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Microfiltration of *Streptomyces rimosus*: Cell Harvesting Process Studies

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ABSTRACT

Process studies were carried out to investigate the feasibility of using tangential crossflow microfiltration to harvest antibiotic-producing cells such as *Streptomyces rimosus* from a whole fermentation broth. The microorganism was grown in a repeated batch fermentation up to the point of secondary mycelium formation in order to simulate broth age and mycelial morphology present in the terramycin production process. Cell separation was carried out in a Millipore Pellicon system equipped with a Durapore 0.45 μm membrane cassette and operated in a batch concentration mode. Permeate fluxes for untreated fermentation broth were measured and compared with those for broth that was pretreated by acidification—a typical process step used to improve antibiotic recovery. Acidification from pH 7 to 4 prior to filtration changed the cell morphology and significantly reduced the inlet feed pressure required to pump the broth. In addition, pretreatment enabled higher crossflow rates and higher steady-state fluxes to be obtained at lower transmembrane pressures. Permeate fluxes for the pH-treated fermentation broth increased with operating temperature but were independent of further changes in pH.

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INTRODUCTION

This paper examines the feasibility of using tangential crossflow microfiltration (MF) as a separation process for harvesting *Streptomyces rimosus* from whole fermentation broth. This multicellular, filamentous organism is one of the industrially important actinomycetes family that is used to produce antibiotics (1). Effects of process parameters, specifically pretreatment by acidification and temperature variation, on flux and concentration are analyzed.

Crossflow MF is utilized as a solids separation step in various biotechnological processes, either to concentrate whole cells for further processing or to separate products from cells and cell lysates. A number of crossflow MF studies for harvesting unicellular organisms such as yeast and bacteria have been published, but very few studies on multicellular organisms are available (2–10). Broth solids from antibiotic fermentations form a slimy, poorly draining, easily compressed cake that tends to limit filtration rate or flux in either conventional or membrane filtration processes (7). MF experiments have been carried out using both rotary and tangential crossflow systems, with higher fluxes reported in the rotary systems (11–14).

Antibiotics are produced as secondary metabolites using filamentous organisms with long, highly branched structures. Commercially useful organisms include *Penicillium chrysogenum* (penicillin G or V), *Cephalosporium acremonium* (cephalosporin C), and various streptomyces species. Historically, these microorganisms have been separated from fermentation broths containing antibiotics using rotary precoat vacuum filtration or centrifugation. Product purification is then obtained via adsorption, extraction, and other downstream separation processes. Studies indicate that membrane filtration is more economical than centrifugation or vacuum filtration for clarification of antibiotic fermentation broths (2, 6, 7).

Several studies have reported on the use of membrane processes to harvest filamentous microorganisms. Merck and Co. investigated crossflow membrane filtration and fluidized bed adsorption as alternatives for whole broth processing in the production of fermentation products such as immunomycin from *Streptomyces hygroscopicus* (8). Merck previously installed a tangential crossflow ultrafiltration (UF) system to clarify *Nocardia lactamdurans* fermentation broth during the production of cephamycin C (7). In the cephamycin process the cell slurry was acidified to stabilize viscosity by lysing cells, ultrafiltered at 20–30°C in a batch recycle mode to approximately one-third of the original volume, then diafiltered at constant volume to increase antibiotic recovery. Flux and concentration data were not reported in either of these papers. Other crossflow MF studies on multicellular organisms include harvesting of *Aspergillus niger* from fermentation broth (9) and recovery of

streptokinase from *Streptococcus equisimilis* fermentation broth (10). The last two papers present flux data for whole fermentation broth but do not consider effects of pH or temperature pretreatment on microfiltration.

In the current investigation, *S. rimosus* was selected as a model system to study separation of antibiotic-producing microorganisms from whole fermentation broth. Typical process conditions for fermentation and cell separation are based on a patent for production of terramycin assigned to Pfizer and Co. (15). Fermentation broths for mycelial organisms form non-Newtonian suspensions with high apparent viscosities (1, 2), where the rheology of the fermentation broth is a strong function of cell morphology and concentration. When grown in submerged cultures, streptomyces species form mycelial pellets with branched hyphae emanating from a growth center; the size of the pellet is shear sensitive and media dependent (1). Other authors state that streptomyces species actually form clumps or cell aggregates instead of pellets, with the clump size dependent on the fermentor geometry and impeller rotation speed (16). In order to release antibiotic from the pellet or cell aggregate, the fermentation broth is generally acidified and heated prior to filtration—steps that significantly improve filtration characteristics by changing morphology and/or decreasing broth viscosity (7, 15).

In conventional filtration of streptomycin fermentation broth, pretreatment by acidification is reported to improve filtration rate (17). As previously mentioned, this observation is explained in the cephamycin process in that acidification stabilizes broth viscosity by lysing cells (7). Since antibiotics are extracellular products of multicellular organisms, "lysing cells" in this case probably means breaking apart microbial aggregates as well as cleaving filamentous structures of microorganisms. Streptomycin broth is also pretreated by heating to 80°C to improve antibiotic recovery (17). Increased fouling and reduced fluxes during microfiltration of heated, lysed, and aged *Candida utilis* fermentation broths were attributed to the presence of cell fragments and released cell contents (18). On the other hand, breaking down *A. niger* pellets into smaller-sized particles had a beneficial effect on flux (9). From these apparently conflicting studies it may be concluded that fluxes are improved by breaking up the cell aggregates and/or filamentous structures in highly viscous mycelial broths, while the opposite is true for unicellular organisms.

