Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Recovery of α-Agarase Enzyme from Fermentation Broths by Membrane Crossflow Filtration

G. Toussaint[a]; L. H. Ding[a]; M. Y. Jaffrin[a]; I. Hassairi[b]; M. Nonus[b]
[a] UMR CNRS 6600, DEPARTMENT OF BIOLOGICAL ENGINEERING, TECHNOLOGICAL UNIVERSITY OF COMPIEGNE, COMPIEGNE, FRANCE [b] DEPARTMENT OF CHEMICAL ENGINEERING, TECHNOLOGICAL UNIVERSITY OF COMPIEGNE, COMPIEGNE, FRANCE

Online publication date: 17 April 2000

To cite this Article Toussaint, G. , Ding, L. H. , Jaffrin, M. Y. , Hassairi, I. and Nonus, M.(2000) 'Recovery of α-Agarase Enzyme from Fermentation Broths by Membrane Crossflow Filtration', Separation Science and Technology, 35: 6, 795 — 809
To link to this Article: DOI: 10.1081/SS-100100193
URL: http://dx.doi.org/10.1081/SS-100100193

SEPARATION SCIENCE AND TECHNOLOGY, **35**(6), pp. 795–809, 2000

Recovery of α-Agarase Enzyme from Fermentation Broths by Membrane Crossflow Filtration

G. TOUSSAINT, L. H. DING, and M. Y. JAFFRIN*
UMR CNRS 6600
DEPARTMENT OF BIOLOGICAL ENGINEERING

I. HASSAIRI and M. NONUS
DEPARTMENT OF CHEMICAL ENGINEERING

TECHNOLOGICAL UNIVERSITY OF COMPIEGNE
BP 20 529, 60205 COMPIEGNE, FRANCE

ABSTRACT

We describe the recovery of extracellular α-agarase enzyme from fermentation broths using a polypropylene hollow fiber filter with 0.5 μm pores normally used for plasma collection from donors. The permeate flux increased with inlet wall shear rate from 30 L/h·m^2 at 1500 s^{-1} to 45 L/h·m^2 at 3000 s^{-1}. But the enzyme activity sieving coefficient (ratio of permeate to inlet activity) decreased with increasing shear rate and transmembrane pressure (TMP). Therefore, the enzyme flux transmitted was found to be maximum at a shear rate of 1500 s^{-1} and a TMP between 20 and 40 kPa. Since enzyme activity decay with time is less at low temperature, the recovered activity was found to be nearly independent of temperature between 5 and 37°C, the maximum occurring at 22°C. The superimposition of flow and pressure pulsations on the inlet and outlet lines of the filter with a modified roller pump produced permeate flux enhancements ranging from 44% at 1000 s^{-1} to 68% at 3000 s^{-1} for a TMP of 3 kPa.

Key Words. α-Agarase; Microfiltration; Enzyme recovery; Membrane separation

INTRODUCTION

The extracellular α-agarase enzyme, which is capable of degrading agar polysaccharides, is used in molecular biology for DNA sequencing (1). It can be produced by culture of a marine bacterial strain *Alteromonas agarlyticus*

* To whom correspondence should be addressed. E-mail: michel.jaffrin@utc.fr

(2) that releases the enzyme in an appropriate culture medium (3). The normal mode of purification is by affinity chromatography on crosslinked agarase and by anion-exchange chromatography (3, 4). Primary purification has been carried out by ultrafiltration with a 100-kDa polysulfone membrane (3). Leon et al. (2) purified extracellular agarase by anion-exchange chromatography and gel filtration with an overall yield of 45%. The purification steps are generally carried out at temperatures between 4 and 8°C since enzyme activity decays rapidly at temperatures above 30°C.

Since anion-exchange and affinity chromatography techniques are time consuming and have limited outputs, membrane crossflow filtration is increasingly used in the downstream processing of enzymes (5–7) and more specifically for extracting solutes from fermentation broths (8–12). Kroner et al. (5) noted that cellular debris produced more fouling than the cells themselves and that fouling induced enzyme retention. Le et al. (6) investigated the recovery of microbial asparaginase from fermentation broths by microfiltration with asymmetric organic membranes of various pore sizes from 0.2 to 0.6 μm. The best permeate fluxes, obtained with 0.45 μm pores, decayed from 50 to 15 L/h·m^2 in 4 hours of filtration. The permeate flux also decayed logarithmically with increasing cell concentration from 1 to 3 g/L in accordance with the classical concentration polarization model. The limiting wall concentration of cells, calculated from extrapolation to zero flux, was found to be near 5 g/L. Enzyme transmission was highly dependent on circulation velocity. At the highest velocity (1.3 m/s), it reaches a peak of 92% after 10 minutes and decayed to 80% after 3 hours. At the lowest speed (0.09 m/s), transmission decreased from a peak of 55% to 10% in 3 hours.

The same group (7) also separated amidase from cell lysate using the same membrane. They observed the same pattern of enzyme transmission with time as in Ref. 6, except that the peak was reached sooner, after about 3 minutes, and exceeded 100% at the highest speed (0.33 m/s) when the more open side of the membrane was in contact with the feed. When the membrane was used with its skin in contact with the feed, its enzyme transmission dropped faster with time, reaching 35% after 30 minutes instead of 95% as in the previous case. Li et al. (9) reported that cell concentration increased when fermentation was carried out on line with filtration through a 0.22-μm pore ceramic membrane connected to the fermentor. Liew et al. (10) observed that fouling resistance increased with aging of the cells due to cell fragments and released cell contents. Parnham and Davis (11), who recovered proteins from bacterial debris using cellulose acetate membranes, found that backpulsing permeate at 4 Hz frequencies increased protein transmission to 100% from 60% without backpulsing and the permeate flux by a factor of at least 3. Bowen and Hall (12) used 0.2 μm polycarbonate membranes for recovery of yeast alcohol hydrogenase enzymes. They observed an important decrease in pore volume due

to enzyme desposition in the pore walls as confirmed by atomic force microscopy. It is therefore important to understand the mechanisms leading to membrane fouling by biological suspensions and to optimize filtration conditions to make enzyme recovery by membrane economically competitive.

This paper investigates the feasibility of recovering α-agarase enzyme from fermentation broths by membrane cross-flow filtration for the purpose of purification and concentration.

