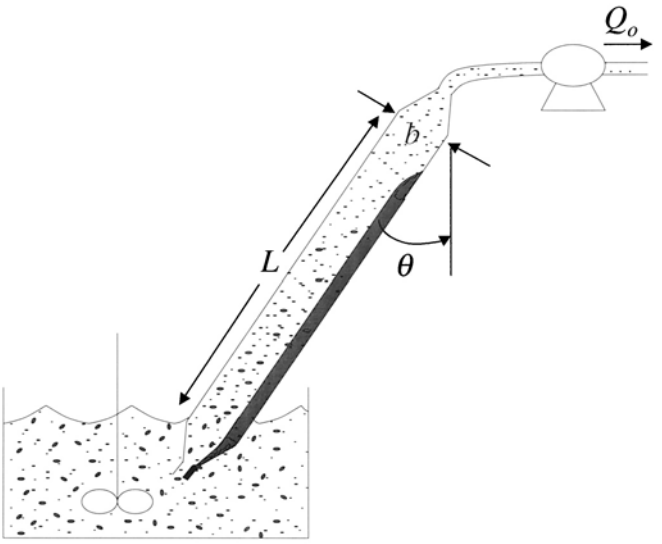

Fig. 1. Schematic of the inclined settler used to remove larger particles from the process stream prior to membrane filtration.

Microfiltration

Dead-End Filtration

Preliminary membrane testing was carried out in a Nuclepore dead-end filtration apparatus (Scientific Products, McGaw Park, IL). The unstirred cell holds up to 70 mL of feed and contains a membrane disk that is 4.7 cm in diameter. In each experiment, about 60 mL of solution was filtered in a single pass through the membrane. The cell is pressurized with nitrogen.

Microfiltration membranes were used to separate cellulase enzymes from lignocellulosic particles. All microfiltration membranes were purchased from Micron Separations (Westborough, MA) and had a nominal pore size of 0.22 µm, including membranes made of cellulose acetate (cat. no. A02SP04700), polysulfone (cat. no. S02SP04700), and nylon (cat. no. N02SP04700). The experiments were run at a lignocellulose concentration of 5% (w/v) in water on a dry weight basis. ABS cellulase (cat. no. C-8546), produced by *Trichoderma reesei*, was obtained from American Biosystems (Roanoke, VA). Its concentration in the permeate was determined using a BCA protein assay kit from Pierce, after calibration. The experiments were run at a cellulase concentration of 0.3% (w/v) in water. All microfiltration experiments were run at a transmembrane pressure of 10 psi and room temperature (22–24°C). The cellulose acetate membranes were used in these short-time experiments only for comparison purposes, because they are degraded by cellulase in long-time experiments.

Backwashing was performed on the microfiltration membranes to determine the effectiveness of cleaning. Backwashing entails reversing the