Cells harvested from antibiotic processes, whether by conventional filtration or crossflow membrane filtration, tend to form a compressible cake that quickly blinds the filter or membrane. Pretreatment prior to conventional filtration, e.g., heating and coagulation, flocculation of cells, and adsorption on filter aids, is a common process step in the recovery of products from mycelial fermentation broths. Filter aid is usually added to improve filtration rates of fermentation broths in rotary vacuum filtration systems, but filter aid increases process costs and solid waste tonnage while eliminating some

potential methods of solid waste disposal such as biological treatment (6, 7). In membrane filtration processes, flux rates of fermentation broths can be improved (without addition of filter aid) by increasing tangential crossflow velocity and/or process temperature.

THEORY

During cell harvesting there is a rapid initial drop in the permeate flux, J, as cells collect and build up a thin cake on the surface of the membrane. The permeate flux at time t may be described by the following equation (19):

$$J(t) = \frac{\Delta V}{A_m \Delta t} = \frac{\Delta P}{\mu_0(R_m + \hat{R}_c \delta_c(t))} \quad (1)$$

where ΔV is the volume passing through the membrane in time Δt, A_m is the membrane surface area, ΔP is the transmembrane pressure, μ_0 is the viscosity of the solution passing through the cake and membrane, R_m is the membrane resistance, $\hat{R}_c$ is the specific cake resistance, and δ_c is the cake thickness which is a function of time. In crossflow microfilters the fluid flowing across the membrane counteracts cake buildup by sweeping away particles from the cake surface. Thus, a cake of constant thickness should develop at a given crossflow velocity, accompanied by a steady-state or nearly constant flux.

For liquids passing through a membrane or through an incompressible cake, flux is expected to increase with pressure as indicated by Eq. (1). For biological cakes which tend to be compressible, however, a number of studies have shown flux to be nearly independent of pressure (9, 19, 20). In prior studies with *Saccharomyces cerevisiae*, the authors found that increased pressure led to a higher initial flux, followed by a more rapid decline in flux during the transient period, resulting in a steady-state value that was comparable to that observed in low-pressure experiments (21).

For whole fermentation broths the flux relationship is generally more complex than indicated by Eq. (1) (22, 23). Between the initial period of rapid flux decline and the final steady-state period, a period of gradual flux decline associated with fouling may occur. Fouling is caused by a number of factors that increase the resistances R_m and $\hat{R}_c$ (Eq. 1), including adsorption of proteins on the membrane surface, internal pore blockage with protein aggregates, blockage of the filter cake with smaller particles, and compression of the filter cake.

All of the experiments in this paper were run is a batch concentration mode, i.e., permeate was removed from the system and retentate was recycled to the feed tank. Therefore, the concentration factor, $\psi(t)$, as defined by the following equation, increases with time.

$$\psi(t) = \frac{C(t)}{C_0} = \frac{\Gamma(t)}{\Gamma_0} \tag{2}$$

In Eq. (2), C_0 is the initial feed concentration, $C(t)$ is the concentration in the feed/retentate tank at time t, Γ_0 is the equivalent turbidity of the initial feed solution, and $\Gamma(t)$ is the equivalent turbidity of the slurry in the feed/retentate tank at time t. Note that samples must be diluted to low concentrations in order to obtain a linear relationship between turbidity and cell concentration. Thus, equivalent turbidity is defined as the value obtained by multiplying the turbidity reading of a diluted sample by the ratio of the diluted sample volume over the initial (undiluted) sample volume.

Two different sets of dependent variables, permeate volume and equivalent turbidity, were measured as functions of time in the experiments. Utilizing a simple mass balance ($C*V = C_0*V_0$) and the concentration factor $\psi(t)$ as defined in Eq. (2), the following equation results:

$$\psi(t) = \frac{V_0}{V(t)} = \frac{V_0}{V_0 - J_{ss}A_m t} \tag{3}$$

where V_0 is the initial feed volume (20 L) and $V(t)$ is the volume at time t. If the transient period is very short so that a steady-state flux, J_{ss}, is immediately reached, then $V(t)$ can be expressed in terms of feed volume, V_0, membrane area, A_m, steady-state flux, and time (Eq. 3).

Setting Eqs. (2) and (3) equal to each other and rearranging, a linear relationship results:

$$\left(1 - \frac{\Gamma_0}{\Gamma(t)}\right) = \frac{J_{ss}A_m}{V_0} t \tag{4}$$

Thus, steady-state fluxes can be determined from the volume/time data (Eq. 1) or regressed from the turbidity/time data (Eq. 4).

In a nonfouling system the steady-state flux, J_{ss}, of a liquid through a membrane and/or a noncompressible cake should increase with temperature. The increased flux results from decreased permeate viscosity at increased temperature. An Arrhenius relationship may be used to relate flux to temperature, as follows (2):

$$J_{ss} = J_0 e^{(-E_a/RT)} \tag{5}$$

In Eq. (5), E_a is the activation (separation) energy, R is the universal gas constant, T is the absolute temperature, and J_0 is a constant at infinite temperature.

EXPERIMENTAL MATERIALS AND METHODS

Culture Conditions

Streptomyces rimosus (ATCC 10,970) was selected as an example of an organism used in industry to produce an antibiotic (terramycin). The freeze-dried cells were reconstituted with ATCC medium 5 and transferred to agar slants. The composition of the sporulation agar was 15.0 g/L agar, 10.0 g/L glucose, 2.0 g/L typtose, 1.0 g/L yeast extract, 1.0 g/L beef extract, plus a trace of Fe_2SO_4, dissolved in distilled water, adjusted to pH 7.0, and sterilized. The composition of the growth medium was the same except that agar was omitted. The agar slants were incubated at 26°C for 3 days.

Incubated cells were transferred to two shaker flasks filled with 0.5 L each of sterile growth medium. Cell slurries were shaken in a water bath at 27°C for 2 days. Microscopic examination at the end of this period showed thick filaments which are characteristic of primary mycelium formation (24). Visually observed morphology showed thick threadlike strands that remained suspended in the medium for some time. This procedure provided the inoculum for the repeated batch fermentation.