MATERIALS AND METHODS

Fermentation

The enzyme α-agarase, of 180 kDa molecular weight, was produced by culture of bacteria *Alteromonas agarlyticus* in an adapted medium (3) in a 2-L fermentor. This medium was chosen because it satisfies the needs of our strain. It is composed of a carbon source (agar), nitrates, and salts which reproduce the natural marine medium of the bacteria. The culture medium at 22°C was composed of NaCl (15 g/L), $MgSO_4$ (3 g/L), KCl (0.1 g/L), $CaCl_2 \cdot 2H_2O$ (0.4 g/L), $FeSO_4$ (0.02 g/L), NH_4NO_3 (2 g/L), $Na_3\ PO_4 \cdot 12H_2O$ (7.6 g/L), bacto-agar E type (2.5 g/L), and extract from yeast (0.4 g/L). The pH was regulated to 7.2 by the addition of 2 mol/L of NaOH. The cellular concentration was 1.6 g/L.

Enzymatic Activity Measurement

The agarolytic activity was determined from measurement of the reducing sugar released during agarase hydrolysis in the presence of enzymes at 40°C during 30 minutes. Reducing sugars were measured by a colorimetric method with ferricyanure based on the reduction of heavy metal salts (Fe^{3+}) in alkaline medium according to the method of Hoffman. A unit of activity of α-agarase corresponded to an increase of 0.1 unit of absorbency at 237 nm (13).

Filtration Setup

The circuit (Fig. 1) consists of a thermostated reservoir feeding the filtration unit through a Masterflex peristaltic pump. The filtration unit consists of a polypropylene hollow fiber filter with a membrane surface area of 0.07 m^2, normally used for donor's plasmapheresis. Fiber length and inner diameter are respectively 14 cm and 330 μm, and mean pore size is 0.5 μm. The inlet flow (Q_i) was measured by an electromagnetic flowmeter (SPECTRAMED model SP 2202B). The permeate was collected in a beaker placed on an electronic scale connected to a microcomputer by a RS 232C interface. The time-aver-

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

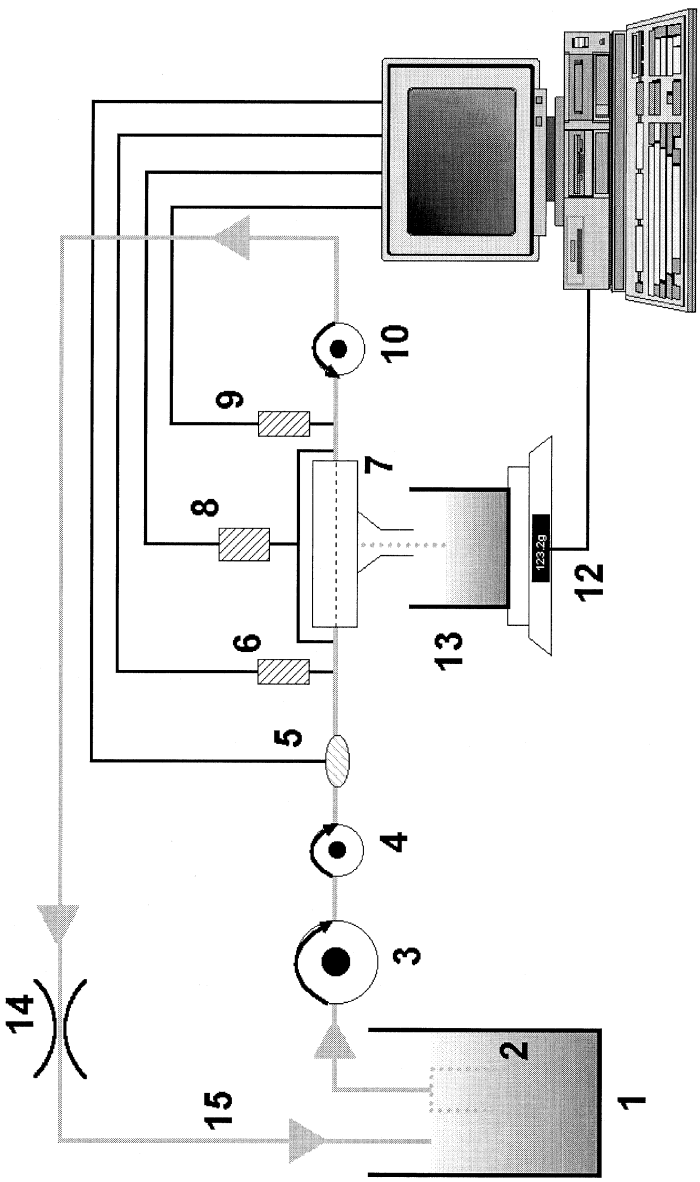

FIG. 1 Schematic of experimental setup.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

aged permeate flow was determined by differentiation of a signal with respect to time. Inlet pressure (P_i) and outlet pressure (P_o) of the filter were measured by Validyne pressure transducers in order to determine the mean transmembrane pressure (TMP) by

$$P_{tm} = (P_i + P_o)/2$$

Since the permeate is collected at atmospheric pressure, and the pressure drop along the filter is

$$\Delta P = P_i - P_o$$

All signals were fed to a microcomputer through a 12 bits A/D converter. In order to increase permeate flux from our previous work on plasmapheresis (14, 15), a pulse generating system, consisting of another peristaltic pump in which one of the two rollers was mounted on the rotor, has been removed. During its rotation the single roller alternately squeezes two lines made of silicone tubing, referred to as 4 and 10 in Fig. 1, which are located upstream and downstream, respectively, of the filter. When the roller squeezes line 3, it produces a forward wave which accelerates the feed fluid through the filter and increase the inlet pressure P_i. When the roller squeezes line 10, it produces a retrograde wave which can reverse the flow direction in the filter and therefore raises the outlet pressure of the filter P_o.

There are, in fact, three possibles modes of operation. In the first mode, only the downstream line 10 is squeezed and the peristaltic feeding pump 2 is connected directly to the filter inlet, bypassing the pulsating generator, as shown in Fig. 1. In the second mode, only the upstream line 4 is squeezed and the filter outlet is directly connected to the reservoir. In the third mode, lines 4 and 10 are both squeezed by the roller. The pulsation frequency is controlled by the speed of a single roller pump (generally 1 Hz) and the volume displaced by the roller is the same for lines 4 and 10, and approximately equal to 2 mL.

