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Purification of Alkaline Phosphatase by Ultrafiltration in a Stirred Batch Cell

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Abstract

The purification of a crude grade of bovine intestinal alkaline phosphatase by ultrafiltration has been studied. The purification process utilized a small stirred ultrafiltration cell operated in a batch mode to purify the enzyme. An Amicon YM100 membrane proved effective by recovering high amounts of enzyme activity and allowing the crude enzyme to be purged of its lower molecular weight impurities, which were mostly albumin. Studies were done utilizing various buffers, operating pressures, and stirrer speeds. Extensive investigation of the latter two parameters showed that stirrer speed was the most significant factor in decreasing flux decline and improving separation efficiency.

INTRODUCTION

This paper focuses on the utilization of ultrafiltration (UF) membrane processes to purify enzyme alkaline phosphatase, specifically the bovine intestinal type. Enzymes, in general, are difficult to purify and usually require elaborate procedures with numerous process steps to obtain small quantities of high-grade material.

Ultrafiltration membrane processes are utilized in a variety of commercial applications. These include treating industrial effluents and process water; concentrating, purifying, and separating macromolecular solutions in the chemical, food, and drug industries; sterilizing, clarify-

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ing, and purifying biological solutions and beverages; producing ultrapure water; and pretreating seawater in reverse osmosis processes. Systems vary from small laboratory-scale models to 1,440,000 gal/d plants (*1*).

The biochemical area seems the most promising for growth of UF process applications. Specific examples of existing uses include: depyrogenating pharmaceutical process water; concentrating and purifying vaccines and blood fractions; concentrating gelatins, albumin, and egg solids; and recovering proteins and starches (*1*). UF processes have been used in the production of leukocyte interferon from white blood cells and fibroblast interferon from cell culture (*2*) and for the production of human insulin (*3*).

In UF, a solution is introduced on one side of a membrane barrier, and water, salts, and low molecular weight materials pass through the unit under an applied pressure. UF membranes are rated in terms of their molecular weight cut-off, with the low end being about 1000 (*4*). Therefore, they are able to separate proteins and other biochemicals according to molecular weight differences. UF membranes are characterized by thinness, mechanical strength, flexibility, low adsorption, and a flat surface texture. Many different membrane materials make wide temperature and pH range processing possible.

The major problem encountered in utilizing this separation process effectively is concentration polarization and subsequent fouling of the membrane (*5*). Membrane fouling is used to describe the reduction in product flux, in general, irregardless of cause. Concentration polarization normally causes deviations from ideal mass transfer, and this phenomena is quite complex. It decreases both selectivity for separation and product flux. While generalizations on operating UF systems can be drawn from past research on protein separation, each separation is unique due to the many parameters that exist between membrane, system design, and, most important, the components of the solution to be separated. Very little work has been done on the separation of proteins in a crude alkaline phosphatase mixture by UF.

Porter and Michaels (*6*) discussed the effects of several process parameters which generally influence system performance in the separation of organics: fluid shear rates, membrane area, concentration, viscosity, temperature, and pressure. Their studies concluded that high fluid shear rates utilizing a large membrane area in a thin-channel UF design minimized concentration polarization. Other researchers have investigated similar process phenomena on the separation of proteins such as bovine serum albumin (*7–11*). In addition to altering the operating parameters so that the gel layer is minimized, another

technique is to immobilize enzymes on the membrane surface to hydrolyze the gel layer. This has been found to be effective, and several models have been proposed (*12*). Electroultrafiltration has been proposed as a method of controlling the effects of concentration polarization by using an electric field to control the build-up of retained proteins at the membrane surface (*13*).

Several techniques have been previously investigated for the purification of alkaline phosphatase. One common procedure involves butanol extraction and acetone precipitation, followed by ion-exchange and affinity chromatography (*14*). Continuous paper curtain electrophoresis has also been studied (*15*). Parametric pumping and preparative-scale polyacrylamide gel electrophoresis (prep-PAGE) are promising alternatives to final purification steps (*16*). Membrane processes are one of the most attractive enzyme separation processes available due to their selectivity, gentle treatment of the enzyme, and the availability of various customed-manufactured membranes (*2*). This should prove to be an attractive alternative for alkaline phosphatase purification.