Repeated Batch Fermentation

The next cell growth step was performed using a Magnaferm 14-L stirred tank bioreactor, manufactured by New Brunswick Scientific. The fermentor was cleaned and assembled; 1 L of water was added and the entire system was sterilized. After sterilization, 5 L of sterile growth medium and 1 L of inoculum were added to the fermentor, bringing the liquid volume to 7 L. Temperature was maintained at 27°C by the fermentor control unit, air was introduced at a rate of 13 L/min, impeller speed was set at 400 rpm, and pH was relatively stable in the 6 to 8 range (minimal titration with 1 N NaOH needed to maintain pH 7). The mechanical foam breaker was turned on periodically to control foaming, and silicone antifoam was added regularly (every 12 hours and during media additions).

The fermentation process was run in a semibatch mode until approximately 20 L of cell slurry was collected, with the bioreactor aerated and agitated continuously. After each 2-day period, which is a sufficient time to produce terramycin (15), a 5–6 L batch of *S. rimosus* cell slurry was removed from the fermentor and refrigerated. The volume removed was replaced with fresh ATCC growth medium and another batch growth period commenced. Visually observed morphology showed irregularly shaped cell masses that appeared lightweight and fluffy early in the fermentation cycle but became denser (more prone to settling) and darker as time progressed, i.e., growth of secondary mycelium occurred and large aggregates formed.

Microfiltration Equipment

A tangential crossflow filtration systems manufactured by Millipore Corporation, the Pellicon cassette system, were selected for this study. The Pellicon system includes a membrane filtration unit, and supports microfiltration or ultrafiltration depending on the type of membrane selected. The system may be purchased with either a peristaltic pump or a rotary vane pump, but the peristaltic pump is not capable of circulating the highly viscous, untreated cell slurry. The Procon rotary vane pump was somewhat challenged for pumping the untreated cell slurry, but provided sufficient feed pressure to do so at lower flow rates. The Procon pump is rated at maxima of 227 L/h, 1750 rpm, and 690 kPa (100 psig), and is fitted with rigid-wall polyethylene tubing to withstand pressures up to 690 kPa. Operation of the Pellicon system is limited at high cell concentrations by the channel design, i.e., the cassette has screen spacers that tend to cause the unit to plug with solids.

For harvesting bacterial or yeast cells, Millipore recommends a Durapore 0.45 μm membrane. Durapore is the trademark for an anisotropic porous membrane, made from the polymer polyvinylidene difluoride (PVDF), that has low protein binding properties. These membranes are surface treated to be hydrophilic. A membrane cassette with an effective filter area of 0.465 m^2 was used for all runs. The cassette is a plate-and-frame type unit with a bonded package of 10 membrane packets (two membranes per packet) separated by coarse polypropylene retentate screens. The cassette is designed to provide parallel flow across the membrane packets. Maximum operating conditions for the membranes are 690 kPa, 50°C, and a pH range of 2–11. The cassette is installed horizontally in the center of the Pellicon unit between upper and lower manifold plates that contain the feed inlet, retentate outlet, and permeate outlet ports. These three items are sandwiched between solid baffle plates, and the entire unit is held together with rods and bolts. The bolts were torqued to 400 in.-lb for all the experiments.

Experimental Methods

A process flow diagram for operating a crossflow microfilter is shown in Fig. 1. The system includes pressure gauges, immediately adjacent to the unit, on the feed and retentate lines. Ball valves installed on the feed, retentate, and permeate lines remained fully open during all the experiments, so the permeate and retentate pressures were at atmospheric conditions. All experiments were run in a batch concentration mode, with retentate recycled to the feed tank and permeate collected in another container. Thus, the concentration in the feed/retentate tank increased throughout each run. Fluxes and pressures were measured at the beginning of each run and at 10-minute intervals thereafter. Prior to each run the membrane was preconditioned by recycling the

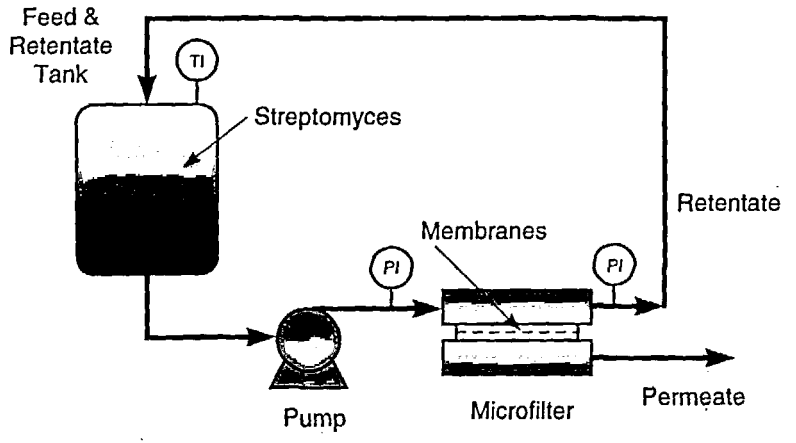

FIG. 1 Process flow diagram for batch concentration mode of operation. TI = temperature indicator, PI = pressure indicator.

fermentation medium (without cells), at the temperature and pH of the feed slurry, through the membrane unit for 10 minutes. After each run the membrane was thoroughly cleaned. A preliminary experiment was carried out with yeast grown in the fermentor in order to test all the equipment.