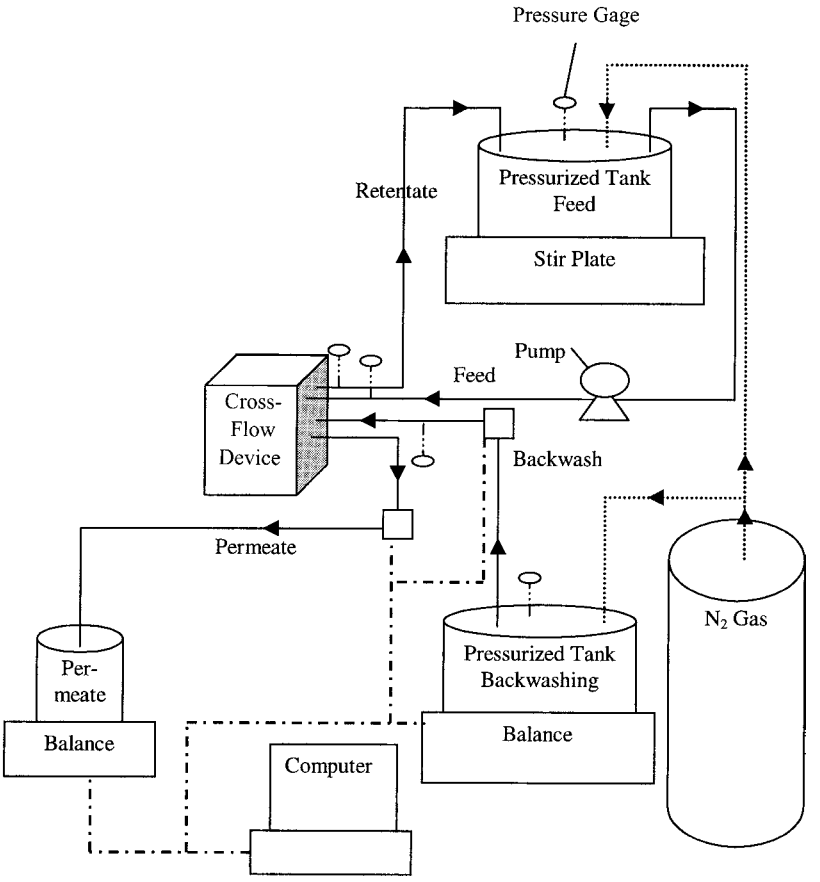

Fig. 2. Schematic of the crossflow membrane filtration apparatus. Solid lines are liquid streams, dotted lines are gas streams, and dashed-dotted lines are electrical connections.

transmembrane pressure to remove the foulant cake from the membrane surface. Membranes were backwashed for 5 min at a reverse transmembrane pressure of 10 psi. Afterward, clean water was filtered through the membrane to determine the flux recovery.

Crossflow Filtration

Lignocellulosic particles (ground yellow poplar, pretreated) were filtered with a Minitan (Millipore) flat-sheet device. This device has three parallel channels, each 7 mm wide by 5 cm long by 3 mm high. A polysulfone membrane with a 0.22-µm nominal pore size (cat. no. S02SP00010) from Micron Separations was employed. A schematic of the crossflow filtration device is shown in Fig. 2. Nitrogen gas is used to provide 10 psi of transmembrane pressure. A peristaltic pump (Millipore) is used to feed the suspension into the filter. The permeate mass is measured by an electronic microbalance (Mettler PG5002) interfaced with a computer. Microfiltration

membranes were cleaned by means of backwashing for 5 min at a reverse transmembrane pressure of 10 psi. As in dead-end filtration, clean water was subsequently filtered through the membrane to determine the flux recovery.

Ultrafiltration

Dead-End Filtration

Ultrafiltration membranes were used to separate glucose sugar from the cellulase enzymes. Experiments were conducted using the same apparatus and procedure as used for microfiltration dead-end filtration. Molecular weight cutoffs of 10,000, 30,000, 50,000, and 100,000 Daltons were chosen for the ultrafiltration membranes to test their ability to retain the cellulase (60,000–90,000 Daltons) while passing the glucose (181 Daltons). The polymeric membranes were made of polysulfone or polyethersulfone. Polysulfone membranes were purchased from Sartorius, and the polyethersulfone membranes were purchased from Millipore. The glucose was purchased as D-(+)-glucose anhydrous from Sigma-Aldrich and used at a concentration of 8% (w/v) in water. The glucose concentration in the permeate was determined by refractive index measurements. The cellulase was the same as that used in the microfiltration experiments, and again, had a concentration of 0.3% (w/v). Cellulase concentrations were measured by absorption at 290 to 296-nm wavelength in a Hewlett Packard 8452A Diode Array Spectrophotometer, after calibration. All ultrafiltration stir-cell experiments were performed at a transmembrane pressure of 46 psi and room temperature (22–24°C).

Crossflow Filtration

Crossflow ultrafiltration experiments were done using a setup similar to that used for crossflow microfiltration, as shown in Fig. 2. Experiments were performed with the Mid-Gee and Xampler hollow-fiber cartridges manufactured by A/G Technology. The hollow fibers have inside diameters of 1 mm and lengths of 27 cm. Feed is passed through the fiber lumens, and permeate is collected on the shell side. The Mid-Gee contains 2 fibers (16-cm^2 total filtration area), and the Xampler contains 13 fibers (110-cm^2 total filtration area). Polysulfone hollow-fiber membranes (30-kDa mol wt cutoff) were used in all crossflow ultrafiltration experiments.

