Ultrafiltration of Endo-Pectinase Solution with a Static Mixer Placed in a Ceramic Membrane

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1. Introduction

Industrially important extracellular hydrolytic enzymes of microbial origin are usually manufactured by solid-state or submerged cultivations, both of them having its own strengths and weaknesses [1]. The main characteristic of submerged productions is that the enzymes are obtained in a much diluted form and therefore, choice of the operations in downstream processing have to allow enzymes concentration and purification that will be economically efficient. Moreover, the chosen technique has also to take care of preservation of enzymes fragile structure that is associated to their biological function.

Application of an ultrafiltration as the one of the downstream processing techniques allowing both concentration and primary purification of enzymes, but there is also a possibility of extensive loss of an enzyme activity due to high shear forces generated in such a system [2,3]. Ultrafiltration systems with spiral-wound membranes are convenient for a large-scale concentration and/or purification of industrial enzymes due to the high area to plant volume and small hold up volume. In spite of such good characteristics, being polymeric in nature, the spiral membranes are strongly influenced by the pH and ionic strength. Because ultrafiltration processes for enzyme recovery in biotechnology are, among various parameters, also influenced by characteristics of the sample such as pH, initial concentration and composition, etc. [4], choice of the type of the membrane must take those requirements into consideration, as well.

For the bioprocess design and economy point of view, it is always desirable to investigate the stability of commercially important enzymes, such as pectinases, under different conditions of downstream processing and application. The objective of this work is to investigate the use of a ceramic membrane system for the ultrafiltration of endo-pectinase solution. Cross-flow ultrafiltration is not among the techniques which are in use for concentration and purification of enzyme solutions due to their possible sensitivity to shear stress, created in such a system. In order to improve the ultrafiltration performance, the Kenics static mixer was placed inside a ceramic membrane tube. The investigation performed in this

work could contribute to finding a proper solution for successful ultrafiltration of shear-stress sensible enzymes.

2. Experimental

2.1. Materials and methods

The enzyme solution used throughout the experiments was prepared from a commercial pectinase solution Vinozym^{TM} (Novozyme, Denmark). Vinozym^{TM}, was diluted 50 times in 10 mmol L^{-1} acetate buffer pH 5.0, and the endo-pectinase (endo-p) activity of the feed enzyme solution was determined to be 30.4 ± 4.5 U mL^{-1}. Endo-pectinase activity of the feed solution as well as the activities of retentate and permeate samples were determined according to Peričin et al. [5].

The experiments were carried out on a laboratory scale cross-flow ultrafiltration unit. The membrane used was a Schumasiv^{TM} (Pall Schumacher GmbH, Germany), a singe-tube membrane 250 mm long with 7 mm inner diameter. The useful membrane area was 4.84×10^{-3} m^2. The membrane had a nominal pore size of 5 nm and was made of aluminium oxide coated with titanium oxide. All experiments were carried out at a temperature of 25 ± 0.2^{o}C and mean transmembrane pressure of 100 ± 5 kPa. The Kenics^{TM} static mixer (FMX8124-AC, Omega, USA) as a static turbulence promoter was used throughout the experiments. The static mixer consisted of 30 mixing elements with a diameter of 6.35 mm and had the ratio of the mixing element length to mixer diameter (aspect ratio) equal to 1. A detailed description of the experimental unit and its schematic representation can be found elsewhere [6].

2.2. Calculations

The retention of endo-pectinase ($R_{endo\text{-}p}$) by the membrane was calculated as:

$$R_{endo\text{-}p}\,(\%) = 1 - \frac{\text{endo-pectinase activity of the permeate}}{\text{endo-pectinase activity of the retentate}} \times 100 \qquad (1)$$

After the permeate flux, the most important parameter from the economic point of view represents the specific hydraulic energy consumption (E), i.e. the hydraulic dissipated power per unit volume of the permeate:

$$E = \frac{\text{Feed flow rate} \cdot \text{Pressure drop along the membrane}}{\text{Permeate flux} \cdot \text{Membrane area}} \qquad (2)$$

Therefore, the efficiency of the static mixer was checked through determination of permeate flux improvement and the reduction of specific energy consumption. These parameters were calculated as a relative improvement/reduction of a certain parameter obtained by using the static mixer (SM mode of operation) compared to the parameter without using the static mixer (NSM mode of operation):

$$\text{Flux improvement}\,(\%) = \frac{\text{Permeate flux}_{SM} - \text{Permeate flux}_{NSM}}{\text{Permeate flux}_{NSM}} \times 100 \quad (3)$$

$$\text{Reduction of } E\,(\%) = \frac{E_{NSM} - E_{SM}}{E_{NSM}} \times 100 \quad (4)$$

3. Results and discussion

3.1. The efficiency of the static mixer

The variations of the permeate flux and the specific energy consumption during ultrafiltration of the endo-pectinase solution obtained by using the static mixer (SM mode) and without the static mixer (NSM mode) are shown in Fig. 1. The feed solution was concentrated to the volumetric concentration factor (*VCF*) of 3 and the operation conditions were chosen in such a way as to provide the same initial values of the permeate flux. Therefore, the experiment without using a static mixer was carried out at a feed flow rate of 60 L h^{-1} (mean cross-flow velocity of 0.43 m s^{-1}) while the similar initial permeate flux was obtained at a feed flow rate of 30 L h^{-1} (mean cross-flow velocity of 0.27 m s^{-1}) by using the static mixer.