The experimental runs are summarized in Table 1. All five runs started with 20 L of fermentation broth and concentrated the slurry to approximately 1 L. As a base case, whole fermentation broth at pH 7.0 (without pretreatment by acidification) was concentrated in the Pellicon microfiltration system at room temperature (Run 1). The filtrate and retentate from Run 1 were recombined and treated with 1 N HCl to lower the pH to 4.0. The flow rate in Run 1 was limited to 61 L/h by the pumpability of the untreated broth (the

TABLE 1
Summary of Experimental Runs

Run	Condition	pH	T (°C)	C_0 (gdw/L)	ψ (Γ/Γ_0)	Pump rate (L/h)
1	Untreated	7.0	23	2.1	16	61
2	pH-treated	4.0	26	1.3	25	176
3	pH-treated	4.0	19	1.4	22	176
4	pH-treated	4.0	30	1.4	25	176
5	pH-treated	7.0	26	1.2	16	176

pump started to cavitate at higher flow rates), and approximately one-third of the cell mass was lost by plugging in the membrane cassette at the end of this run. In Runs 2–5 it was possible to use higher crossflow rates at much lower transmembrane pressures. In Runs 2–4, acidified slurry was microfiltered at several different temperatures. In Run 5 the acidified cell slurry was treated with 1 N NaOH to return the pH to 7.0. Visually observed morphology showed large irregularly shaped cell masses remaining at the end of Run 1 (although their size may have been reduced somewhat by pumping through the microfilter), as compared with small sandlike solids in the broth after pH treatment (Runs 2–5).

The concentration factors, ψ, in Table 1 and in the figures that follow were calculated from ratios of equivalent turbidity values (Eq. 2). Equivalent turbidity, Γ, is defined in this study as the number obtained when the turbidity reading of a diluted sample is multiplied by the volume of the diluted sample and divided by the initial (undiluted) sample volume. The turbidity of the slurry in the feed/retentate tank (MF experiments) was measured at the initial and final points, and at 20-minute intervals during each experiment with a DRT-100B turbidimeter (manufactured by HF Scientific Inc.). Turbidity readings become nonlinear, approaching a constant value at higher cell concentrations; therefore, each sample was diluted with media (1:2, 1:4, 1:8, etc.) until two successive dilutions resulted in the same equivalent turbidity value.

Two calibration curves were prepared, one for the whole cells (Run 1) and another for the pH-treated cells (Runs 2–5), by measuring turbidities of samples with various cell concentrations. The linear portions of the calibration curves were used to determine the feed concentrations, C_0 (Table 1). Linearity occurs when the cell concentration is low enough for absorbance to follow Beer's law (25). Turbidities were measured in NTU (normal turbidity units), and cell concentrations in grams dry weight (gdw) per liter. To prepare the calibrations, turbidities were recorded for all of the calibration samples (without dilution). Then the samples were centrifuged and rinsed several times to remove soluble solids, dried in aluminum pans in an oven at 95°C for 24 hours, and weighed to determine dry cell weights. A more direct method of determining C_0 would be to measure the dry weight of each feed sample following procedures given in the literature (9, 16, 18). Other authors generate linear calibrations with a spectrophotometer; specifically, absorbance at 632 nm versus hemacytometer cell count (25) or optical density at 660 nm versus dry cell weight (12).

A sodium hypochlorite solution was used for cleaning and sanitizing the membranes between runs. The solution was prepared by diluting fresh household bleach to 300 ppm, lowering the pH to the 6–8 range with 1 N HCl, then heating the solution to 40–50°C. The permeate valve was closed and

the membrane cassette was flushed with approximately 20 L of distilled water. Then, the membranes were cleaned with hypochlorite solution which was recycled through the system at maximum pump speed until the retentate was clear. Next, the permeate valve was opened and fresh hypochlorite solution was pumped through the system to finish cleaning the membrane. After the cleaning procedure was completed, the cassette was flushed thoroughly with large volumes of distilled water to remove the hypochlorite solution.

Clean water fluxes were measured for the new membrane and after each cleaning (between runs) using distilled water at room temperature and 69 kPa transmembrane pressure (TMP). The clean water flux of the new membrane was 344 L/m^2·h. After the preliminary yeast run the membrane was cleaned by exposure to hypochlorite solution for 50 minutes, and the clean water flux was restored to 343 L/m^2·h. After completion of Run 1 the membrane cassette was heavily fouled and extremely difficult to clean. It was cleaned five times, with exposure to the hypochlorite solution lasting 3–5 hours for each cleaning. In between cleanings the cassette was rinsed with distilled water and flipped over so that the hypochlorite solution was pumped through in the opposite direction during the next cleaning. The final clean water flux after Run 1 was 305 L/m^2·h. Since the remaining runs were stopped before plugging with solids occurred, water fluxes of 306 to 308 L/m^2·h were obtained after a single cleaning.

RESULTS AND DISCUSSION

Whole Fermentation Broth

The whole (untreated) fermentation broth contained large amounts of visible filamentous cell masses. The first run was relatively difficult because the broth was so viscous that it was hard to pump. The highest pump setting that could be used without inducing cavitation was 61 L/h. As shown in Fig. 2, the inlet feed pressure to the membrane started at 260 kPa gauge and rose to >690 kPa gauge (the system maximum). Thus, the transmembrane pressure (TMP) increased from 130 kPa to >345 kPa. At the last data point (Fig. 2), the pressure and flux both increased sharply. Retentate stopped flowing completely shortly after the last data point was recorded, indicating that the channels in the cassette were plugged with solids. To prevent heavy fouling in subsequent runs (Runs 2–5), experiments were stopped immediately if a sharp rise in permeate flux or operating pressure was observed near the end of a run (around the 1-L mark).