EXPERIMENTAL

The experimental UF system is shown in Fig. 1. Its maximum capacity for batch operation is 2000 cm^3. It includes a cooling system to regulate solution temperature and is pressurized by a nitrogen gas source. An agitator or stirring bar sits over the membrane surface to impart a velocity to the solution above the membrane surface. The stirrer speed can be varied from 0 to 550 rpm. The unit accepts 15.0 cm flat circular membranes, with a net effective area, upon assembly, of 165 cm^2. Amicon Diaflo membranes (Amicon Corporation, Danvers, Massachusetts) were used exclusively in the studies. The YM series membranes were chosen because of their nonspecific protein binding properties and outstanding resistance to biochemical solvents. Amicon recommends the YM series when maximum solute recovery is the desired function (*4*). YM100 membranes with a molecular weight cut-off of 100,000 were utilized because the alkaline phosphatase molecular weight is 140,000 (*17*) and the protein impurities are less than 100,000 MW. Bovine serum albumin of molecular weight 69,000 is the major impurity. The membranes were cleaned between runs with an enzymatic detergent and flushed several times with distilled water.

The three different buffers utilized in the experimental studies were acetate, Tris/HCl, and phosphate. The acetate buffer was used at a pH of 4.0 to 4.2. It was prepared by mixing a 0.05-*M* solution of acetic acid with a

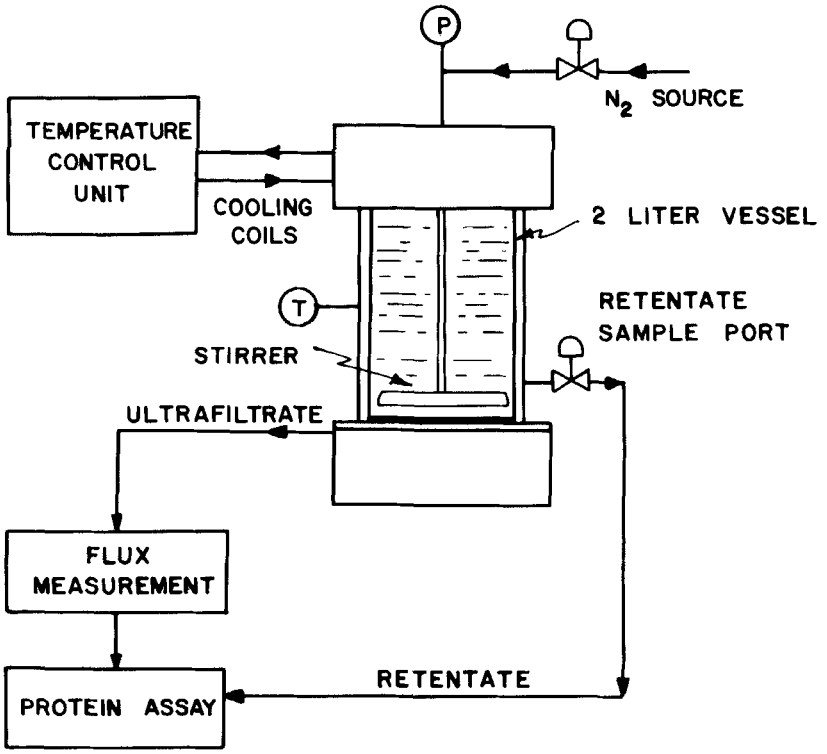

FIG. 1. Ultrafiltration system utilized for the alkaline phosphatase purification studies.

0.05-*M* solution of sodium acetate. The Tris/HCl buffer consisted of a 0.05-*M* solution of Tris (hydroxymethyl) aminomethane (THAM) and a 0.05-*M* solution of HCl mixed to pH's of 7.6 to 7.8. The phosphate buffer had a pH range of 6.8 to 7.0 and was prepared by mixing equal-molar solutions, 0.05 *M* of monobasic phosphate and dibasic phosphate, until the desired pH was obtained (*18*).

The albumin used to check the permeability of the YM100 membrane in the buffer studies was human serum albumin (Cooper Biomedical). Albumin concentration, and that for total protein in the alkaline phosphatase studies, was measured at 280 nm on a Bausch & Lomb spectrophotometer. The alkaline phosphatase employed in these studies was crude bovine intestinal alkaline phosphatase (Sigma Chemical Co.) with an activity of 1.7 units/mg. According to the Worthington assay procedure (*19*), one unit of enzyme activity will hydrolyze 1 μmol of *p*-

nitrophenyl phosphate per minute at 30°C at a pH of 10.15. Analysis of this reaction was monitored at 405 nm on a spectrophotometer. A breakdown of the impurities present in the crude grade of alkaline phosphatase was not attempted. The majority of the protein impurities are believed to be albumin (*14*).