Results and Discussion

Sedimentation

Guided by the theory of Davis et al. *(11)*, the flow rate through the settler and the angle of inclination were varied to give a desired concentration and particle size in the clarified overflow stream. As shown in Fig. 3, particle concentration in the overflow (θ_o/θ_f) increased with increasing overflow rate, Q_o, and decreasing angle of inclination, θ. As Q_o increased,

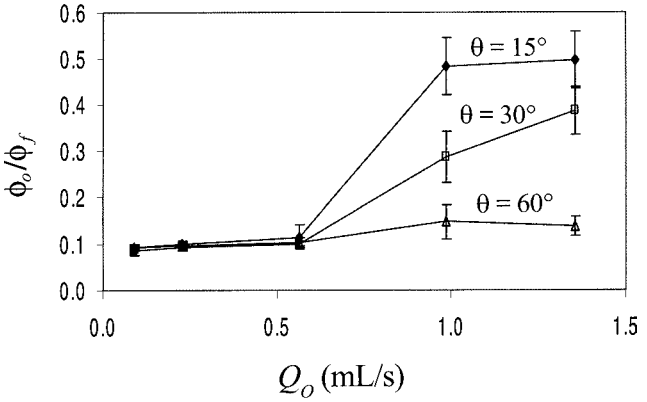

Fig. 3. Overflow particle concentration (θ_o) divided by feed particle concentration (θ_f) vs overflow rate, Q_o, for inclined settling. Values are given for angles of inclination of 15°, 30°, and 60° from vertical. Lignocellulose particles were used at room temperature (22–24°C) at a concentration of 1.25% (w/v) dry weight.

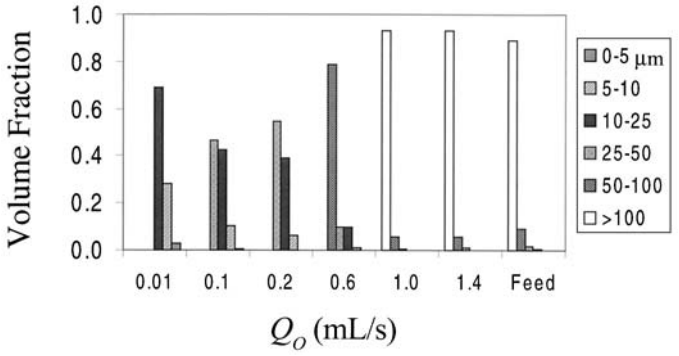

Fig. 4. Overflow particle size distributions by volume for various overflow rates, Q_o, for inclined settling. Particles are grouped according to their length. Lignocellulose particles were used at room temperature (22–24°C) at a concentration of 1.3% (w/v) dry weight. LMH, L/(m^2·h).

the particles had less time to settle down the column. As θ decreased, the horizontal projected area for settling decreased, and greater concentrations of particles were drawn into the overflow.

The particle distribution in the sedimentation overflow was also determined as a function of the overflow rate. Figure 4 depicts the volume fraction of particles in different ranges of length (0–5, 5–10 µm, and so on) at various overflow rates. The volume fraction of particles was determined assuming cylindrical particles with a length-to-diameter ratio of 2:1. Particle lengths were measured visually using a Nikon microscope. For overflow rates of 1.0 mL/s and higher, the overflow particle distribution was virtually identical to that of the feed, indicating that no particles settled out during the short holdup time at high flow rates. As the overflow rate

Table 1
Final Flux (L/[m²·h]) During Dead-End Microfiltration of 60 mL of Solution at 10-psi Transmembrane Pressure Through a Membrane Disk with 4.7 cm Diameter and 0.2-µm Nominal Pore Size[a]

Membrane	Water	Lignocellulose	Cellulase	Mixture
Cellulose acetate	7300 ± 900	2000 ± 1000	1800 ± 600	400 ± 300
Polysulfone	13,300 ± 600	1500 ± 100	300 ± 200	100
Nylon	2900	800	NA	NA

[a]With ±1 SD provided for repeated experiments with different membranes. NA, not available.

decreased, so did the average particle length in the overflow, owing to more particles settling during the longer holdup times. For an overflow rate of 0.01 mL/s, there were virtually no particles in the overflow >25 µm long. An angle of inclination of 30° from the vertical and a flow rate through the settler of 0.2 mL/s were selected as providing the desired performance; under these conditions, the clarified overflow had a particle concentration of <10% of that in the feed, and nearly all the particles reaching the overflow were <50 µm long.

Microfiltration

Dead-End Filtration

The results of the microfiltration dead-end tests are summarized in Table 1. The clean water flux (defined as the volume of permeate collected per time per surface area of the membrane) was highest for the polysulfone membrane, but the flux in the presence of foulants (lignocellulose and cellulase) was higher for the cellulose acetate membrane. Unfortunately, cellulase gradually degrades cellulosic membranes, and thus cellulose acetate is not an acceptable material for the present application. The nylon membrane gave the lowest flux, so tests with it were discontinued. The flux was significantly lower when lignocellulose and cellulase were mixed together than when filtered separately, possibly owing to the lignocellulose particles becoming sticky when enzyme bound to them. However, the flux improved dramatically by backwashing, increasing from the fouled flux of 100 L/(m²·h) to a cleaned flux of 11,200 L/(m²·h). The flux recovery by backwashing indicates that periodic cleaning by reverse filtration may be effective in maintaining high average fluxes. During forward filtration, the enzyme recovery in the permeate was high (>80% transmission), as desired. Considerable variation between different membranes from the same lot was observed.