Membranes were cleaned after each test with chlorine at 0.025 mol/L in a closed circuit for 1 hour and rinsed with pure water so as to recover a hydraulic permeability within 15% of its normal value of 4000 L/(h·m^2·bar). Prefiltration of the feed solution was carried out in a dead-end mode by inserting a bubble trap from a dialysis blood line equipped with a 0.1-mm mesh screen at the inlet of the pump.

RESULTS

Effect of Prefiltration

The effect of prefiltration of the feed solution on the time variation of permeate flux and pressure drop along the filter is illustrated on Fig. 2. In the absence of prefiltration, the permeate flux decays rapidly from 21.6 to 7.2 L/h·m^2 in 75 minutes while the pressure drop along the fiber length increases from 2.7

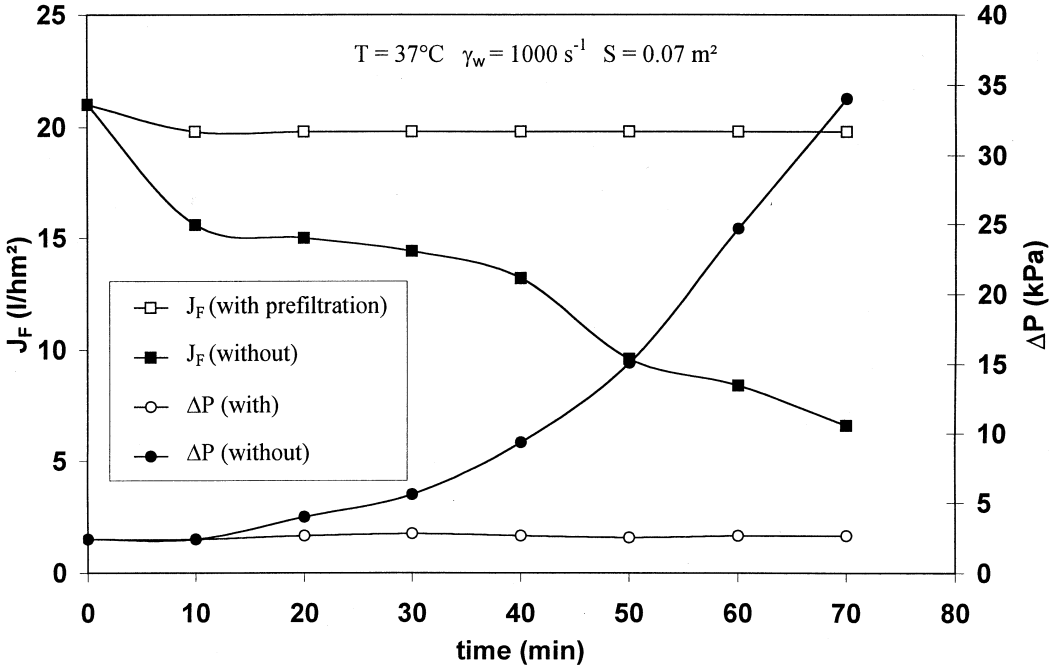

FIG. 2 Comparison of permeate flux and pressure drop along membrane with and without prefiltration.

to 37.3 kPa and the TMP increases from 14 to 25 kPa. Removal of cell aggregates by the screen almost entirely prevents permeate flux decay and pressure drop increase, while the TMP remains at its initial value of 14 kPa. Therefore, all subsequent tests were carried out with prefiltration of the enzyme solution.

The pressure drop increase without prefiltration could have two possible causes: 1) a reduction in the number of fibers N due to blocking of lumen by cell aggregates and 2) a partial obstruction of a large number of fibers by adsorption of debris equivalent to a reduction in lumen diameter. Since the permeate flux hardly decreases with prefiltration, we can assume that permeate flux decay without prefiltration is mostly due to a decrease in functional membrane area caused by fiber obstruction. The ratio of final to initial number of open fibers should be

$$N_f/N_0 = (J_{f\,\text{without}}/J_{f\,\text{with}})_{\text{final}} = 0.33 \tag{1}$$

An obstruction of $\frac{2}{3}$ due to fibers will cause the pressure drop to increase by 3 instead of by 13.8 as observed on Fig. 2. Therefore, partial obstruction by the remaining open fibers is necessary to explain the large pressure drop observed without prefiltration. Using Poiseuille's law:

$$\Delta p = (128\mu L\, Q)/(\pi d^4 N) \tag{2}$$

we can write for the ratio of initial to final pressure drop

$$\Delta p_0/\Delta p_f = (d_f/d_0)^4 \, (N_f/N_0) \tag{3}$$

and, for the ratio of final to initial lumen diameter, using Eqs. (1) and (3):

$$\frac{d_f}{d_0} = \sqrt[4]{3\frac{\Delta P_0}{\Delta P_f}} = 0.68 \tag{4}$$

The observed reduction in permeate flux and increase in pressure drop can be explained by a complete obstruction of two-thirds by the fibers and a partial obstruction of the remaining third, which reduces the lumen by 32%.

Variation of Permeate Flux and Sieving Coefficients with Transmembrane Pressure (TMP) and Wall Shear Rate

The variation of permeate flux with TMP (Fig. 3) presents the classical pattern of flux limitation by concentration polarization with a pressure-independent plateau. The maximum permeate flux increases nonlinearly with increasing wall shear rate γ_w as $35\gamma_w^{0.65}$, reaching 48 L/h·m^2 at a TMP of 68 kPa, a temperature of 37°C, and a wall shear rate of 3000 s^{-1} (inlet flow = 300 mL/min). These permeate fluxes are similar to those reported by Li et al. (9).

