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Optimization of enzymatic hydrolysis of haemoglobin in a continuous membrane bioreactor

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Abstract

This paper describes the enzymatic hydrolysis of haemoglobin in a continuous membrane bioreactor which was equipped with either a microfiltration membrane (Carbosep M14) with an average pore diameter of 0.14 μm or an ultrafiltration membrane (Carbosep M5) with 10 kDa molecular weight cut-off. These membranes gave similar performances indicating that the virgin porosity was modified by adsorption of proteins and enzymes, and by the formation of a dynamic membrane on the mineral membranes. Practically both membranes presented identical optimum parameters corresponding to an average transmembrane pressure of 1.25 bars and a tangential velocity of 0.7 m s^{-1}. A simple theoretical kinetic model based on the Michaelis–Menten relation and the substrate mass balance was successfully used for the enzymatic hydrolysis of the haemoglobin in a continuous membrane bioreactor.

Keywords: Enzymatic hydrolysis; Membrane bioreactor; Haemoglobin; Papaine

1. Introduction

A considerable research has been conducted during this past decade to investigate the new possibilities offered by enzyme in waste valorization and/ or treatment. The literature concerning this research directed towards developing enzymatic treatment systems for solid, liquid and hazardous wastes has been extensively reviewed recently [1]. Proteases are a group of hydrolases, which are widely used in the food industry processing, and meat waste [1–4]. The enzymatic hydrolysis is usually performed in batch type processes involving high costs due to the large quantity of enzyme required, high energy, long reaction, large volume reactors and the loss of enzymes after the inactivation operation at the procedure end [4–6].

The development of the membrane technology, particularly mineral membranes, improved the utilization of the enzymatic hydrolysis operation in a continuous membrane bioreactor since these membranes can work under temperatures exceeding 60°C in a large pH range (1–14) and can be sterilized after each operation. In this case, the enzyme is recycled and reused while the product of the enzymatic hydrolysis is continuously withdrawn as the permeate.

Among the many animal by-products, animal blood from slaughterhouses has probably the highest potential value. Bressolier et al. [7], Piot et al. [8] and Cempel et al. [9] have discussed the enzymatic hydrolysis of plasma and haemoglobin proteins in a continuous ultrafiltration reactor.

The purpose of this study was to optimize the membrane bioreactor for the enzymatic hydrolysis of haemoglobin blood and to compare the performances of mineral microfiltration membranes to those of ultrafiltration membrane.

2. Materials and methods

2.1. Enzyme and substrate

An industrial papain enzyme (Merck, Clevenot-France) extracted from latex of Carica papaya plant was used. Papaine enzyme has a molecular weight of 23.7 kDa and is most stable in the pH range of 6 to 9. Enzyme solutions of about $C_E = 4\ g\ dm^{-3}$ were made with distilled water. The enzyme has a reported activity of 30,000 units mg^{-1} of protein (caseine, pH 6, $T = 40$°C and 60 min).

The substrate was a bovine haemoglobin obtained after centrifugation (11,200*g*, 6°C, J 21 Beckman centrifuge) of bovine blood collected from the slaughterhouse of El-Harrach (Algiers) in sterilized bottles containing EDTA

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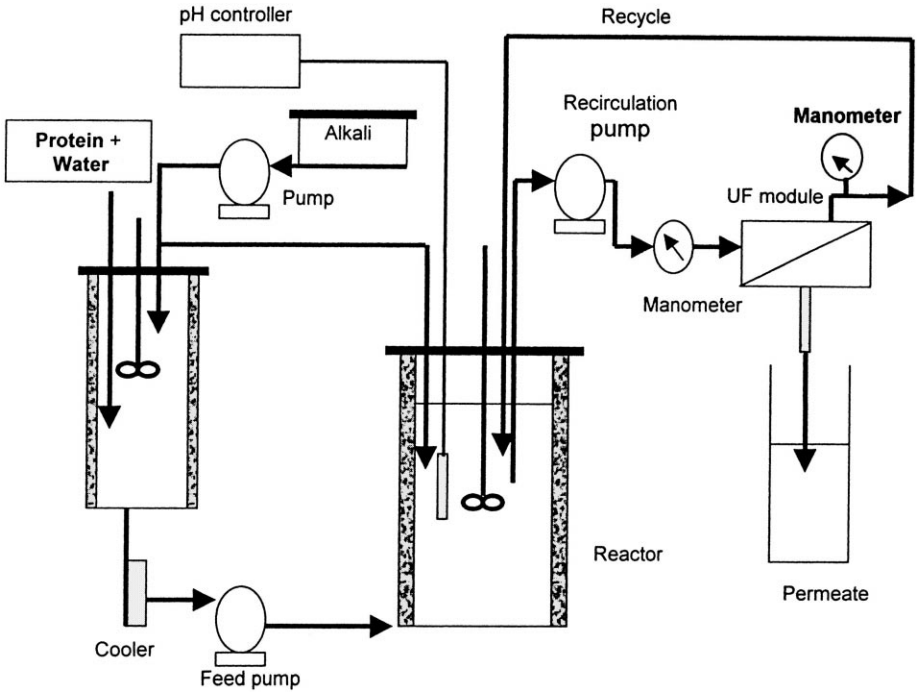

