ULTRAFILTRATION FIBERS LIKE BIOREACTORS

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Abstract

A polysulfone ultrafiltration membrane with pectinase physically immobilized on it by a dynamic formation method was used to examine the potential of these reactive membranes in applications involving solutions containing pectin. The effect of various operational parameters such as: pH of enzyme and pectin solutions, NaCl, retentate flow rate, and enzyme (C_e) and substrate (C_p) concentrations on the production of reducing compounds expressed as galacturonic acid (C_a), was investigated. It was found that the maximum C_a values were obtained when: (i) enzyme solution to immobilize, without NaCl, had pH values between 4.2 and 4.6 and enzyme concentration from 5.0 to 7.5 mg/mL; (ii) pectin solution had initial pH values between 4.2 and 5.0 and a concentration of 3 mg/mL; and (iii) retentate flow rate was 0.025 L/min.

1. Introduction

Pectic substances, a group of complex acidic polysaccharides, are the major component of the middle lamella and cell wall of fruits and vegetables. Pectins are basically linear polymers made by D-galacturonic acid units coupled by glicosidic 1,4-α links (Sakai *et al*., 1993). Clarified apple juice concentrate ranks second in fruit juice consumption in the world. Problems in clarification of apple juice are caused mainly by the presence of pectic substances, which suspend toward insoluble (pulp) particles. Commercial pectin enzymes, or "pectinases", are used in apple juice manufacturing to depectinize pressed juices in order to remove turbidity and prevent cloud-forming. The available commercial preparations generally contain a mixture of pectinesterase, polygalacturonase (PG) and pectin lyase (PL). Complete pectin breakdown in apple juices can only be ensured if all the three types of these enzymes are present in the correct proportion. (Belitz and Grosch, 1997). While PG hydrolyzes pectin 1,4-α glicosidic linkage, PL generates (C_4-C_5) products by non-hydrolytic de-polymerization (Ceci and Lozano, 1998).

At the present fruit juice industry employs important amounts of pectin enzymes, both as powder or liquid state. On the other hand, enzyme immobilization by physical adsorption is simple and well established technique (Szaniawski, 1996; Gekas, 1986). However, immobilized pectinase enzymes are not currently available commercially. In view of the high molecular weight and viscosity of pectin, the use of immobilized pectinase in most fruit processing applications may be rather limited. While different immobilization techniques have been proved, neither reliable kinetics models nor adequate immobilized pectinase reactors readily applicable to the juice industry are available (Govind *et al*., 1989; Alkorta *et al*., 1995a).

The term "membrane reactor" has been used to describe several catalytic reactor configurations. The role of membranes may be only to prevent catalyst leakage or to avoid contact between catalyst and solid particles. However, when catalyst is an enzyme, membrane are commonly used as a support (Afani *et al*., 1979; Gekas, 1986; Gille and Staude, 1994; Giorno *et al*., 1998).

Major problem with enzyme immobilization deals with the enzyme activity. Under standard operation conditions, immobilized enzymes show lower activity than in the free state. Enzyme inactivation after immobilization was attributable to both configurational restrictions and mass transfer limitations (Alkorta *et al*., 1996). Reactor configuration and operational conditions should be carefully considered during commercial application of immobilized enzymes.

The objective of the present work was to study the enzymatic reaction of pectinase immobilized on a hollow fiber ultrafiltration membrane (HFUF), with pectin solution as substrate. Moreover, the effect of: (1) NaCl during physical immobilization of the enzyme; (2) pH of buffers and solutions used for immobilization and washing (pH_i); (3) retentate flow (Q); (4) pH of the pectin solution (pH_p); and (5) the concentrations of both enzyme (C_e) and pectin solution (C_p) on ultrafiltration yield and enzyme activity, were considered.

2. Materials and Methods

A lab HFUF unit with a single hollow fiber (cutoff 50,000) consisting of a peristaltic pump,

manometers, reservoir, flowmeter, thermostatic bath, operated in batch mode was used. Experimental runs were performed by total permeate and retentate recycling.