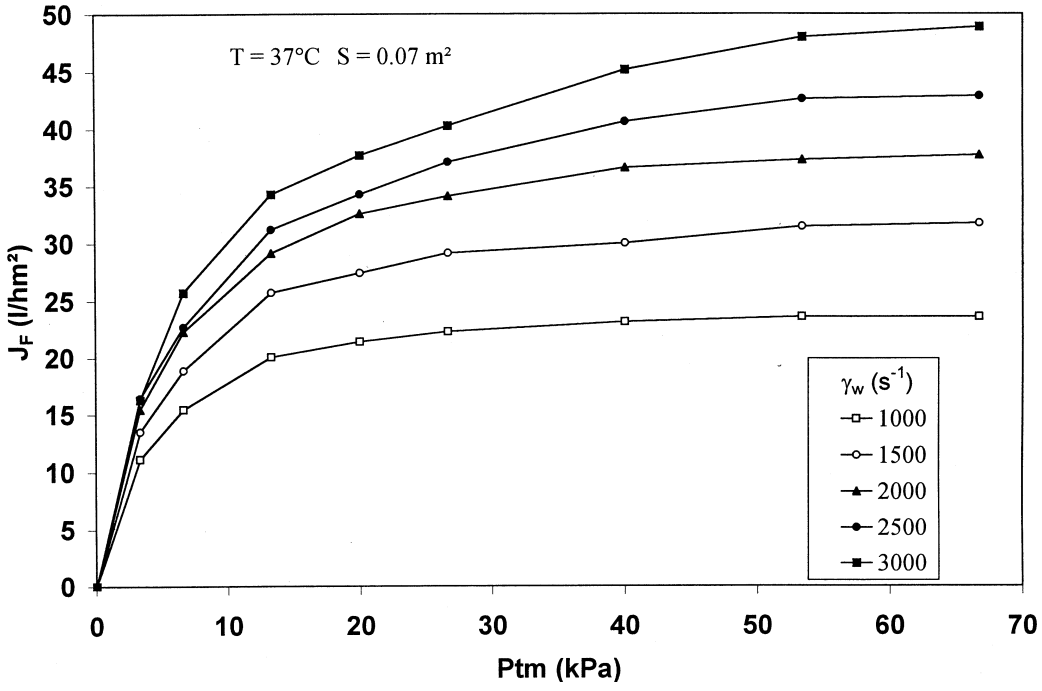

FIG. 3 Variation of permeate flux with transmembrane pressure at various wall shear rates.

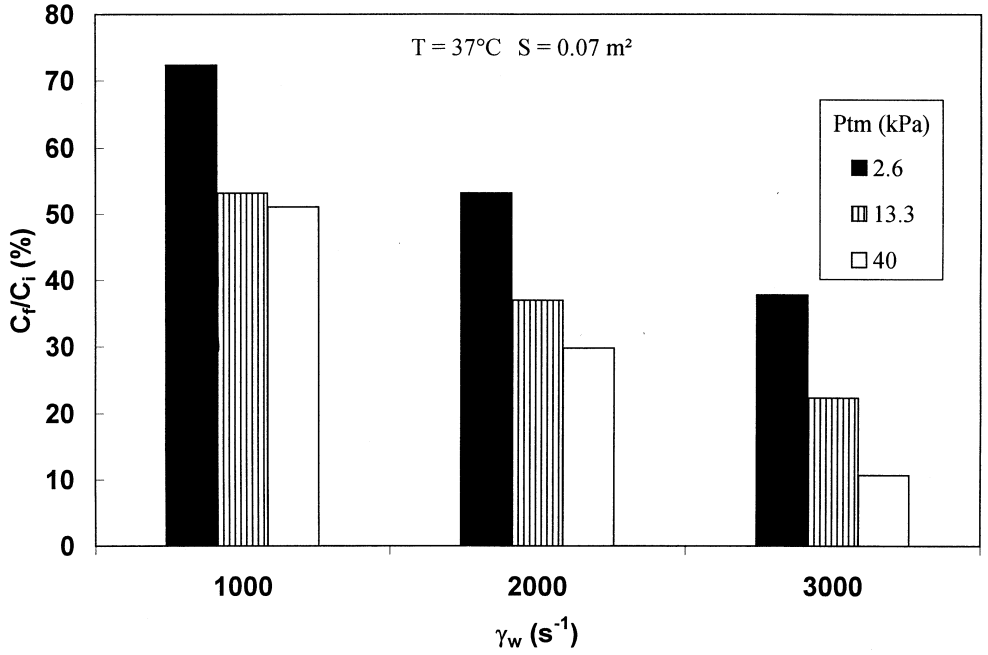

FIG. 4 Enzyme sieving coefficients at various transmembrane pressures and wall shear rates.

The sieving coefficient of α-agarase (ratio of permeate to inlet enzymatic activity C_f/C_i measured simultaneously) is presented in Fig. 4 after 1 hour of filtration for three values of wall shear rates (1000, 2000, and 3000 s^{-1}) and three transmembrane pressures. This sieving coefficient decreases when TMP increases, presumably because the layer of rejected cells deposited on the membrane becomes less permeable when it is packed by pressure. The observed decay of sieving coefficient with increasing wall shear rate is surprising and difficult to explain since the deposited layer is generally thinner at high shear rates. A possible explanation may be a degradation of the enzyme at high shear during its passage through the filter.

These results are confirmed by Fig. 5 which displays the continuous variation of permeate flux and sieving coefficient with wall shear rate γ_w from 500 to 3000 s^{-1} for a TMP of 40 kPa. The permeate flux at this pressure increases as $\gamma_w^{0.61}$ while the sieving coefficient decays linearly with increasing shear rate. Since the permeate flux and the α-agarase sieving coefficient vary in opposite directions as a function of wall shear rate, the α-agarase flux through the membrane J_{RA}, defined as the product of permeate flux by the sieving coefficient of enzymatic activity, will reach a maximum at a certain value of shear rate which, from Fig. 6, can be seen to be between 1400 and 1800 s^{-1} for the three transmembrane pressures considered.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