Fig. 1. Schematic diagram of continuous membrane bioreactor system for continuous hydrolysis of haemoglobin proteins.

solution (1 g dm^{-3}) as an anticoagulating agent. After mixing with distilled water, the substrate concentration (S_0), ranging from 4.5 to 37.5 g dm^{-3}, was adjusted to the required value.

2.2. Analytical methods

2.2.1. Measurement of the enzyme activity and peptide concentration

The enzyme activity of the papaine in reaction volume and permeate solution was determined during the experiments. Casein protein (Merck, Clevenot-France) was chosen as a reference using the Kondo et al. method [10].

Total proteins on each side of the membrane which refer to the peptide and amino acids contents were determined by the total Kjeldahl nitrogen (APHA-2540) method [11].

2.2.2. Measurement of the degree of hydrolysis

The degree of hydrolysis (D_H) representing the broken peptides and/or total peptide bonds, was determined by the pHstat technique [12] according to equation:

$$D_H = \frac{V_B N_B}{M_p} \frac{1}{\alpha} \frac{1}{N_P} \quad (1)$$

where V_B and N_B are, respectively, the volume and the normality of the base used. M_p and N_p are, respectively, the mass and the number of peptides bonds of the substrate proteins. α is the degree of dissociation of NH_4^+.

2.3. Continuous membrane bioreactor (CMB)

The membrane bioreactor system is illustrated in Fig. 1. Continuous enzymatic hydrolysis of haemoglobin was achieved by first loading the bioreactor (Setric 2l, Toulouse-France) with the amount of substrate required. Provisions were made for control of temperature, pH and agitation in the reaction vessel. After adjusting the temperature and pH optimal values (55°C and pH 8), previously determined [13], in both feed tank and reaction vessel, papain solution was added. During the first hour the CMB was operated in permeate recycling (i.e. permeate was returned to the reaction vessel). After this period, permeate recycling was stopped and a fresh haemoglobin solution was pumped into the reaction vessel at a velocity equal to the permeate flux. Thus, a constant reaction volume was fixed during the experiments.

The membrane module was equipped with either a Carbosep M5 ultrafiltration membrane with a molecular weight cut-off of 10 kDa or a Carbosep M14 microfiltration membrane with an average pore diameter of 0.14 μm. Both Carbosep membranes are composed of ultrafine pore ZrO_2 supported on porous carbon. The filtration area of these modules was 0.012 m^2. The hydraulic permeabilities of the membrane M5 and M14 with prefiltered water were 41 and 403 dm^3 h^{-1} bar^{-1} m^{-2}, respectively. The filtration

experiments were performed under an average transmembrane pressure $[P_1 + P_0]/2$ in the range of 0.5 to 2×10^5 Pa.

3. Results and discussion

3.1. Influence of hydrodynamic parameters on the permeate flux with a complete permeate recycling

The influence of the average transmembrane pressure (ΔP) and the tangential velocity (U) on the permeate flux during ultrafiltration was studied with a permeate recycling. The enzymatic hydrolysis was conducted in a batch bioreactor under optimum parameters ($T = 55^\circ$C, pH = 8, $S_0 = 12.5\,\mathrm{g\,dm^{-3}}$, $C_E = 4\,\mathrm{g\,dm^{-3}}$ and $t_f = 4$ h) previously determined [13]. The hydrolyzate obtained was then pumped in the concentrate compartment and the filtration operation was performed with mineral membranes.

The compositions of the hydrolyzate solution remained constant with the permeate recycling. The results obtained, presented in Fig. 2, indicate that for the two membranes, concentration polarization occurred at all tangential velocities. The curves obtained for Carbosep M14 microfiltration membrane showed that for low velocities the permeate flux was independent of the average applied transmembrane pressure for values greater than 1.25×10^5 Pa. The curves obtained for the Carbosep M5 ultrafiltration membrane also indicated that the permeate flux reached a limit at $\Delta P = 1.6 \times 10^5$ Pa for the lowest tangential velocity U. These average transmembrane pressures were used for the enzymatic hydrolysis operation in a continuous membrane bioreactor.