Crossflow Filtration

Figure 5 shows the permeate flux vs time for typical crossflow microfiltration experiments with feeds of lignocellulosic particles, cellulase enzyme, and a mixture of particles and enzyme. The lignocellulosic

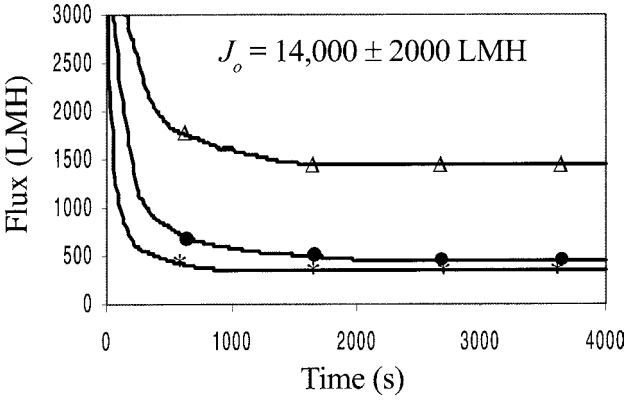

Fig. 5. Flux vs time for crossflow microfiltration with 0.2-µm polysulfone membranes for a feed with 0.2% (w/v) lignocellulose (△), 0.3% (w/v) cellulase (●), and a mixture of both (*). Experiments were conducted at room temperature. The clean water flux, J_o, was 14,000 ± 2000 L/(m²·h) (LMH).

Table 2
Preliminary Crossflow Microfiltration Fluxes[a]

	J_o (L/[m²·h])	J_s (L/[m²·h])	J_r (L/[m²·h])
Lignocellulose	14,000 ± 2000	1300 ± 200	4000 ± 500
Cellulase	15,000	400	2300
Mixture	16,000	360	11,300

[a]J_o is the initial water flux, J_s is the long-term flux after fouling, and J_r is the recovered water flux after a 5-min backwash. For lignocellulose, the results are shown as the average ±1 SD for three experiments.

particle concentration in the feed to the settler was 2.5% (w/v), which resulted in a settler overflow particle concentration of 0.2% (w/v) at the overflow rate of 0.2 mL/s. The cellulase enzyme concentration of 0.3% (w/v) was the same in both the feed to and the overflow from the settler. This settler overflow was used directly as the microfiltration feed. The recirculation rate of feed to the filter was 6 mL/s, which corresponds to a wall shear rate of 190 s^{-1}. In all three cases, the permeate fluxes started at the initial flux of J_o = 14,000 ± 2000 L/(m²·h) and then declined rapidly owing to particle deposition on the membrane surface, before reaching nearly steady values after approx 1000 s. As in the dead-end filtration experiments, the most dramatic fouling was found in the mixed system, in which the flux declined to a value of J_s = 360 L/(m²·h).

In all cases, membranes were backwashed with water for 5 min at 10 psi of reverse transmembrane pressure after 4000 s of filtration, to determine cleaning effectiveness. The results are shown in Table 2. The cleaning resulted in a flux recovery in the lignocellulose system from J_s = 1300 ± 200 L/(m²·h) to J_r = 4000 ± 500 L/(m²·h), reported as the mean ± 1 SD for three repeats. The cellulase system showed less improvement, with a

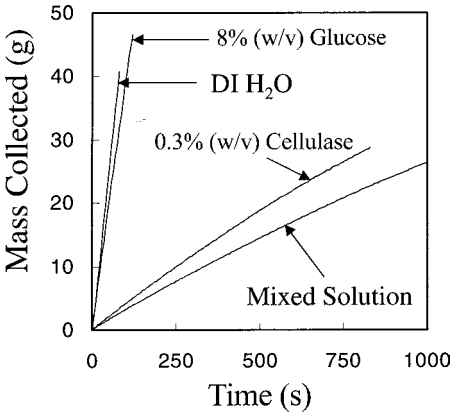

Fig. 6. Permeate mass vs time for dead-end ultrafiltration experiments using a 30-kDa polysulfone membrane and 46-psi transmembrane pressure. DI, deionized.

recovered membrane flux of J_r = 2300 L/(m²·h). However, the mixture of cellulase and lignocellulosic particles showed a very high flux recovery with backwashing, possibly because the cake layer of lignocellulosic particles effectively serves as a secondary membrane that prevents cellulase aggregates and very small particles from fouling the primary membrane *(12)*. These potential foulants are removed with the lignocellulose during backwashing, increasing the effectiveness of cleaning.