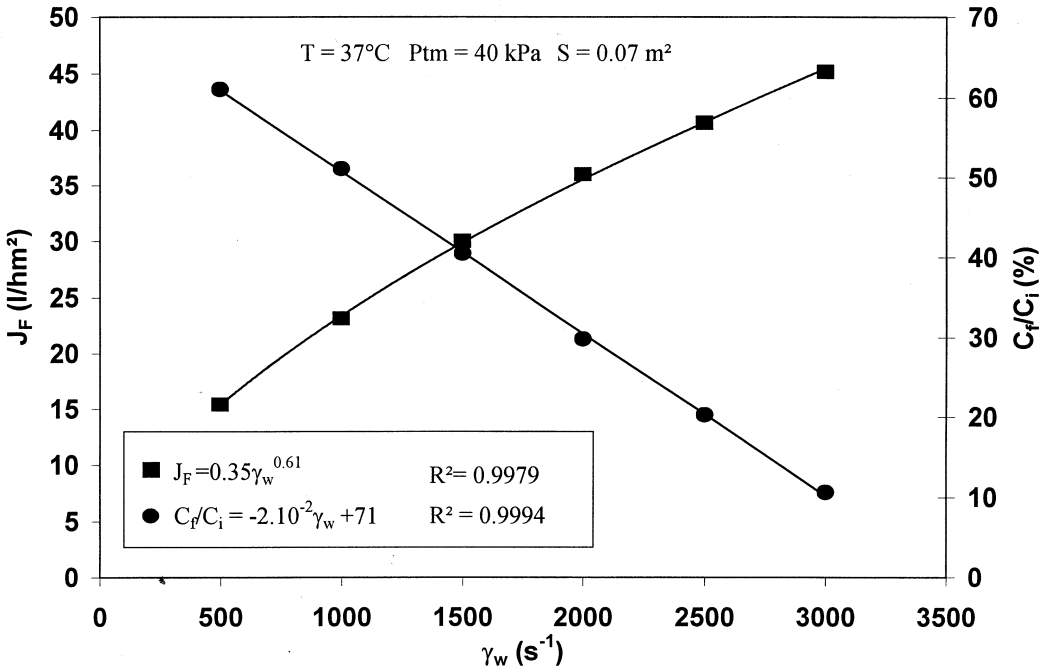

FIG. 5 Variation of permeate flux and sieving coefficient with wall shear rate.

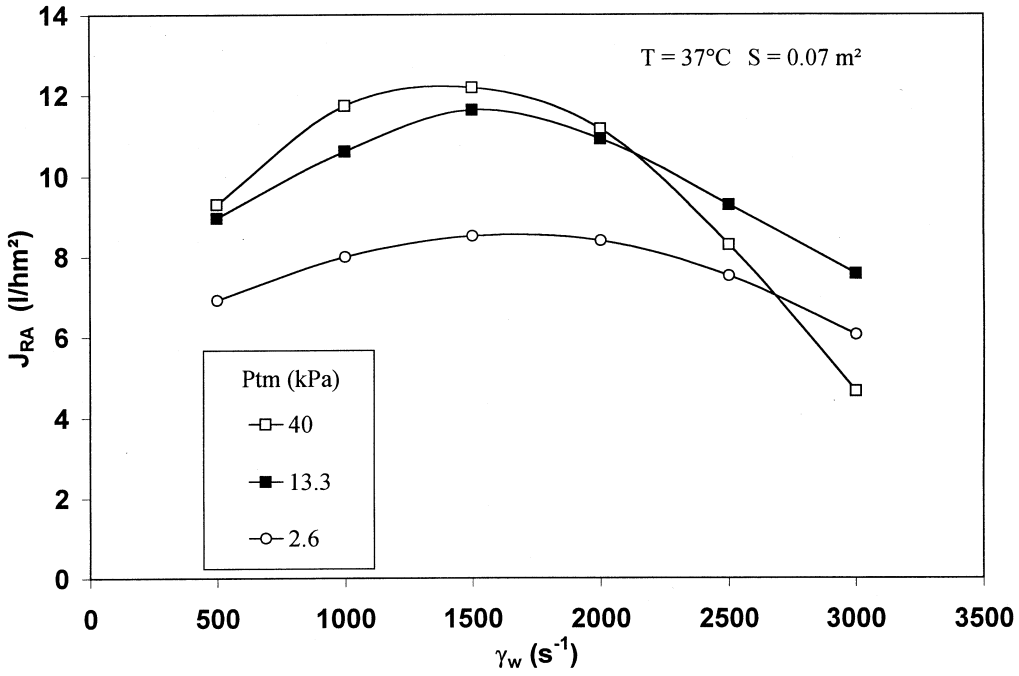

FIG. 6 Variation of α-agarase flux with wall shear rate for three different transmembrane pressures.

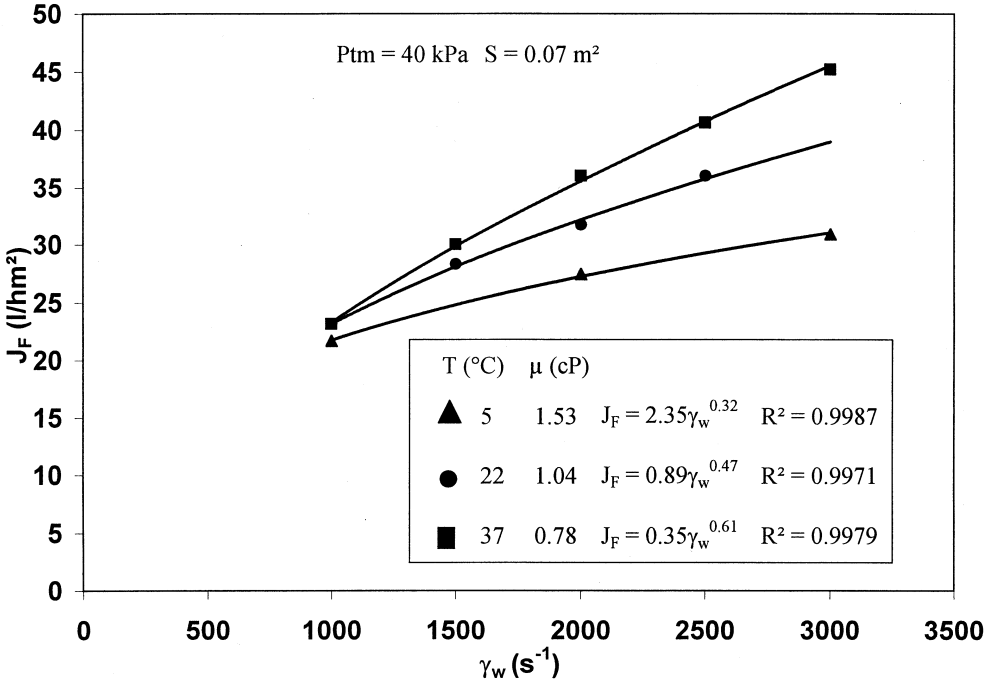

FIG. 7 Variation of permeate flux with wall shear rate at three temperatures and a TMP of 40 kPa.