Reducing compounds, colorimetrically detectable in the bulk of the reservoir solution with the Nelson-Somogyi method (Somogyi, 1952) and expressed as galacturonic acid content (C_a), were considered as final products. Initial parameters were based on the C_a production with free enzyme in solution: 5 mg/mL of enzyme in acetate buffer at a pH= 4.6 with 0.05 M NaCl and 2 mg/mL pectin in water. The pH was finally adjusted to 4.6 with NaOH 0.5 M (Carrín *et al*., 1999*).*

Before each experimental run the hollow fibers were washed in reverse flux with NaOH (0.1N) and distilled water. Finally, fibers were washed again with distilled water but in normal flux. After the cleaning process, water permeability of fibers was determined at selected retentate flows.

Experiences were performed at 50°C.

2.1 Immobilization steps

Enzyme was dissolved in the appropriate buffer under stirring. Physical immobilization was performed by ultrafiltering the enzyme solution during 1 h at a retentate flow Q= 0.045 L/min and a transmembrane pressure difference ΔPTM= 9.8 kPa. Then, fiber was washed at low Q (0.01 L/min) during 5 min to avoid enzyme lost by dragging.

2.2 Effect of NaCl

NaCl (0.05M) was added to the buffer (pH_i= 4.6) in which enzyme (C_e= 5.0 mg/mL) was dissolved and to the washing solution. Operational conditions were C_p= 2.0 mg/mL and Q= 0.045 L/min.

2.3 Retentate in-flow (Q)

Effect of retentate flow in the range 0.025 y 0.075 L/min on permeate flux, was analyzed. Ultrafiltration conditions were C_e= 5.0 mg/mL, pH_i= 4.6 (0 M NaCl) and C_p= 2.0 mg/mL with pH_p= 4.6.

2.4 Immobilization pH (buffer pH)

Immobilization pH_i was modified in the range 3.8 to 5.0. Ultrafiltration conditions were Q= 0.045 L/min, C_e= 5.0 mg/mL, (0 M NaCl) and C_p= 2.0 mg/mL with pH_p= 4.6.

2.5 Pectin solution pH_p

Pectin solutions (C_p= 2 mg/mL) with pH in the range 3.88 to 5.45 were ultrafiltred in HFUF membranes with immobilized pectinase. Immobilization conditions were C_e= 5.0 mg/mL (0 M NaCl) and pH_i= 4.6. Retentate flow was fixed to 0.045 L/min.

2.6 Enzyme concentration (C_e)

The influence of enzyme solutions in the range C_e= 1.0 to 10.0 mg/mL (pH_i= 4.6, 0 M NaCl) on the immobilization and ultrafiltration of pectin solution was investigated. Ultrafiltration conditions were Q= 0.045 L/min and C_p= 2.0 mg/mL with pH_p= 4.6.

2.7 Pectin concentration (C_p)

After immobilization of pectinase (C_e= 5.0 mg/mL, pH_i= 4.6, 0 M NaCl) on the hollow fiber membrane, pectin solutions with different concentration (C_p= 1.0 to 4.0 mg/mL) were ultrafiltred at a retentate flow of 0.045 L/min.

3. Results and Discussion

Study of the ultrafiltration of pectin solutions through an enzyme-HFUF membrane system was complicated by the complex mass transfer mechanism occurring at a microscopic level. This phenomenon includes mass transfer by diffusion and convection, membrane fouling, concentration-polarization of gel on the membrane surface, and enzymatic reactions. However, the whole system can be macroscopically considered as a “black box” operating as a batch reactor. From that point of view, variation of solution concentration in the reservoir gives useful information on the immobilized enzyme-HFUF membrane system.

3.1 *Influence of NaCl*

Table 1 lists the variation of reaction product concentration (C_a) as influenced by NaCl. It can be observed that the influence of NaCl on the enzymatic reaction is negligible. This result is in disagreement with those obtained during pectin hydrolysis with soluble enzyme (Carrín *et al*., 1999). Difference was attributable to interactions between enzyme-membrane and enzyme-substrate. Snir *et al*. (1995) studied the influence of NaCl on the enzyme permeability during UF. Results indicated that enzyme retention was practically total in the absence of the salt.

Table 1. Galacturonic acid formation as a function of UF time, with and without NaCl.

t [min]	C_a [μmole/mL]	
	0 M NaCl	0.05 M NaCl
60	1.1866	1.0782
120	1.6883	1.5925

3.2 *Influence of Q (retentate flow).*

Fig.1 shows product concentration decreases when Q increases. This behavior was attributable to a lower drag force at low retentate flow, increasing

both the rate of formation of the gel layer and the residence time of pectin molecules on the immobilized pectinase (Alkorta *et al*., 1995b). It was also attributed to the curve dragging of enzymes, due to the week forces involved in immobilization (Sheu *et al*., 1987). However, this assumption should be confirmed by an enzyme inactivation, not observed in this work.