Flux measurements of both ultrafiltrate or permeate and total protein were made by continuously monitoring the output of the UF cell. Unless otherwise specified, flux refers to ultrafiltrate flux:

$$J = \frac{\text{ultrafiltrate flow}}{\text{membrane surface area}} \tag{1}$$

Flux of protein through the membrane is obtained by multiplying the ultrafiltrate flux by the protein concentration in the ultrafiltrate. Samples of initial feed, final retentate, and total ultrafiltrate were taken for total protein and enzyme activity assays. Since the system was operated in a batch concentration mode, a ratio of initial to final volume, V_0/V_f, was measured for each run.

Separation effectiveness is denoted by several methods. Purification factor, P.F., is a measure of the enzyme activity per mg of retentate divided by the activity per mg of feed:

$$\text{P.F.} = \frac{(a_f/C_f)}{(a_0/C_0)} \tag{2}$$

where enzyme activity in the initial feed and final retentate are denoted as a_0 and a_f, respectively. The total protein concentration in the initial feed and final retentate are represented by C_0 and C_f, respectively. The maximum possible P.F. for a given run is equal to the volumetric concentration factor for that run, V_0/V_f. Enzyme activity yield, Y, is the percent enzyme activity remaining in the final retentate with respect to the initial feed:

$$Y = \frac{(a_f)(V_f)}{(a_0)(V_0)} \times 100\% \tag{3}$$

Ideally, the yield on enzyme activity should be as close to 100% as possible. Rejection of both enzyme activity and total protein are based on overall system separation (*20*). The rejection of enzyme activity, R_a, is defined as

$$R_a = \frac{\ln (a_f/a_0)}{\ln (V_0/V_f)} \tag{4}$$

and the rejection of total protein, R_p, is

$$R_p = \frac{\ln (C_f/C_0)}{\ln (V_0/V_f)} \tag{5}$$

RESULTS AND DISCUSSION

Initial testing with a series of YM100 membranes yielded pure water fluxes of 0.90 to 1.3 $\times$ 10^{-2}cm^3/cm^2-s at 10°C under an applied pressure of 10 psi. Pure water fluxes at pressures from 5 to 40 psi were also examined. Utilizing a representative YM100 membrane, water flux increased at a rate of 8.23 $\times$ 10^{-4} (cm^3/cm^2-s)/psi.

Buffer solutions containing the protein, albumin, were utilized in the next group of studies. These studies were done to see if any buffer and albumin membrane interactions were present and if any albumin was retained in the feed solution. This is important, because in the alkaline phosphatase runs it is desired to pass the protein impurities, which are mostly albumin, through the membrane and eliminate any gel layer accumulation or any other mass transfer interactions that result from the albumin or the buffer system used. The buffers tested were Tris/HCl, acetate, and phosphate at concentrations of 0.05 *M* and albumin concentrations of 0.2 g/L.

Studies on the Tris/HCl system were characterized by a reduction in flux rates. The reduction in ultrafiltrate flux was rather significant at all three operating pressures with a stirrer speed of 180 rpm and a temperature of 10°C. At 20 psi the ultrafiltrate flux decreased by 43.7%, whereas the albumin flux rate decreased by 44.5% in 5 min of operation. A gel layer was noticed forming on the membrane surface. At 5 and 10 psi the ultrafiltrate and albumin flux losses, respectively, were 8.6 and 9.8%, and 11.1 and 11.5% after 10 min of operation. Increasing the stirrer speed decreased the reduction in flux loss.

The acetate buffer was analyzed with results similar to those found for the Tris/HCl case. When the pressure was increased from 10 to 20 psi with a stirrer speed of 180 rpm and a solution temperature of 10°C, the loss in ultrafiltrate and albumin flux increased from 26.8 and 24.4% to 66.7 and 69.9%, respectively. This large reduction in flux rates after 10 min of operation, even at a low pressure of 10 psi, was the determining factor in deciding not to use this buffer in later studies.

The phosphate buffer system was the final one analyzed in this preliminary study. The ultrafiltrate flux is shown in Fig. 2 and the albumin flux in Fig. 3. The major finding in this buffer evaluation was that only a slight reduction in both ultrafiltrate and albumin fluxes was evident with respect to time. At 10 psi, 10°C, and 180 rpm stirrer speed, a 13.6 and 15.8% drop in ultrafiltrate and albumin flux was noted after 10 minutes (Figs. 2 and 3). Even at a higher pressure of 20 psi, flux losses did not increase. The effect of increased pressure, which tended to enhance the formation of the gel layer on the membrane surface in the Tris/HCl and acetate studies, was not evident.