Ultrafiltration

Dead-End Filtration

Figure 6 shows the mass of permeate collected vs time for dead-end filtration using a 30-kDa polysulfone membrane. A high rate of water collection was observed, which was reduced by about 30% when glucose was added (owing to the higher viscosity of the glucose solution). When cellulase was added, a much lower collection rate resulted (owing to membrane fouling by the rejected cellulase).

The cellulase concentration in the permeate for the different polysulfone and polyethersulfone membranes was monitored using a feed containing 0.3% (w/v) cellulase and 8% (w/v) glucose. As expected, the cellulase transmission (defined as concentration in the permeate divided by that in the feed) increased with increasing molecular weight cutoff, from 2 to 5 to 40% for polysulfone membranes with 10-, 30-, and 100-kDa molecular weight cutoffs, respectively, and from 2 to 6% for polyethersulfone membranes with 30- and 50-kDa molecular weight cutoffs, respectively. Fortunately, the cellulase transmission was very low (<10%) for all but the 100-kDa polysulfone membrane. Moreover, the glucose showed essentially 100% transmission for all membranes, as desired. Thirty-kilodalton polysulfone, 30-kDa polyethersulfone, and 50-kDa polyethersulfone membranes proved to be the best candidates for crossflow testing, with average

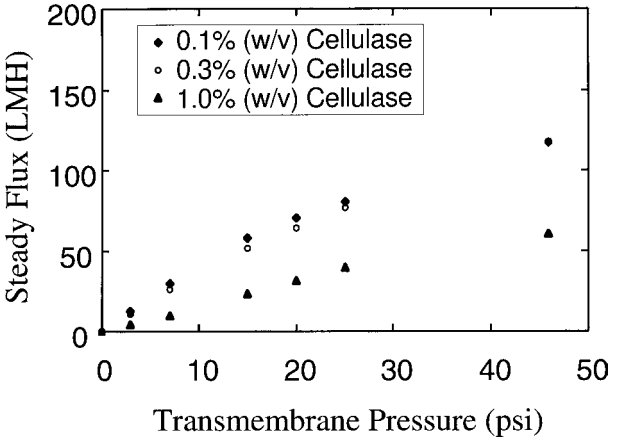

Fig. 7. Long-term flux vs transmembrane pressure for crossflow ultrafiltration of cellulase solutions using hollow-fiber polysulfone membranes with a 30-kDa molecular weight cutoff. LMH, L/(m^2·h).

fluxes of 53 ± 6, 49 ± 1, and 64 ± 0 L/(m^2·h), respectively, shown with ±1 SD for two experiments. These membranes are stable, give relatively high flux, and reject cellulase while passing sugar and other small molecules.

Crossflow Filtration

Figure 7 shows preliminary results with the Mid-Gee hollow-fiber cartridge containing 30-kDa polysulfone membranes. For these experiments, the wall shear rate was 1500 s^{-1}. Cellulase concentrations of 0.1, 0.3, and 1.0% (w/v) were used, and the transmembrane pressure varied from 0 to 46 psi. The long-term flux increased with increasing transmembrane pressure, indicating that the limiting flux was not reached over the ranges of concentration and pressure studied. At low cellulase concentrations, a steady flux of about 115 L/(m^2·h) was obtained at the highest transmembrane pressure (46 psi) investigated. Essentially 100% enzyme retention was achieved with the 30 kDa polysulfone membranes, as desired.

Economic Analysis

Micro- and ultrafiltration fluxes have been used in a preliminary economic analysis of the cost of implementing a membrane-based enzyme recycling system. The total annualized cost of each membrane separation step will depend on the membrane area required and, hence, on the permeate flux achieved. By using an economic model for membrane filtration of fermentation broth described by Kuberkar et al. *(13)*, researchers have made preliminary estimates that indicate that the reduction in enzyme costs when enzyme recovery and recycle are used will significantly outweigh the added cost of employing membrane separation with values of the permeate flux comparable with those obtained in our preliminary tests.