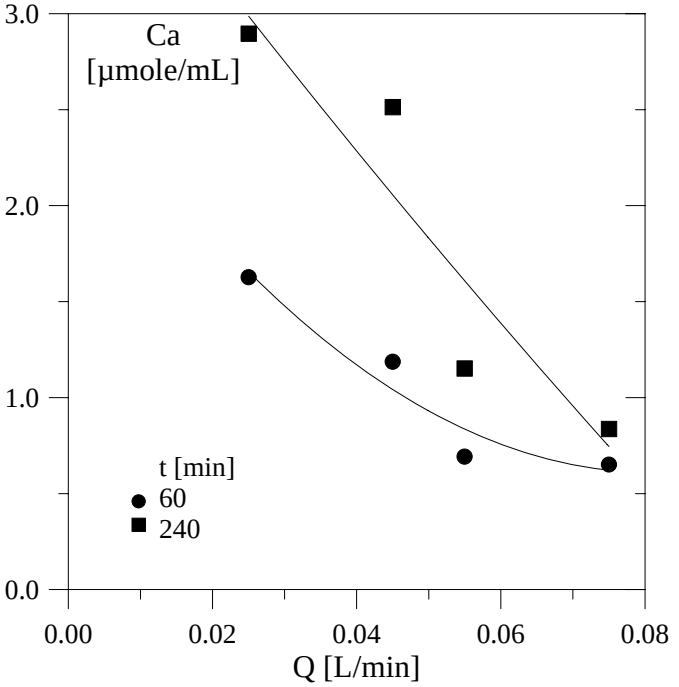

Figure 1. Effect of Q on C_a at different times of ultrafiltration.

3.3 *Effect of pH_i on immobilization-ultrafiltration*

As Fig. 2 shows, there is an optimal pH_i range (4.2-4.6) for the immobilization of pectinase on HFUF membrane. It also was observed that the optimal pH range was constant during the whole reaction period. Snir *et al*. (1995) reported pH_i is critical in order to define the enzyme retention on a membrane.

3.4 *Influence of the pectin solution pH_p on the enzymatic reaction*.

It was found that the behavior of the immobilized pectinase changed with the substrate pH_p. An optimal range from 4.2 to 5.0 also was defined in this case (Fig.3). pH_p sensibility increased with time of reaction, resulting critical after 4 h of pectin hydrolysis

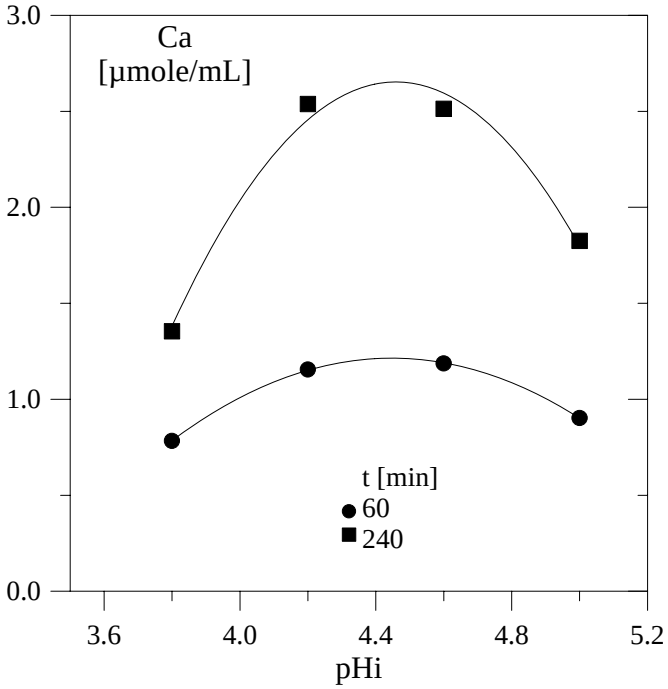

Figure 2. C_a - immobilization pH_i curve.