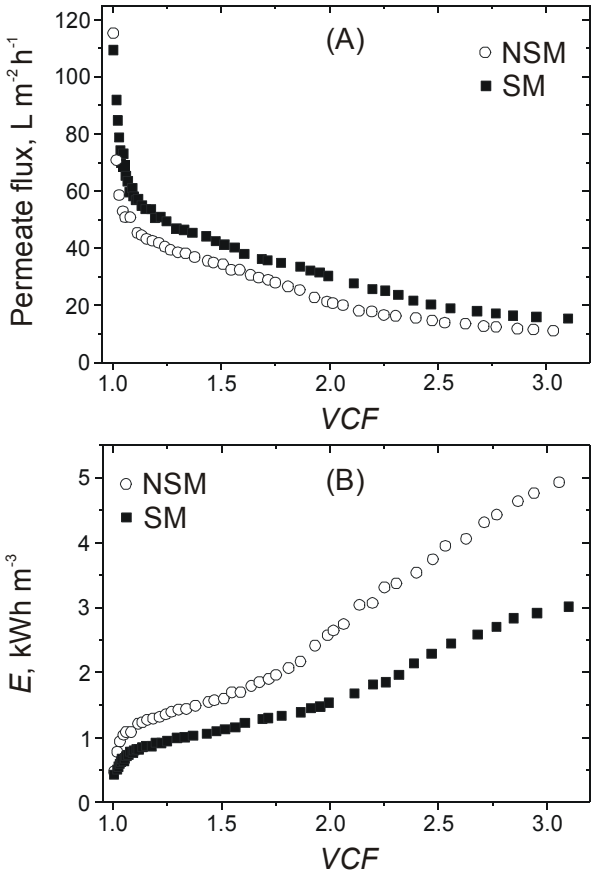

Fig. 1. The variations of permeate flux (A) and specific energy consumption (B) with *VCF*. Feed flow rate: 60 L h^{-1} (NSM); 30 L h^{-1} (SM).

The insertion of the static mixer promoted a degree of turbulence in the membrane tube and increased shear stress in the vicinity of the membrane providing similar initial flux at double the lower feed flow rate compared to the flow rate without using the static mixer. However, inserting a static mixer into a membrane tube causes an increase in pressure drop along the membrane length, leading to increased energy consumption. The pressure drop along the membrane length increased from 16 kPa to 26 kPa by inserting the static mixer despite the fact that the operation was at a lower flow rate. It should be emphasised that the hydraulic dissipated power (product of feed flow rate and pressure drop along membrane length) was about 15% lower during the operation with the static mixer providing good initial conditions for achieving energy saving compared to the operation without the static mixer.

Fig. 1A shows the rapid decline of the initial permeate flux during both modes of operation. The retentions of endo-pectinase ($R_{endo\text{-}p}$) were similar for both modes of operations: around 95% and 98% for NSM and SM modes, respectively. These results indicate that the rapid flux decline could be attributed to concentration polarization and formation of relatively thick gel layer which cannot be thinned at such low cross-flow velocities. Nevertheless, despite that the operation was at a lower feed flow rate, the permeate flux was higher using the static mixer compared to the flux without the static mixer, leading to an additional reduction of the specific energy consumption (Fig. 1B)

The effectiveness of the static mixer as a turbulence promoter can be easily quantified by calculation of the flux improvement and the reduction of specific energy consumption obtained by operating in SM mode instead of NSM mode. Values of these parameters, shown in Table 1, clearly prove the improvement of the process performance: the flux improvement of 45% at a *VCF* of 3 with the energy saving of about 40%.

Table 1. The efficiency of the static mixer during feed concentration.

VCF	Flux improvement (%)	Reduction of *E* (%)
1.2	25	32
1.5	29	34
2.0	39	41
2.5	42	41
3.0	45	40

However, the extensive loss of endo-pectinase activity was observed during both modes of operation: around a half of the enzyme activity was lost during concentration to a *VCF* of 1.5. After concentration to a *VCF* of 3 the solution did not show almost any endo-pectinase activity: the loss of activity was 92% and 87% for NSM and SM modes, respectively. To check the sensitivity of endo-pectinase to the surrounding conditions, the sample of the solution was left in a thermostated water bath overnight at 25^{o}C. The loss of the activity was around 15% after 12 hours of staying in the room temperature indicating a demand

for as short as possible duration of the ultrafiltration process. Furthermore, the obtained results proved high enzyme sensitivity on shear forces even during mild operation conditions.