As shown in Fig. 2, the permeate flux in Run 1 started at 44 L/m^2·h, dropped sharply to 32 L/m^2·h, then declined gradually as fouling occurred to a steady-state value of 14 L/m^2·h. These fluxes are reasonable compared to values reported in the literature for tangential crossflow microfiltration of fermented broths of *S. equisimilis*, i.e., initial and final fluxes of 26 and 19 L/m^2·h, respectively (10). In Run 1, cell concentration increased exponentially to

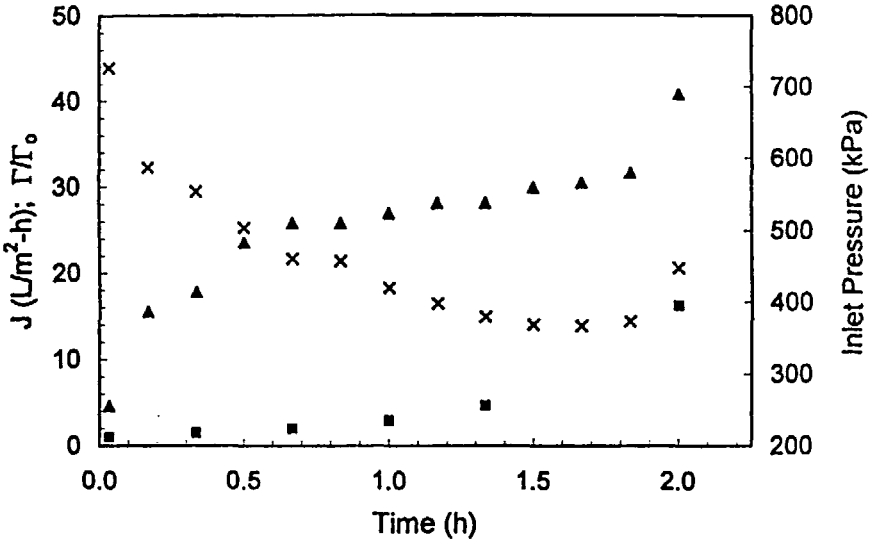

FIG. 2 Process studies on whole fermentation broth prior to acidification (Run 1): (▲) inlet feed pressure to the membrane; (×) permeate flux (J); (■) concentration factor (Γ/Γ_0).

the final data point at 2.0 hours where the concentration factor was 16. By comparison, 10 L of yeast slurry that was prepared by fermentation in the preliminary study reached a concentration factor of 15 and a steady-state flux of 34 L/m^2·h in only 0.5 hour. The yeast slurry (2.4 gdw/L initial concentration) was filtered at a pump setting of 227 L/h and a TMP of 75 kPa without any problems. Again, the steady-state flux compared favorably with literature values for traditional crossflow microfiltration systems (20 to 50 L/m^2·h) (11, 12). Higher steady-state fluxes (100 to 395 L/m^2·h) may be obtained by using rotary crossflow systems (11, 12).

As mentioned above, the Pellicon cassette is designed with screen spacers between the membranes which are more susceptible to plugging with solids than plate-and-frame systems with solid spacers (such as Millipore's Minitan system). In a concentration experiment on *A. niger* with a tubular crossflow system, Sims and Cheryan reported a significant increase in TMP and permeate flux at the end of the experiment (9), which agrees with data from Run 1. Gravatt and Molnar recognized the problem of clogging with suspended solids and said that the concentrated slurry becomes a sludge that will not circulate in a hollow fiber system, even at the maximum pressure allowed by the unit (7). They recommend use of either tubular or flat-leaf membrane systems to minimize problems associated with concentrated cell slurries.

Newer designs having an open channel with a wave pattern support plate are available to address these limitations (5, 22, 26).

pH-Treated Fermentation Broth

For Runs 2–5 the *S. rimosus* fermentation broth was acidified from pH 7.0 to 4.0 prior to being concentrated in the MF system. In the case of streptomycin recovery from a *S. griseus* broth, recovery improved as pH decreased from 7 to 2 with an optimum product stability at pH 4 (17). As discussed above, acidification reduces the viscosity of the suspension by lysing cells or breaking up cell aggregates. All five experiments (Runs 1–5) were operated with the permeate and retentate outlets at atmospheric pressure. Inlet feed pressure was extremely high in Run 1, but relatively low (in the range generally observed for microfiltration) for Runs 2–5. The pump was stable (no cavitation) up to 227 L/h at the beginning of Run 2; however, the crossflow rate was set at 176 L/h in order to make sure that the three subsequent experiments (Runs 3–5) could be operated at the same setting.

Improvement in downstream separations process conditions was dramatic after acidification as illustrated in Fig. 3. The inlet feed pressure to the mem-

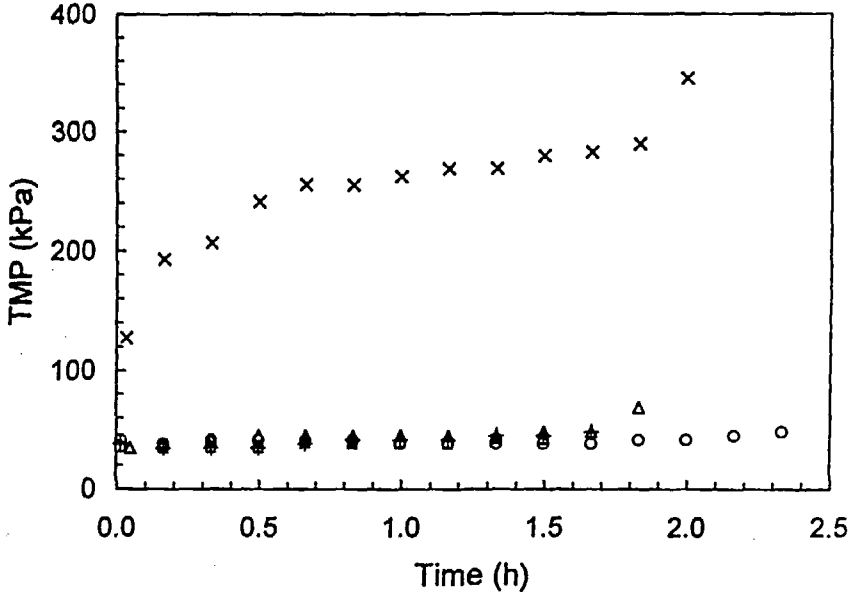

FIG. 3 Comparison of transmembrane pressures (TMP) for whole broth (×, Run 1), and pH-treated broth (△, Run 2; ○, Run 3; □, Run 4; +, Run 5).