The effect of stirrer speed was examined for the phosphate buffer system (Figs. 2 and 3). The speed was varied from 0 to 310 rpm at 10 psi and 10°C. At 0 rpm the ultrafiltrate and albumin flux losses were substantial, 24.0 and 26.5%, respectively. At 180 rpm the losses in ultrafiltrate and albumin fluxes were 13.6 and 15.8%, respectively, and at 310 rpm the losses were slightly less. Ultrafiltrate flux declined 11.4% and the albumin flux declined by 9.1% at 310 rpm. The rejection of total protein in all runs with the phosphate buffer was less than 2%. The

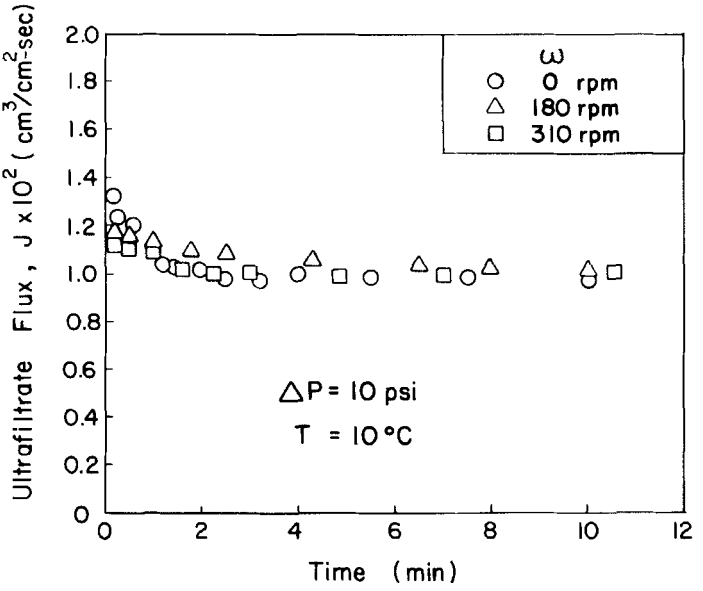

FIG. 2. Ultrafiltrate flux vs time for albumin in a phosphate buffer at various stirrer speeds.

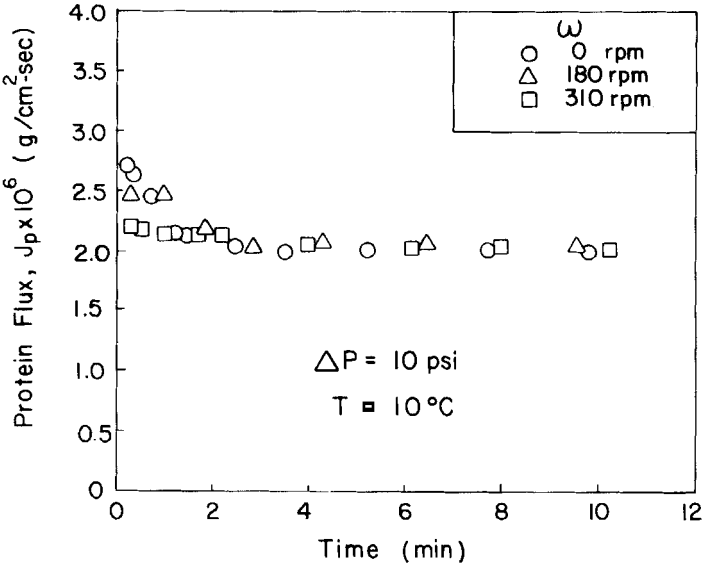

FIG. 3. Protein flux vs time for albumin in a phosphate buffer at various stirrer speeds.

membrane successfully passed almost all of the albumin under the optimal processing conditions.

The phosphate buffer was chosen as the one to utilize with the alkaline phosphatase because it displayed several beneficial characteristics. These were: 1) small reductions in ultrafiltrate flux rates over the course of a run, 2) small reductions in albumin flux rates over the course of a run, and 3) the rejection of total protein was very close to zero, so most of the albumin passed through the system.

The alkaline phosphatase studies focused on batch operation for purifying alkaline phosphatase in a phosphate buffer. Process parameter studies were done again to examine the problems associated with effective separation and flux loss.