3.2. Modification of the feed

In order to "protect" the enzyme activity, 2% of pectin (Grindstedt pectin LC 950, Danisco, Denmark) was added to the original endo-pectinase solution. The viscosity of such modified feed increased more than 24 times, from $9 \cdot 10^{-4}$ Pa s to $2.18 \cdot 10^{-2}$ Pa s. Higher viscosity of the modified feed required selection of different operation conditions to obtain similar initial fluxes like those during the operation with the original feed. Fig. 2 shows the variations of the permeate flux and the specific energy consumption during ultrafiltration of the modified feed by using the static mixer (SM+P mode) and the original feed solution without using the static mixer (NSM mode). While the experiment on the original feed without the static mixer was carried out at a feed flow rate of 60 L h^{-1} (mean cross-flow velocity of 0.43 m s^{-1}), the concentration of the modified feed with the static mixer had to be carried out at a feed flow rate of 85 L h^{-1} (mean cross-flow velocity of 0.77 m s^{-1}) to provide similar initial flux values.

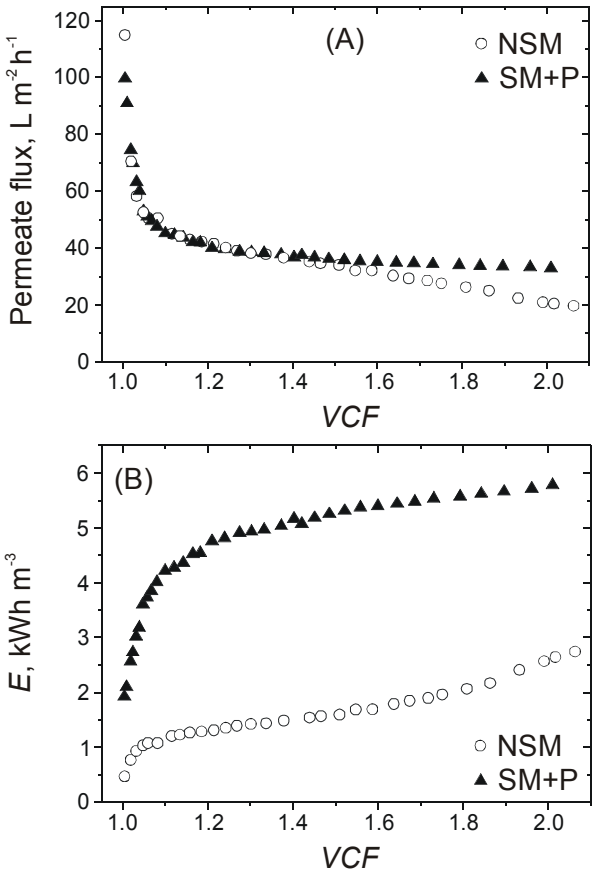

Fig. 2. The variations of permeate flux (A) and specific energy consumption (B) with *VCF*. Feed flow rate: 60 L h^{-1} (NSM); 85 L h^{-1} (SM+P).

Fig. 2A clearly shows the different flux decline patterns for the modified and the original feed. Up to a *VCF* of around 1.5, the flux behaviour was almost identical but then while the flux during operation with the original feed gradually declined, the pseudo-steady flux was reached in the case of the modified feed. The retentions of endo-pectinase ($R_{endo\text{-}p}$) were around 95% for both cases, thus different flux behaviours could be attributed to the different structure of the layer created on the surface of the membrane. Similar initial fluxes were obtained at the expense of higher energy consumption (more than 3.5 times at the beginning of operation) due to higher viscosity of modified feed (Fig. 2B). Table 2 shows the variations of flux improvement and reduction of specific energy consumption by using modified feed instead of the original feed. Negative values of reduction of *E* indicate higher energy consumption during the operation with the modified feed. However, less pronounced flux decline by using the modified feed led to the flux improvement of 60% and consequently reduction of energy consumption at a *VCF* of 2. These results indicate that the use of the static mixer could be particularly beneficial in ultrafiltration of viscous solutions.

Table 2. The efficiency of the static mixer during concentration of modified feed.

VCF	Flux improvement (%)	Reduction of *E* (%)
1.1	0	- 250
1.2	0	- 240
1.4	11	- 218
1.6	29	- 180
2.0	60	- 115

Addition of the pectin resulted in more energy-consuming ultrafiltration process due to more viscous feed. However, the primary objective, to prevent the enzyme inactivation due to shear stress, was completely accomplished: at a *VCF* of 1.5 the loss of the activity was reduced from around 70% to a negligible 8%, proving that cross-flow ultrafiltration of endo-pectinase solution is possible by adequate modification of the original solution.

4. Conclusions

The experimental results presented in this work demonstrate the improved performance of ultrafiltration of endo-pectinase solution by inserting the Kenics static mixer into a ceramic membrane tube. The turbulence promotion and increased wall shear rate by using the static mixer resulted in permeate flux improvement and energy saving. Moreover, the addition of pectin to the original feed significantly reduced the enzyme inactivation during the operation showing that the ultrafiltration of shear-stress sensible enzymes is possible by adequate modification of an enzyme solution. The use of the static mixer enabled ultrafiltration of the more viscous modified feed. However, this was at the expense of higher energy consumption.

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