Effect of Temperature on Permeate Flux and Enzymatic Activity

Since enzyme activity deteriorates with time at temperatures above 5 to 10°C, it is important to investigate filtration characteristics at various temperatures. As usual, the permeate flux decreases when the fluid temperature is decreased from 37 to 22 to 5°C (Fig. 7). The dependence of permeate flux on shear rate is also modified by temperature. The observed correlations are given in Table 1 at a TMP of 40 kPa.

TABLE 1
Variation of Viscosity, Permeate Flux, Reynolds Number, and Transmitted Activity with Temperature

T(°C)	μ (mPa·s)	J_F (L/h·m^2)	Re at γ_w = 1500 s^{-1}	J_F (L/h·m^2) at γ_w = 1500 s^{-1}	J_{RA} (L/h·m^2)
5	1.53	$2.35\gamma_w^{0.32}$	13.5	24.4	24.4
22	1.09	$0.89\gamma_w^{0.47}$	18.9	27.7	24.9
37	0.78	$0.35\gamma_w^{0.65}$	26.3	30.0	24.2

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

The increase in the exponent of γ_w from 0.32 to 0.61 with increasing temperature seems to suggest that flow ceases to be laminar at high temperature when viscosity is smaller, since an exponent of 0.32 corresponds to the laminar theory of Leveque (16) and 0.61 is close to the exponent of the Chilton–Colburn correlation (0.66) for turbulent flow. However, this explanation is not supported by the Reynolds number values given in Table 1 which remains below 30.

Figure 8 represents the enzymatic activity decay with time for a stagnant solution in a reservoir. It is negligible at 5°C but increases noticeably at 37°C where it reaches 22% after 140 minutes. Therefore we can speculate that the gain in permeate flux obtained by heating the solution to 37°C as observed in Fig. 7 may be offset after a certain time of filtration by a decrease in enzymatic activity. Thus the value of α-agarase mass flux J_{RA} at 1500 s^{-1}, which is also indicated as a function of temperature in Table 1, remains almost independent of temperature. Even though this product is slightly higher at 37°C than at 22°C (room temperature), this gain would certainly disappear after a longer period, and so we have decided to conduct future tests at room temperature (22°C).

A concentration test was carried out without recycling permeate at a wall shear rate of 1500 s^{-1}. The initial volume was 2500 mL and the temperature was 22°C. The final volume was 285 mL, giving a volumetric concentration factor of 8.77. The enzymatic activities in the retentate (A_{tank}), in the permeate (A_f), and in the control are represented as functions of time in

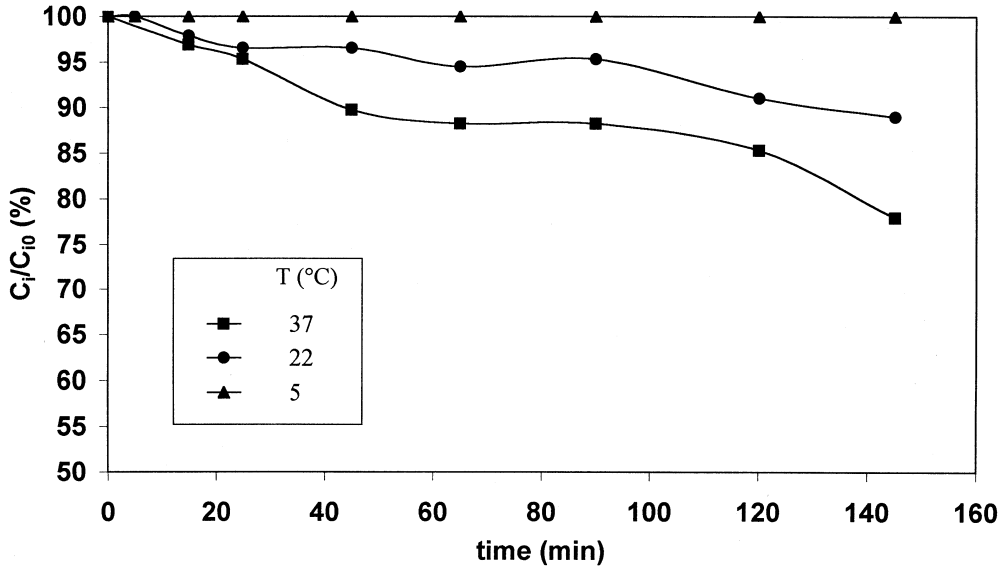

FIG. 8 Enzyme activity in percent of initial one, showing decay with time in stagnant solution for three temperatures.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