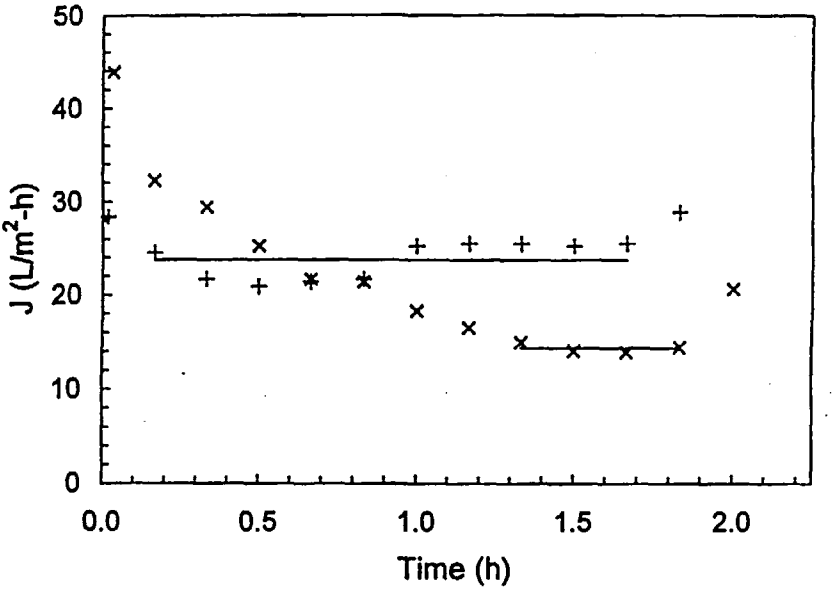

FIG. 4 Comparison of fluxes (J) of whole broth (×, Run 1) and pH-treated broth (+, Run 5), both at pH 7.0; (——) average steady-state values.

brane was fairly constant during the four pH-treated runs, resulting in an average TMP of 44 ± 4, 40 ± 2, 37 ± 3, and 39 ± 4 kPa for Runs 2–5, respectively. These pressures are not significantly different, but all four are much lower than any of the TMP values observed in Run 1. There was a sharp rise in TMP at the end of Run 1 (as previously discussed), and a slight rise at the end of Runs 2, 3, and 5. Therefore, the last pressure reading was excluded from the averages for Runs 2, 3, and 5. Higher transmembrane pressures could be obtained in all of the runs with pH-treated slurry by partially closing the retentate valve, but steady-state fluxes for compressible cakes exhibit little or no improvement with increased pressure, as discussed above.

Figure 4 examines the effect of pH on permeate fluxes. Run 1 represents the untreated fermentation broth at pH 7.0. In Run 5 the slurry was treated by acidification to pH 4.0 but titrated back to pH 7.0 prior to filtration. Both runs were performed at room temperature. If the only effect of acidification were to disassociate cell aggregates, then restoration of neutral pH could be expected to reform the aggregates. In this case, Runs 1 and 5 would be identical. However, the shape of the flux curves are quite different, indicating heavy fouling in Run 1 and minimal fouling in Run 5. Actually, the flux curve for

Run 5 is identical to Run 2 which has the same temperature but a different pH. Thus, the major effect of acidification is to irreversibly cleave cell aggregates and improve the rheological properties or pumpability of the broth.

The steady-state flux is 23.7 ± 2.0 L/m^2·h in Run 5 as compared with 14.4 ± 0.5 L/m^2·h in Run 1, as indicated by the horizontal lines in Fig. 4. A high flux point at the beginning and end of Run 5 was excluded from the average as shown. During the initial transient period, the flux for the untreated broth in Run 1 is higher than the flux in the treated run due to the higher operating pressure in Run 1. Higher TMP provides some advantage to flux while the filter cake is still developing, but only serves to compress the fully developed cake, thus negating the advantage at steady-state. The permeate flux in Run 1 at steady-state falls well below that of the pH-treated run due to the lower maximum crossflow velocity (pumpability limited by slurry viscosity). The temperature in Run 1 (23°C) is slightly less than the temperature in Run 5 (26°C), but this difference alone does not account for the lower flux. The steady-state flux in Run 1 is even less than in Run 3 which has a colder temperature (19°C).

Figure 5 examines the effect of temperature on filtration of the pH-treated slurries. Run 2 was performed at 26°C, the cell suspension was cooled to

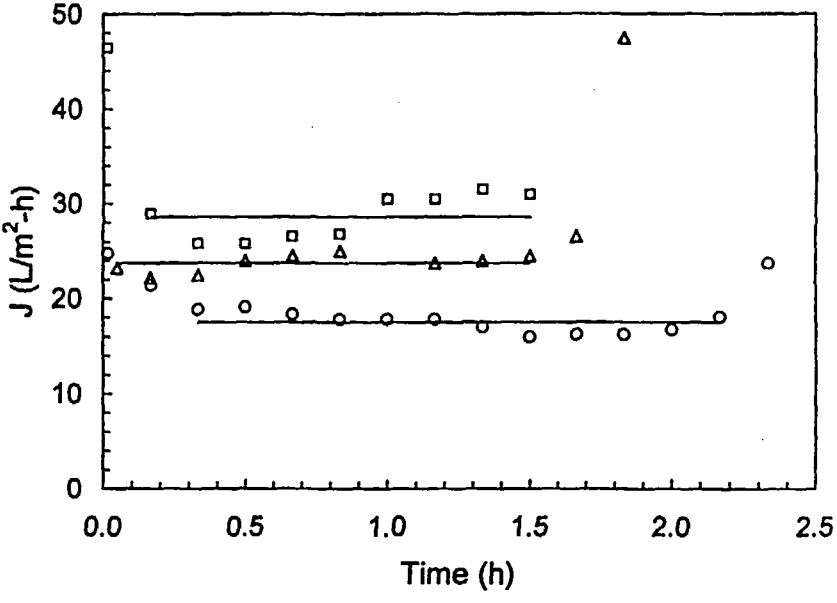

FIG. 5 Effect of process temperature on permeate flux (J) for pH-treated broth at pH 4.0: O, 19°C (Run 3); Δ, 26°C (Run 2); □, 30°C (Run 4); (——) average steady-state values.