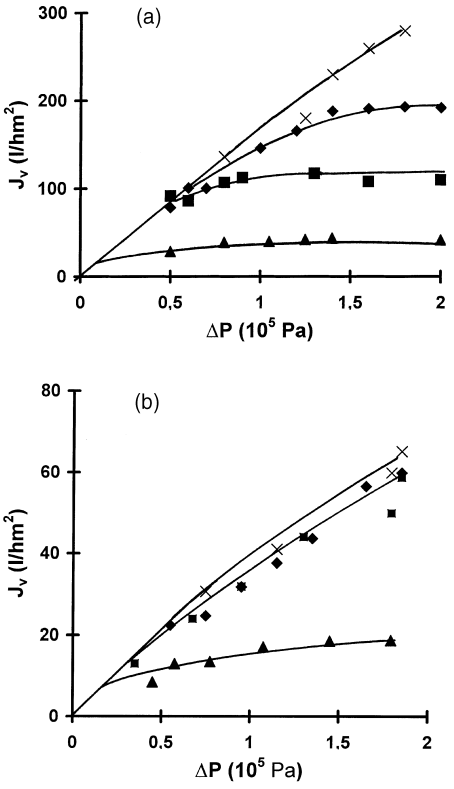

Fig. 2. Influence of hydrodynamic parameter on permeate flux. $S_0 = 12.5\,\mathrm{g\,dm^{-3}}$, pH = 8, $C_E = 4\,\mathrm{g\,dm^{-3}}$, $\Delta P = 1.25 \times 10^5$ Pa and $T = 55^\circ$C; (a) Carbosep M14 membrane. (▲) $U = 0.15\,\mathrm{m\,s^{-1}}$, (■) $U = 0.40\,\mathrm{m\,s^{-1}}$, (◆) $U = 0.70\,\mathrm{m\,s^{-1}}$ and (×) $U = 1.5\,\mathrm{m\,s^{-1}}$ (b) Carbosep M5 membrane (▲) $U = 0.15\,\mathrm{m\,s^{-1}}$, (■) $U = 0.40\,\mathrm{m\,s^{-1}}$, (◆) $U = 0.70\,\mathrm{m\,s^{-1}}$ and (×) $U = 1.5\,\mathrm{m\,s^{-1}}$.

Considering that the permeate flux is the parameter characterizing the performance of the membrane process, the best experimental conditions are those at which a plateau is reached for the applied pressure with the highest value of tangential velocity. A number of experimental parameters should be included to determine the optimum conditions of membrane techniques coupled to a bioreactor. The residual activity of the papain and the membrane rejection of the enzyme may represent these parameters, which are important parameters for quantifying the process efficiency.

3.2. Membrane rejection of enzyme

The residual enzymatic activity of papain in the bioreactor loop and permeate solution were determined. The results obtained, presented in Fig. 3, indicate that within 4 h, the papain activity in the bioreactor loop decreased by approximately 30 and 15% for respectively the Carbosep M14 and the Carbosep M 5 membranes. The papain activity loss with the Carbosep M14 membrane was practically twice that for the Carbosep M5 ultrafiltration membrane.

The enzymatic activity loss may be attributed to the blocking of the biocatalysts at the membrane surface or in the membrane pores [6,14,15]. To diminish enzyme adsorption onto Carbosep M14 membrane, a fresh haemoglobin solution was filtered through M14 membranes during 30 min [16] with CMB operating in a permeate recycling mode. After this period, the enzymatic hydrolysis in CMB was started.

Papain activity was encountered in the permeate solution for both membranes. The enzymatic activity increases slightly for the Carbosep M5 ultrafiltration membrane and reached a limiting value of about 20%. For the Carbosep M14 membrane, the enzymatic activity of the permeate was more important attaining a value of about 40%. The important increase and decrease of the permeate and retentate residual activity during the first 60 min may be explained by the low retention of the papain enzyme until the formation of the dynamic membrane and the adsorption on the pores of the membrane M14 occurred. The determination of the residual activity was achieved with an error precision in the range of $\pm 10\%$.

These results indicate that microfiltration (M14) and ultrafiltration (M5) membranes did not completely reject the papain enzyme.