Table 3 shows the cost basis employed and the calculated costs of one membrane separation (micro- or ultrafiltration) for two different permeate

Table 3
Economic Analysis for Enzyme Recovery by Membrane Separation[a]

Category	Cost basis	Cost (cents/gal EtOH) (flux = 10 L/[m²·h])	Cost (cents/gal EtOH) (flux = 50 L/[m²·h])
Capital	\$200/(m²·yr)	14.6	2.9
Membranes	\$175/(m²·yr)	12.7	2.5
Power	\$28/(m²·yr)	2.0	0.4
Cleaning	\$25/(m²·yr)	1.8	0.4
Maintenance	\$20/(m²·yr)	1.5	0.3
Labor	0.2 person/d	0.01	0.01
Total cost	\$450/(m²·yr)	32.6	6.5
Savings with 75% enzyme recycled	—	24.0	24.0

[a]The cost basis is taken from Kuberkar et al. *(13)* and is based on a biotechnology plant capacity of 40,000 L/d. A linear scale-up with membrane area is assumed.

flux values. In both cases, the largest costs are for capital equipment and membranes, with power, labor, maintenance, and cleaning representing only a small fraction of the total. The cost of membrane separation (per gallon of ethanol produced) decreases with increasing flux, owing to the reduced membrane area and equipment size required. Also shown is the cost savings that would occur by recovering and reusing 75% of the cellulase enzyme. The amount of cellulase that can be recycled will depend strongly on its adhesion to solid particles and its deactivation. Both of these factors must be investigated in future work. Fortunately, previous work by Roseiro et al. *(14)* showed no deactivation of cellulase during batch concentration by ultrafiltration for 8 h. The data on the enzyme cost (\$0.32/gal of EtOH) and the volume ethanol produced per total volume processed (0.05 gal of EtOH/gal total) are taken from Wooley et al. *(4)*. These data are for a simultaneous saccharification and cofermentation (SSCF) process. Although the proposed membrane system is more directly applied to a process with sequential steps, it may also be adapted to an SSCF process.

When using a cost/benefit analysis, a critical flux must be exceeded so that the cost required for adding membrane separation is more than offset by the cost savings of reducing the total enzyme requirements by recovery and recycle. The tradeoff is illustrated in Fig. 8, where the separation cost for one membrane step is plotted vs the average permeate flux. The total cost of implementing a membrane system is the sum of the individual micro- and ultrafiltration costs, each of which is shown in Fig. 8. Assuming that 75% of the cellulase enzyme is recovered in active form by membrane separation, an average flux of 14 L/(m²·h) must be achieved for a single membrane separation step, and 30 L/(m²·h) must be achieved when two membrane separation steps with equal flux are required. Fortunately, the

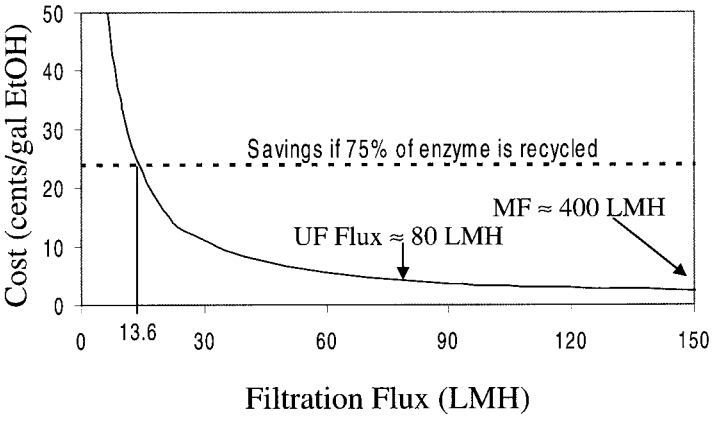

Fig. 8. Cost of one membrane separation step vs average flux during membrane filtration. MF, microfiltration; UF, ultrafiltration; LMH, L/(m²·h).

preliminary fluxes presented herein significantly exceed the critical flux values. Indeed, using typical values of 80 L/(m²·h) for ultrafiltration and 400 L/(m²·h) for microfiltration, the combined cost of the two membrane separation steps is only $0.06/gal of EtOH, whereas $0.24/gal of EtOH would be saved if 75% of the enzyme were reused. The added cost of inclined settling is expected to be small compared with the filtration cost. Although this cost estimate is preliminary, it does encourage further investigation into implementing a membrane system for cellulase recovery.

Acknowledgments

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