Studies were done at pressures of 5, 10, and 20 psi at a temperature of 10°C. Within each pressure study, an examination of stirrer speeds of 0, 180, and 310 rpm was done. The initial concentration of alkaline phosphatase for each study was 0.2 g/L, and this was varied to 0.3 g/L in several studies to examine the effects of increased enzyme concentration. All studies started out with between 1500 to 2000 cm^3 of solution with final volumetric concentrations (V_0/V_f) of 2.0 to 2.67. In most cases a

TABLE 1
Separation and Flux Data for Alkaline Phosphatase Runs

ΔP (psi)	ω (rpm)	V_0/V_f	P.F.	Y (%)	R_a	R_p	$J_f \times 10^3$ (cm^3/cm^2-s)
5	0	2.00	1.09	54	0.10	0	0.9
	180	2.67	1.47	75	0.71	0.31	1.3
	310	2.00	1.50	94	0.90	0.32	2.1
10	0	2.00	1.32	71	0.50	0.08	0.9
	180	2.67	1.65	80	0.77	0.27	1.2
	310	2.00	0.96	67	0.41	0.48	2.0
20	0	2.67	1.08	41	0.09	0.01	0.8
	180	2.67	1.54	94	0.93	0.48	1.2
	310	2.00	1.44	99	0.98	0.46	2.0

limiting flux was observed before the run was over. Results of enzyme and protein separation data and final flux values for the process parameters studied appear in Table 1.

At 5 psi the effect of stirrer speed on final ultrafiltrate flux values was evident (Fig. 4). At all stirrer speeds, initial flux values were 6.5×10^{-3} cm^3/cm^2-s. At 0 rpm the final flux was 9.0×10^{-4} cm^3/cm^2-s. This represented an 86% drop in the product flux by the end of the run, a 2.0 volumetric concentration factor. Ultrafiltrate flux declined rapidly; after 10 min of operation it had dropped 60%. This shows that the gel layer build-up was very rapid. The purification factor in this study was 1.09. Because of the extensive development of the gel layer, the separation effectiveness of the membrane was hindered. Rejection of the enzyme was low, as was that of the proteins. Loss of enzyme in the final concentrate is the result of the enzymes being compressed into the gel layer. This was analytically verified.

As the stirrer speed was increased, so were the final flux values. At 180 rpm the final flux recorded was 1.3×10^{-3} cm^3/cm^2-s. A higher purification factor, 1.47, was also noted along with a greater alkaline phosphatase rejection, 0.71. At a stirrer speed of 310 rpm the gel layer was further minimized. The ultrafiltrate flux after a 50% volumetric concentration was 2.1×10^{-3} cm^3/cm^2-s, 133% higher than with no stirring action. The purification factor had increased to 1.5 out of a possible 2.0, and the system had an enzyme rejection of 0.90 with a total protein rejection of 0.32. Enzyme activity yield was 94%. This was the most promising run of

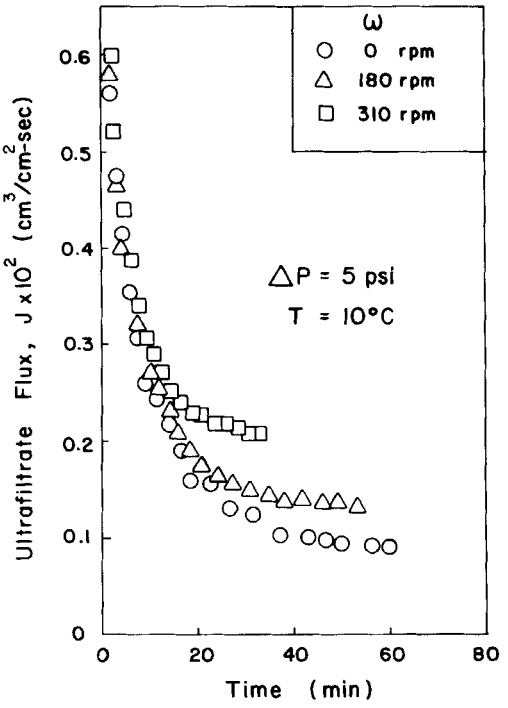

FIG. 4. Ultrafiltrate flux vs time for alkaline phosphatase purification at various stirrer speeds at 5 psi.

the 5-psi studies. Although the flux decline in the 310 rpm study is still high, the final flux value is significantly better than with no agitation, and the membrane's separation effectiveness has been greatly enhanced due to limiting the gel layer formation. Enzyme purification factors should be increased by running higher volumetric concentration ratios in a dialysis mode; these investigations are in progress.