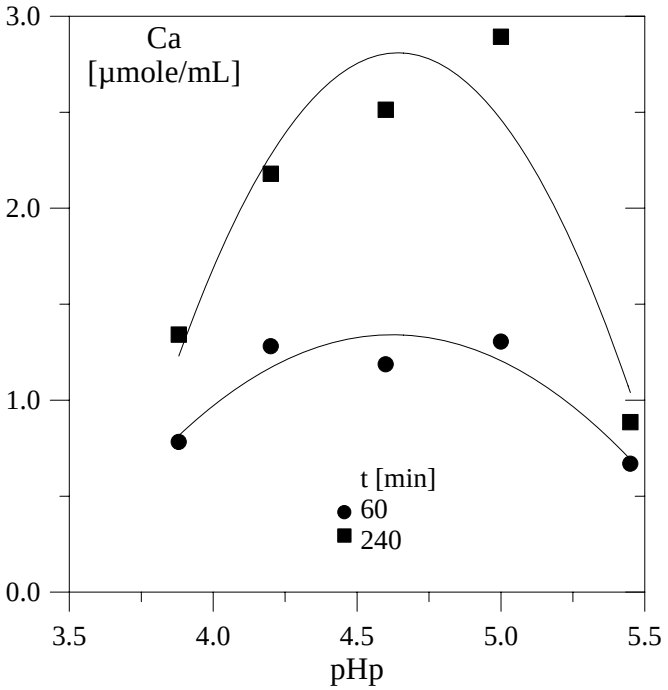

Figure 3. C_a – initial ultrafiltration pH curve.

3.5 *Influence of enzyme concentration (C_e)*

In a typical catalyzed reaction, an increase on catalyst implies an increase in reaction products, until a saturation point is reached. Beyond this point, rate of product formation remains practically constant, independently the amount of catalyst used. However, in the case under study, an optimal pectinase concentration on the solution ultrafiltred to immobilize the enzyme on the hollow fiber was found (Fig. 4). It was assumed that enzymes in excess only inactivate each other, occupying the

active sites of previous enzyme layers (Alkorta *et al*., 1996; Hultin, 1974).

3.6 *Effect of pectin concentration (C_p)*

Reaction product concentration, like C_e, also showed a maximum when pectin concentration was increased in the range studied (Fig. 5). Maximum value and behavior depend on time of ultrafiltration. While after 1 h production of galacturonic acid practically reaches a plateau for pectin concentration > 3 mg/mL, after 4 h of ultrafiltration a very well defined absolute maximum was observed. This behavior was attributed to the compaction of the pectin layer on the pectinase layer, modifying the enzymatic reactions sequence. It was also proposed for similar situations that substrate (pectin) when in excess, could show an inhibitory effect on the enzyme (Alkorta *et al*., 1995b).

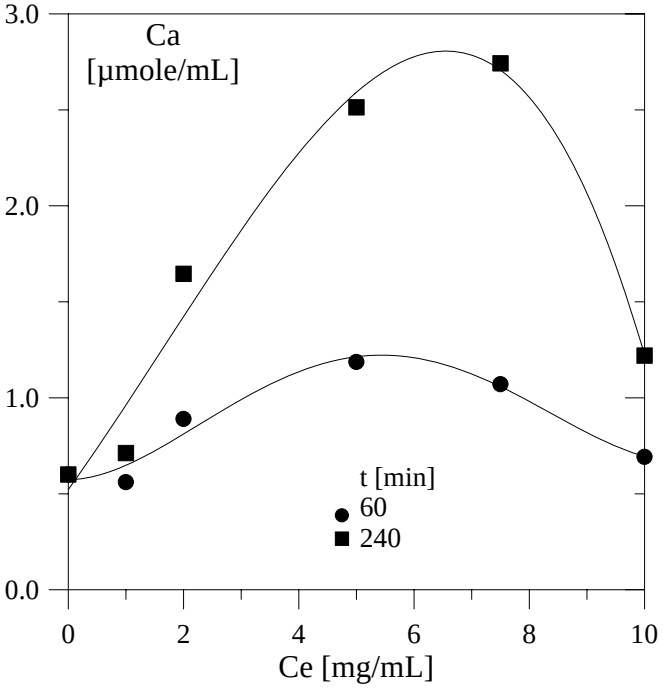

Figure 4. Influence of enzyme concentration on C_a.