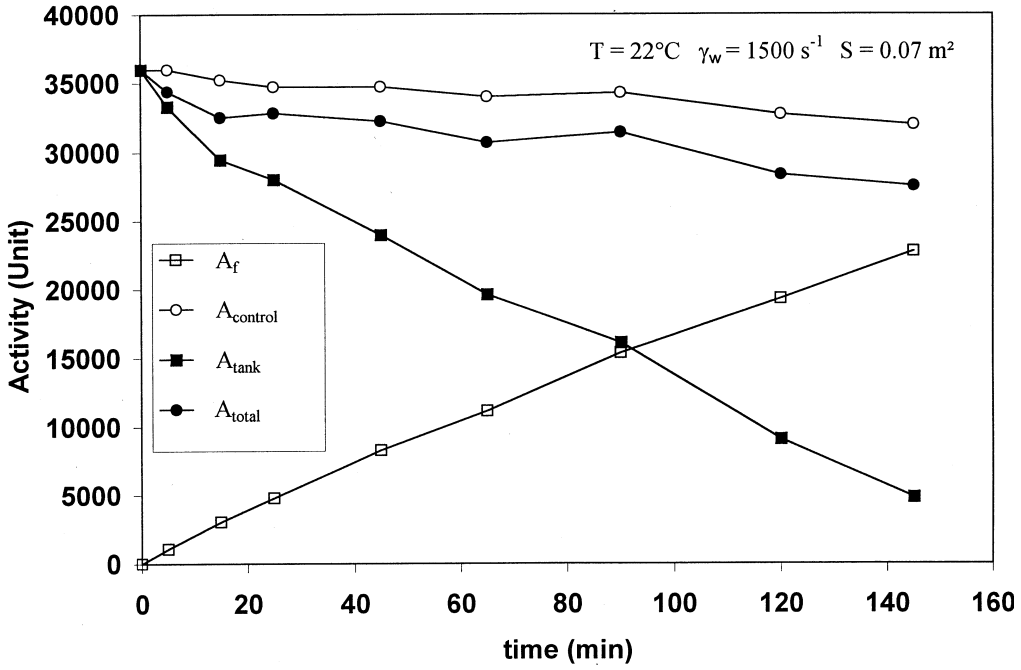

FIG. 9 Variation of enzymatic activity with time in the tank (A_{tank}), in the filtrate (A_f), in the control sample ($A_{control}$), and total activity (A_{total}) at 22°C and a wall shear rate of 1500 s^{-1}.

Fig. 9. The control is a closed tank containing the same solution. It is interesting to note that the sum (A_{total}) of activities in the permeate and the retentate is less than in the control. The difference, representing 4440 activity units at the end of the test or 12.2% of initial activity (36,170 units), corresponds to the quantity adsorbed in the filter and the circuit. After rinsing the filter with distilled water, only 1.1 % of adsorbed enzyme was recovered.

Effect of Pulsatile Flow on Permeate Flux

Figure 10 presents a comparison between stabilized permeate fluxes at a mean TMP of 3 kPa and a frequency of 1 Hz for three values of inlet wall shear rate collected during steady flow and the three modes of pulsatile flow described in the Materials and Methods section. The permeate flux enhancement relative to steady flow filtration is maximum in mode 3 pulsation when both the inlet and out lines are squeezed by the roller. The percent enhancements are 44% for an inlet shear rate of 1000 s^{-1}, 54% at 2000 s^{-1}, and 68% at 3000 s^{-1}. Even through the present solution does not contain large particles, as in the case of blood, pulsations are effective for disturbing the fouling layer deposited on the membrane. The superiority of the third mode over the other two, which was also observed in the case of plasma separation from whole blood (13), because there are two transmembrane pressure peaks per roller revolution instead of one for the two other modes. In addition, the velocity goes through sudden forward and backward accelerations during each period.

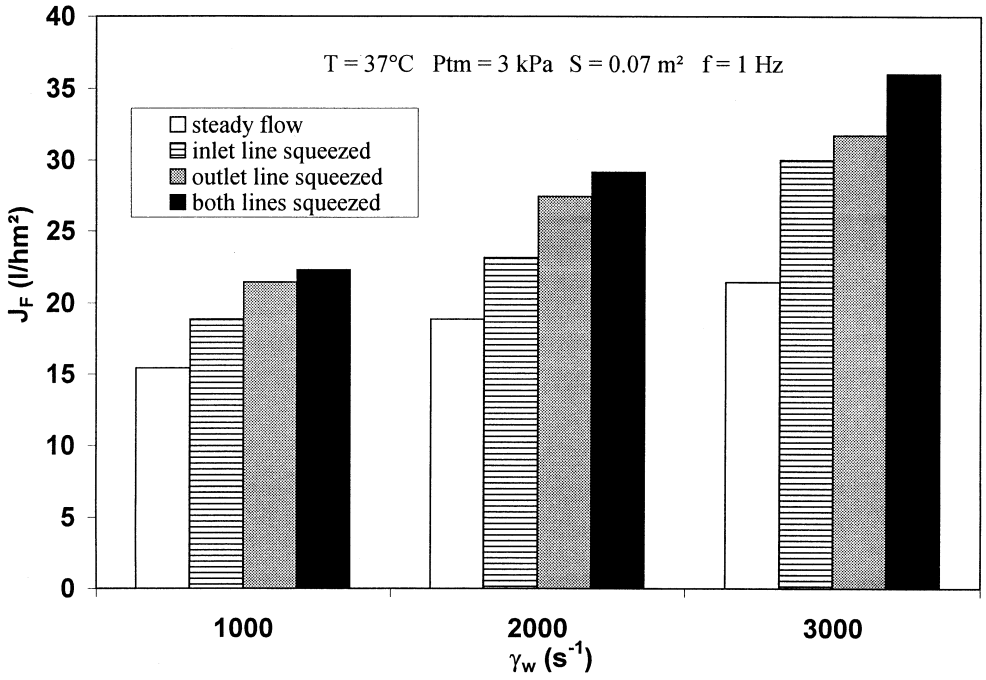

FIG. 10 Comparison of permeate flux for steady flow and three modes of pulsatile flow at 1 Hz, a TMP of 3 kPa, 37°C, and three time-mean wall shear rates.

The permeate fluxes in this case were limited by the low mean transmembrane pressure. Higher permeate fluxes would have been obtained at higher pressure in accordance with Fig. 3. The enzyme activity sieving coefficients, measured after 10 minutes of filtration, are listed in Table 2 for flow rates of

TABLE 2
Activities in Filtrate (C_f), at Inlet (C_i) and at Outlet (C_r) and Sieving Coefficient (C_f/C_f) for Steady Flow and Various Modes of Pulsatile Flow after 10 Minutes of Filtration

Flow rate (mL/min)	Mode	C_f (activity/L)	C_i (activity/L)	C_r (activity/L)	C_f/C_i (%)
100	Steady	8.9	10.3	9.7	86.4
200	Steady	9.5	10	9.8	95.0
300	Steady	9.5	10.2	9	93.1
100	Inlet line squeezed	8.9	9.8	9	90.8
200	Inlet line squeezed	9	9.9	9.1	90.9
300	Inlet line squeezed	9	10.2	9	88.2
100	Outlet line squeezed	8.8	9.9	9.7	88.9
200	Outlet line squeezed	8.9	9.8	9.7	90.8
300	Outlet line squeezed	9	10.2	9.8	88.2
100	Both lines squeezed	9.6	10.2	9	94.1
200	Both lines squeezed	9	10.3	8.9	87.4
300	Both lines squeezed	9.2	9.8	8.8	93.9

100, 200, and 300 mL/min for steady and for various modes of pulsatile flow. The sieving coefficients are similar for steady and the three types of pulsatile flows, and they do not vary with flow rate.