Studies varying the stirrer speed at 10 psi were done in the next series of runs (Fig. 5). While an increase in the initial ultrafiltrate flux of all runs was noted as compared with the 5-psi studies, the final values were similar at 5 and 10 psi. Increasing stirrer speed enhanced flux profiles and generally increased separation. Initial flux values ranged from 0.95 to 1.05×10^{-2} cm^3/cm^2-s and dropped so rapidly that initial values at time zero were taken as those after 2 min of operation. After approximately 60 min at 0 rpm, flux dropped 90% with a final value of 9.0×10^{-4} cm^3/cm^2-s. This was the same value observed in the 5-psi study.

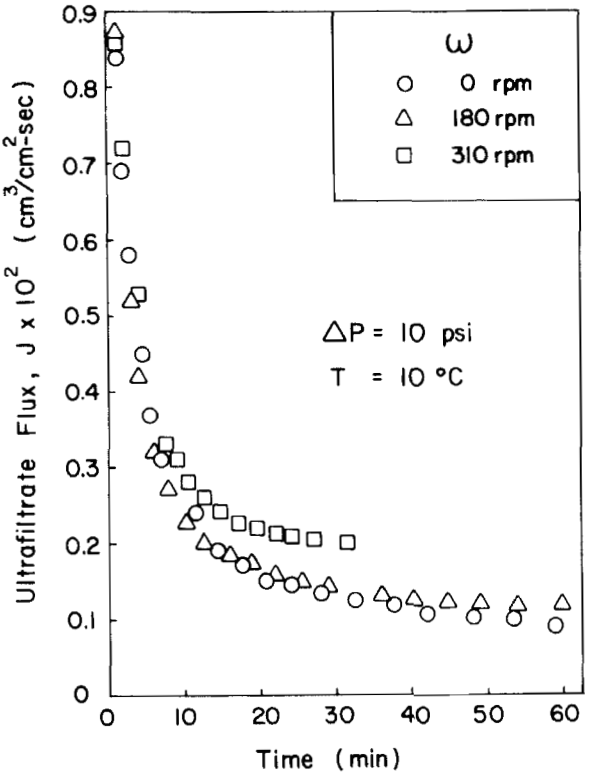

FIG. 5. Ultrafiltrate flux vs time for alkaline phosphatase purification at various stirrer speeds at 10 psi.

When stirrer speed was increased to 180 rpm, the final ultrafiltrate flux value was 1.2×10^{-3} cm^3/cm^2-s, and at 310 rpm, final flux was 2.0×10^{-3} cm^3/cm^2-s. Separation effectiveness increased in the 180-rpm study. The purification factor increased from 1.32 to 1.65. Rejection of the enzyme increased from 0.50 to 0.77 while protein rejection increased from almost zero to 0.27. Separation results were lower than expected in the 310-rpm study, and these values are not representative of the trends observed at higher and lower pressures (Table 1).

A study of the effect of initial alkaline phosphatase concentration on ultrafiltrate flux and separation was also examined in a study at 10 psi. When the initial concentration was varied within a range of 0.2 to 0.3 g/L, there were no significant changes observed.

At 20 psi a promising improvement in final ultrafiltrate flux at higher

stirrer speeds was again demonstrated over those with no agitation. Initial flux values at 20 psi were, as expected, higher than the 10-psi runs, with values ranging from 1.58 to 1.65 × 10^{-2} cm^3/cm^2-s (Fig. 6). Due to the sharp drop in flux, it was difficult to get an accurate initial flux value. At 0 rpm the limiting flux approached 8.0 × 10^{-4} cm^3/cm^2-s. This was close to the same value reported in the studies at 5 and 10 psi. It was thought that the higher pressure would have compressed the gel layer further, driving the final flux values lower, but it seems that the higher pressure only influences the initial flux. After 10 min of operation at 0 rpm, the flux had already dropped by 87%; final flux loss was 95%. The purification factor was 1.08, similar to that in the 5-psi study. Enzyme and total protein rejection were also similar to those observed in the 5-psi, 0-rpm run.