3.7 Kinetics study.

Fig. 6 shows a Lineweaver-Burk plot comparing pectin hydrolysis by applying both immobilized on HFUF membrane, and free enzyme methods.

It is well known that slope in this type of plot increase due to diffusional effects. The extremely high slope (Michaelis-Menten constant) observed when immobilized pectinase was employed, is characteristic of a process with severe diffusion limitation (Lee and Tsao, 1974).

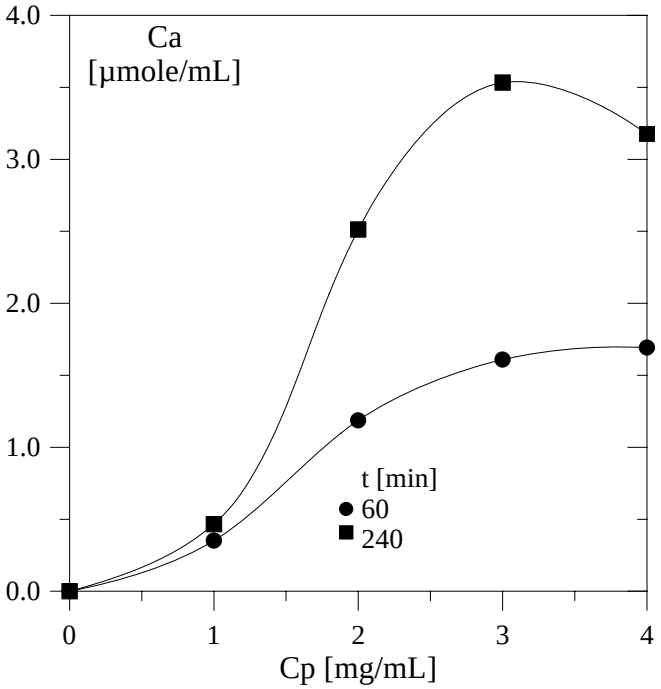

Figure 5. Influence of pectin concentration on C_a.

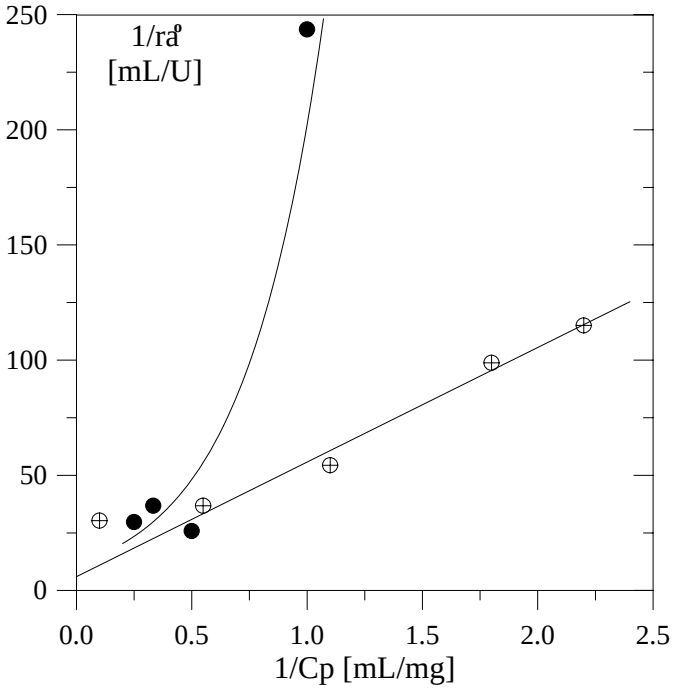

Figure 6. Lineweaver-Burk plot to (•, —) immobilized enzyme (C_e=5 mg/mL) and (⊕, ---) free enzyme (C_e=0.091 mg/mL). (r_a^o: initial reaction rate, U=µmole/min)

4. Conclusions

As a conclusion, enzymatic degradation of pectin using commercial pectinase immobilized on a hollow fiber ultrafiltration membrane was affected in different way by the pH of buffers and solutions used for immobilization and washing, the retentate flow, the pH of the pectin solution, and the concentrations of both enzyme (C_e) and pectin solution (C_p). Contrarily to that found in the case of

soluble (free) pectinase hydrolysis of pectin, the adding of NaCl during immobilization did not affect the enzymatic reaction. It was also found that the complex immobilized pectinase-HFUF membrane system was subject to severe diffusional limitations.

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