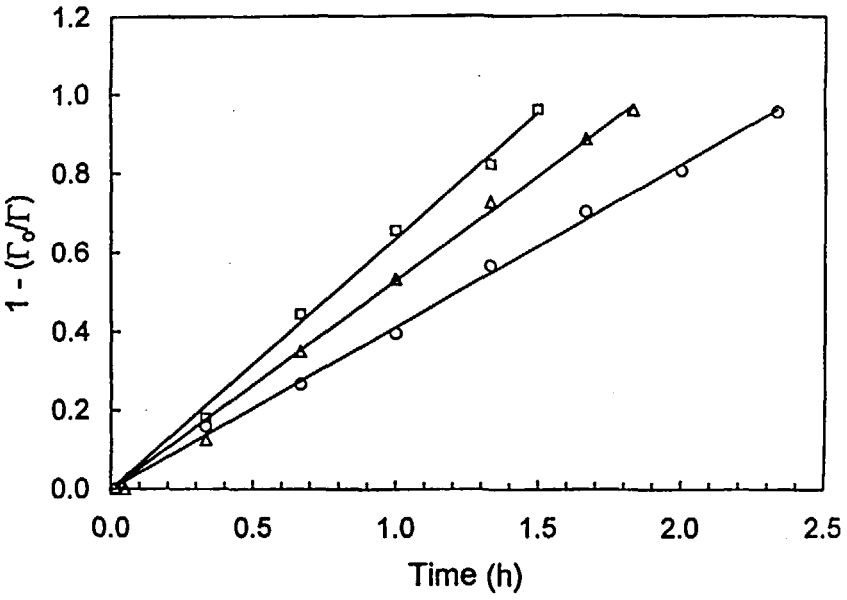

FIG. 6 Calculation of steady-state flux (J) from equivalent turbidity data: ○, 19°C (Run 3); △, 26°C (Run 2); □, 30°C (Run 4); (——) linear regressions.

19°C for Run 3, then it was heated to 30°C for Run 4. As shown in the figure, permeate fluxes drop immediately from clean-water values to steady-state values that are relatively constant throughout each run (horizontal lines represent steady-state averages). One or two high flux points at the beginning and/or end of each run were excluded from the averages. As expected, steady-state flux increased with temperature, resulting in values of 17.5 ± 1.0 L/m^2·h at 19°C (Run 3), 23.7 ± 1.0 L/m^2·h at 26°C (Run 2), and 28.6 ± 2.3 L/m^2·h at 30°C (Run 4). The standard deviation is higher in the 30°C run because it was more difficult to maintain the feed/retentate tank at an elevated temperature than at ambient conditions. Due to small temperature variations, the steady-state fluxes in Runs 2–5 all form wave patterns that slowly oscillate above and below the averages (Figs. 4 and 5).

Steady-state fluxes for the temperature studies were also calculated from Eq. (4) and the regressed slopes, as shown in Fig. 6 (all data points included in regressions). As before, steady-state fluxes increased with temperature, giving values of 17.7 ± 0.4 L/m^2·h at 19°C (Run 3), 22.8 ± 0.9 L/m^2·h at 26°C (Run 2), and 27.3 ± 1.1 L/m^2·h at 30°C (Run 4). As summarized in Table 2, steady-state flux values determined by the two different methods

TABLE 2
Relationship between Flux and Temperature

Run	T (°C)	J_{ss} (Fig. 5)[a]	J_{ss} (Fig. 6)[b]	R^2
2	26	23.7 ± 1.0	22.8 ± 0.9	0.9952
3	19	17.5 ± 1.0	17.7 ± 0.4	0.9976
4	30	28.6 ± 2.3	27.3 ± 1.1	0.9962

[a] Statistical average and standard deviation of sample.
[b] Calculated from linear regression slope and 95% confidence interval.

(volume/flux data and equivalent turbidity data) are very close at a specific temperature.

Modeling Temperature Effects

In order to model the relationship between flux and temperature, a logarithmic plot (Fig. 7) was prepared using Eq. (5) and the data points in Table 2.

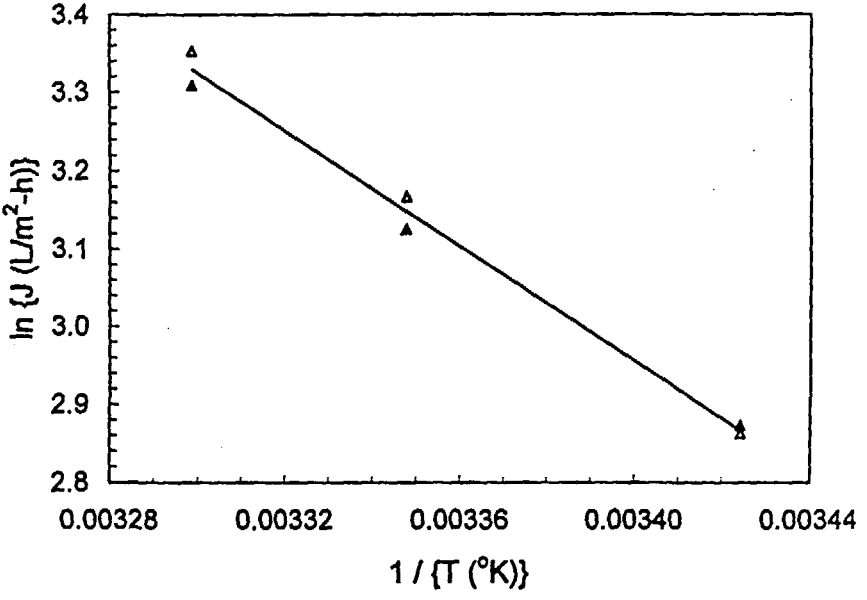

FIG. 7 Arrhenius plot of steady-state flux (J) versus temperature: (△) values from Fig. 5; (▲) values from Fig. 6; (——) linear regression.