CONCLUSIONS

As noted by other investigators (17, 18), the main difficulty in solute extraction from fermentation broths by a membrane lies in the decay of the sieving coefficient with filtration time, which is more severe when TMP is high and, in this case, at high shear rates. Enzyme activity also decreases faster at higher temperatures. Thus, operating conditions which are favorable to permeate flux are unfavorable to enzyme transmission. It is therefore necessary to seek a compromise, and the optimal conditions in our case seem to be a shear rate of 1500 s^{-1}, a TMP of 40 kPa, and a temperature of about 22°C. However these conditions are not very critical, and Table 1, for instance, shows that the enzyme activity flux recovered at 5°C is very close to that at 22°C. The superposition of pulsations on the feed flow increases the permeate flux by reducing membrane fouling. According to Parnham and Davis (11), it should also facilitate enzyme transmission. Table 2 shows that at the beginning of the test, enzyme transmission was high and the same as in steady flow. Presumably the sieving coefficient will decay with time as observed in steady flow, but, unfortunately, due to a technical problem, they were not measured at later times.

ACKNOWLEDGMENT

This research has been supported by the Regional Council of Picardy.

SYMBOLS

C_i	inlet concentration (activity unit/L)
C_{i0}	initial concentration (activity unit/L)
C_f	permeate concentration (activity unit/L)
C_r	retentate concentration (activity unit/L)
d	inner fiber diameter (m)
J_F	filtration flux (L/hm^2)
J_{RA}	α-agarase flux, product of permeate flux by percentage of residual activity (L/hm^2)
N	number of fibers
P_i, P_o	inlet (outlet) pressure
P_{tm}	transmembrane pressure (Pa)
Q_i	inlet flow (mL/min)
S	membrane surface area (m^2)
T	temperature (°C)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

ΔP pressure drop (Pa)
γ_w wall shear rate (s^{-1})

REFERENCES

1. R. Armisen, "Agar and Agarose Biotechnological Application," *Hydrobiologia*, *221*, 157–166 (1991).
2. O. Leon, L. Quintana, G. Peruzzo, and J. C. Slebe, "Purification and Properties of an Extracellular Agarase from *Alteromonas* sp Strain C-1," *Appl. Environ. Microbiol.*, *58*, 4060–4063 (1992).
3. P. Potin, C. Richard, C. Rochas, and B. Kloareg, "Purification and Characterization of the α-Agarase from *Alteromonas agarlyticus* (Cataldi) Comb. Nov., Strain GJIB," *Eur. J. Biochem.*, *214*, 599–607 (1993).
4. I. Yamura, T. Matsumoto, M. Funatsu, H. Shigeiri, and T. Shibata, "Purification and Some Properties of Agarase from *Pseudomonas* sp. PT-5," *Agric. Biol. Chem.*, *55*, 2531–2536 (1991).
5. K. H. Kroner, H. Schutte, H. Hustedt, and M. R. Kula, "Cross-flow Filtration in the Downstream Processing of Enzymes," *Process Biochem.*, *4*, 67–74 (1984).
6. M. S. Le, L. B. Spark, P. S. Ward, and N. Ladwa, "Microbial Asparaginase Recovery by Membrane Processes," *J. Membr. Sci.*, *21*, 307–319 (1984).
7. M. S. Le, L. B. Spark, and P. S. Ward, "The Separation of Aryl Acylamidase by Cross Flow Filtration and the Significance of Enzyme/Cell Debris Interactions," *Ibid.*, *21*, 219–232 (1984).
8. S. Huang, X. Wu, C. Yuan, and T. Lin, "Application of Membrane Filtration to Glutamic Acid Recovery," *J. Chem. Tech. Biotechnol.*, *64*, 109–114 (1995).
9. S-L. Li, K.-S. Chou, J-Y. Lin, H-W. Yen, and I-M. Chu, "Study on the Microfiltration of *Escherichia coli*-containing Fermentation Broth by a Ceramic Membrane Filter," *J. Membr. Sci.*, *110*, 203–210 (1996).
10. M. K. H. Liew, A. G. Fane, and P. L. Rogers, "Hydraulic Resistance and Fouling of Microfilters by *Candida utililis* in Fermentation Broth," *Biotechol. Bioeng.*, *48*, 108–117 (1995).
11. C. S. Parnham and R. H. Davis, "Protein Recovery from Bacterial Cell Debris Using Crossflow Microfiltration with Backpulsing," *J. Membr. Sci.*, *118*, 259–268 (1996).
12. W. R. Bowen and N. J. Hall, "Properties of Microfiltration Membranes: Mechanisms of Flux Loss in the Recovery of an Enzyme," *Biotechnol. Bioeng.*, *46*, 28–35 (1994).
13. D. K. Kidby and D. J. Davidson, "A Convenient Ferricyanid Estimation of Reducing Sugars in the Nanomole Range," *Anal. Biochem.*, *55*, 321–325 (1973).
14. L. H. Ding, J. M. Laurent, and M. Y. Jaffrin, "Dynamic Filtration of Blood: A New Concept for Enhancing Plasma Filtration," *Int. J. Artif. Organs*, *14*, 365–370 (1991).
15. L. H. Ding, C. Charcosset, and M. Y. Jaffrin, "Albumin Recovery Enhancement in Membrane Plasma Fractionation Using Pulsatile Flow," *Ibid.*, *14*, 61–65 (1991).
16. J. Leveque, *Ann. Mines*, *12*, 365 (1928).
17. C. Harscoat, M. Y. Jaffrin, P. Paullier, B. Courtois, and J. Courtois, "Extraction of Microbial Polysaccharides from Fermentation Broths by Microfiltration on Ceramic Membranes," *J. Chem. Tech. Biotechnol.*, *74*, 571–579 (1999).
18. E. Drioli and L. Giorno, *Biocatalytic Membrane Reactors*: *Applications in Biotechnology and the Pharmaceutical Industry*, Taylor and Francis, London, UK, 1999.

Received by editor April 29, 1999
Revision received September 1999

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!