When stirrer speed was increased to 180 rpm, final flux values increased to 1.2 × 10^{-3} cm^3/cm^2-s. The purification factor increased and the rejection coefficient for the enzyme increased to 0.93. At the same time, the rejection of total protein was 0.48. The best run for flux of the 20-psi studies was at 310 rpm. After a 50% volumetric concentration, the final flux was 2.0 × 10^{-3} cm^3/cm^2-s, almost the same as in the 5-psi study. The enzyme rejection was 0.98 while the total protein rejection was 0.46. Total

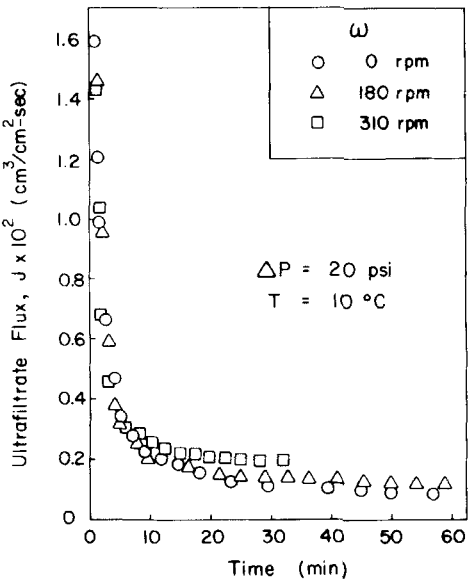

FIG. 6. Ultrafiltrate flux vs time for alkaline phosphatase purification at various stirrer speeds at 20 psi.

enzyme activity yield was 98%. The overall purification factor for this run was 1.44 out of a possible 2.0.

These results suggest that the gel layer resistance forms and builds up rapidly, and that the same limiting ultrafiltrate flux is reached in almost all cases of moderate to no stirrer action regardless of pressure (Fig. 7). Along with this, the relative initial flux values do not seem to predict final flux. In the studies at a stirrer speed of 310 rpm and 10°C, the ultrafiltrate flux at operating pressures of 5, 10, and 20 psi approached the same value at the conclusion of both runs. Even though the initially recorded values for the 20- and 5-psi studies were 1.44×10^{-2} and 0.60×10^{-2} cm^3/cm^2-s, respectively, final values were 0.21×10^{-2} and 0.20×10^{-2} cm^3/cm^2-s, respectively. This phenomenon was also evident for the studies at 0 and 180 rpm, although a lower final flux was approached.

A comparison of ultrafiltrate fluxes for the alkaline phosphatase purification studies and the albumin studies shows the vast difference in the final flux values (Fig. 8). Since the albumin in phosphate buffer readily passes through the YM100 membrane both as a single solute and in the protein mixture, the product fluxes in these studies are evidently only slightly affected by the presence of albumin under optimal

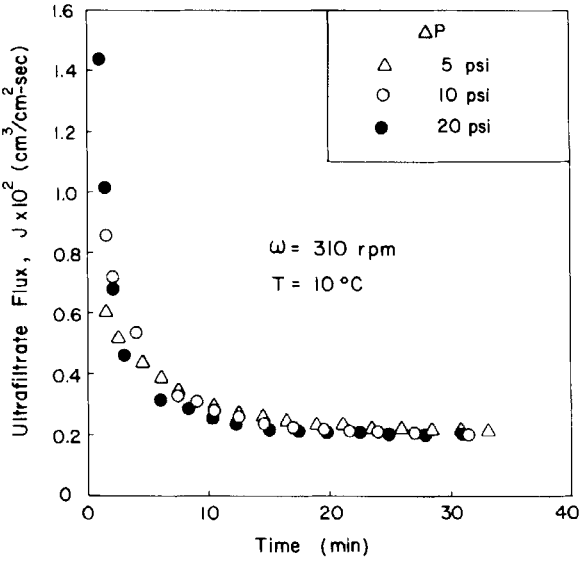

FIG. 7. Ultrafiltrate flux vs time for alkaline phosphatase purification at various pressures at a stirrer speed of 310 rpm.

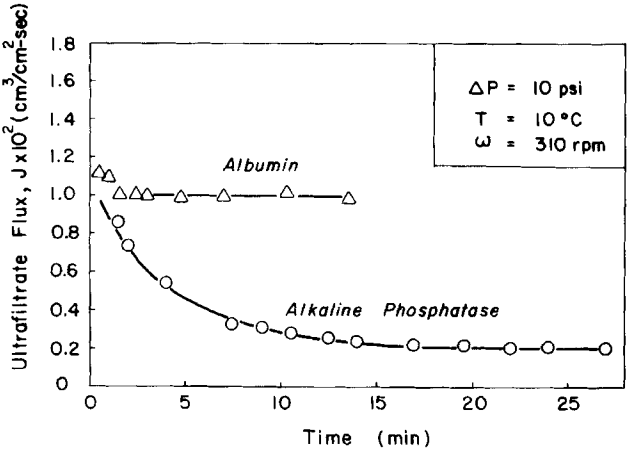

FIG. 8. Ultrafiltrate flux for pure albumin and the crude alkaline phosphatase vs time under the same processing conditions.

processing conditions. The predominate factor yielding the large flux drop is the mass-transfer inhibiting effects associated with retaining the alkaline phosphatase in the retentate during ultrafiltration.