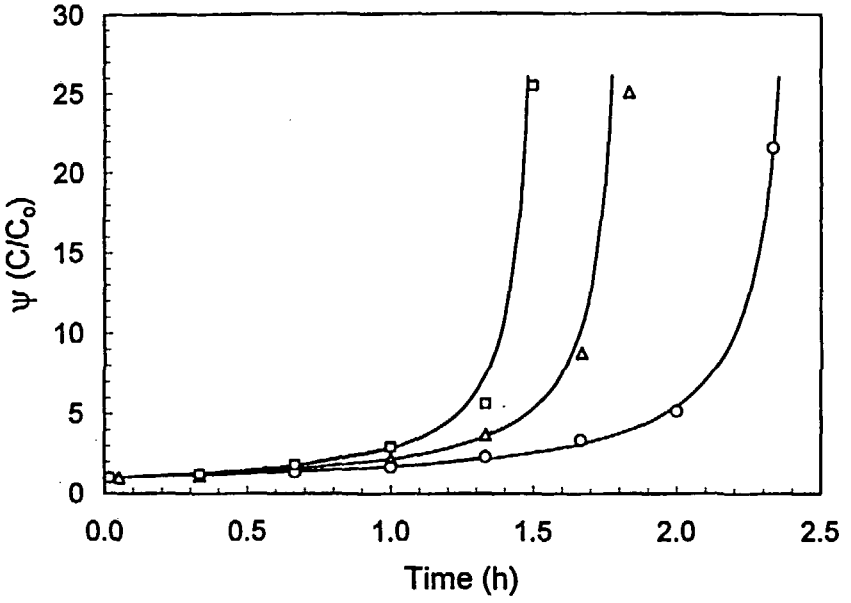

FIG. 8 Comparison of calculated concentration factors (C/C_0) with experimental data: ○, 19°C (Run 3); △, 26°C (Run 2); □, 30°C (Run 4); (——) calculated values.

This graph is possible since Runs 2–4 were carried out at the same crossflow velocity and TMP. The crossflow velocity and slurry properties determine the cake thickness δ_c in Eq. (1). Thus, at steady state everything is constant in Eq. (1) for these three runs except for the solution viscosity which decreases as temperature increases. The Arrhenius plot (Fig. 7) results in an R^2 value of 0.9912, with linearly regressed values at the 95% confidence level of 15.5 $\pm$ 1.6 for the intercept ($\ln J_0$) and -3690 ± 370 for the slope ($-E_a/R$). From the slope, an activation (separation) energy of 7.8 kcal/mol was calculated.

In Fig. 8 the experimental concentration factors are plotted as a function of time for the three temperature runs. The concentration data in Runs 2–4 all form smooth curves which appear to increase exponentially toward the end of the runs. Concentration factors were also calculated from the Arrhenius constants determined above, by using Eq. (5) to calculate steady-state flux at a given temperature and Eq. (3) to calculate the concentration factor when steady-state flux is known. The calculated curves in Fig. 8 follow the experimental trends fairly well. Examination of the graph indicates that the time required to reach a concentration factor of 20 decreases from 2.3 to 1.5 hours as the operating temperature increases from 19 to 30°C. Thus the improvement

in flux with operating temperature translates to reduced batch processing time. Temperatures up to 50°C, the maximum permitted for the PVDF membrane, should further improve the processing time.

CONCLUSIONS

Streptomyces rimosus fermentation broth was prepared and grown to the point where secondary mycelium are formed and the antibiotic terramycin is normally produced. Crossflow microfiltration experiments on the whole fermentation broth resulted in a relatively high concentration factor and reasonable permeate fluxes. However, the high slurry viscosity of the untreated broth necessitated a high inlet feed pressure to the membrane, rising to the system maximum of 690 kPa. High viscosity also limited the maximum crossflow velocity possible for concentrating whole fermentation broth.

Pretreatment of the whole broth by acidification, a typical step for improving antibiotic recovery, lysed the cellular aggregates and stabilized slurry viscosity, thus improving system performance. When the whole broth was acidified from pH 7.0 to 4.0, steady-state flux values increased; however, there was no change in flux when the acidified broth was titrated back to 7.0. The treated broth could be microfiltered at lower transmembrane pressures (37 to 44 kPa) and higher crossflow velocities than the untreated broth. The treated broth displayed relatively constant fluxes with minimal fouling throughout each run. Steady-state flux values increased from 18 to 28 $L/m^2 \cdot h$ as temperature increased from 19 to 30°C. Processing time decreased as temperature increased.

The method that was presented to calculate concentration factors as a function of temperature agrees well with experimental data. Concentration factors of 16 to 25 were reached in experiments on both the untreated and pH-treated cell slurries. Above that point the channels in the membrane cassette either became blocked with solids or were approaching the point of blockage as evidenced by flux and/or operating pressure surges. Industrial-scale systems are available with better channel designs to solve this problem. Overall, the data indicate that acid treatment of whole broth followed by tangential crossflow microfiltration at elevated temperature is a viable method for removing mycelial organisms from antibiotic fermentation broths.

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