CONCLUSIONS

Purification of the enzyme bovine intestinal alkaline phosphatase by UF seems to be a promising method. While the problems of flux and separation efficiency degradation by concentration polarization and fouling were observed in some studies, increasing the flow of the solution past the membrane lessened these problems. The most critical process parameter in a stirred cell system appears to be the agitator speed. A YM100 membrane proved effective in the separation procedure by retaining high amounts of alkaline phosphatase activity and allowing the system to be purged of the lower molecular weight protein impurities. Optimal results for both final flux and separation of a 0.2-g/L alkaline phosphatase solution in a 0.05-*M* phosphate buffer were obtained at an agitator speed of 310 rpm. Utilizing process conditions of 10°C and 5 psi, the ultrafiltrate flux at a 2.0 volumetric concentration was 2.1×10^{-3} cm^3/cm^2-s. Enzyme activity yield was 94% and the purification factor was 75% of the maximum possible (V_0/V_f). Similar performances were obtained at higher operating pressures.

SYMBOLS

a	enzyme activity (units/mg)
C	protein concentration (g/cm^3)
J	ultrafiltrate flux (cm^3/cm^2-s)
J_f	final ultrafiltrate flux (cm^3/cm^2-s)
J_p	protein flux (g/cm^2-s)
P.F.	purification factor
ΔP	applied pressure gradient (psi)
R_a	enzyme activity rejection
R_p	protein rejection
V	volume (cm^3)
Y	enzyme activity yield (%)
ω	stirrer/agitator speed (rpm)

Subscripts

f	final retentate
0	initial feed

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REFERENCES

1. L. E. Applegate, *Chem. Eng., 91,* 64 (1984).
2. T. J. O'Sullivan, A. C. Epstein, S. R. Korchin, and N. C. Beaton, *Chem. Eng. Prog., 80,* 68 (1984).
3. A. S. Michaels, *Ibid., 80,* 19 (1984).
4. Amicon Products for Separations Technology, Amicon Corp., Danvers, Massachusetts, 1983.
5. R. P. Ouellette, *Biotechnology Handbook*, Technomics, 1985.
6. M. C. Porter and A. S. Michaels, *Chemtech., 1,* 56 (1971).
7. A. A. Kozinski and E. N. Lightfoot, *AIChE J., 18,* 1030 (1972).
8. W. F. Leung and R. F. Probstein, *Ind. Eng. Chem., Fundam., 18,* 274 (1979).
9. J. J. S. Shen and R. F. Probstein, *Ibid., 16,* 459 (1977).
10. R. F. Probstein, W. F. Leung, and Y. Alliance, *J. Phys. Chem., 83,* 1228 (1979).

11. L. A. Errede and P. D. Mortinucci, *Ind. Eng. Chem., Prod. Res. Dev., 19,* 573 (1980).
12. J. A. Howell and O. Velicangil, in *Ultrafiltration Membranes and Applications,* Plenum, New York, 1980, pp. 217–229.
13. J. M. Radovich, N. S. Mason, and R. E. Sparks, *Sep. Sci. Technol., 15,* 1491 (1980).
14. L. E. Seargeant and R. A. Stinson, *J. Chromatogr., 173,* 101 (1979).
15. N. K. Ghosh and W. H. Fishman, *Biochem. J., 108,* 779 (1968).
16. J. F. Chao, V. P. Rollan, H. C. Hollein, and C. R. Huang, *Sep. Sci. Technol., 18,* 999 (1983).
17. P. G. Righetti and T. Caravaggio, *J. Chromatogr., 127,* 1 (1976).
18. S. P. Colowick and N. O. Kaplan, *Methods in Enzymology,* Academic, New York, 1955.
19. Worthington Biochemical Corp., *Worthington Enzyme Manual,* Freehold, New Jersey, 1977.
20. W. F. Blatt, in *Membrane Separation Processes,* Elsevier, New York, 1976, pp. 81–120.

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