Knowing that the average pore diameter of the Carbosep M14 membrane is important, it is quite surprising to find a membrane rejection of papain with a molecular weight of

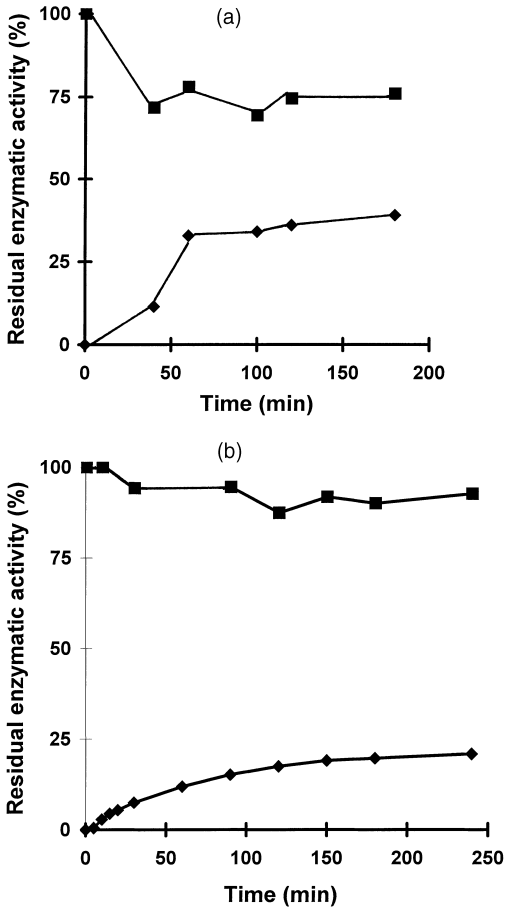

Fig. 3. Membrane rejection of papaine enzyme $S_0 = 12.5\,g\,dm^{-3}$, pH = 8, $C_E = 4\,g\,dm^{-3}$, $\Delta P = 1.25\ 10^5$ Pa and $T = 55°C$; (a) Carbosep M14 membrane. (■) concentrate residual enzymatic activity and (◆) permeate residual enzymatic activity, (b) Carbosep M5 membrane. (■) concentrate residual enzymatic activity and (◆) permeate residual enzymatic activity.

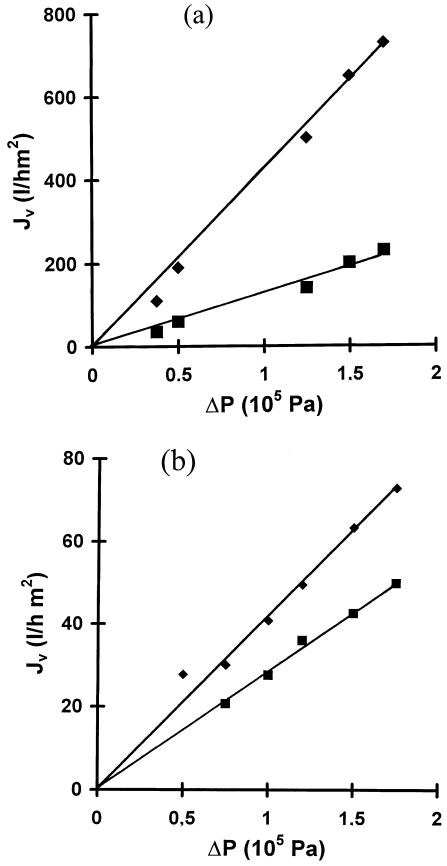

Fig. 4. Solvent curves before and after filtration; (a) Carbosep M14 membrane at $T = 25°C$. (◆) before microfiltration and (■) after microfiltration, (b) Carbosep M5 membrane at $T = 25°C$ (◆) before ultrafiltration and (■) after ultrafiltration.

23.7 kDa. It is clear that the molecular weight cut-off of the virgin membrane was altered by adsorption of proteins and enzymes.

To evaluate the importance of adsorption, we measured the hydraulic permeability of the membrane before and after enzymatic hydrolysis of haemoglobin in CMB with prefiltered water (Fig. 4). The large drop in permeability after filtration was estimated to 70% for the microfiltration membrane (M14) and to 30% for the ultrafiltration membrane (M5), as calculated by the ratio of the slopes (membrane-solvent permeabilities) obtained before and after filtration.

The ultrafiltration membrane appeared to be less affected by the adsorption phenomenon than the microfiltration membrane. For the Carbosep M5 membrane, the molecular weight cut-off of 10 kDa was smaller than the papain molecular weight (23.7 kDa) and as a result, steric fouling could be neglected.

For the microfiltration membrane M14, the pore radius was greater than the solute radii and steric fouling could occur. Assuming that adsorption onto a microfiltration membrane leads to a permeability variation resulting from a modification of the average pore radius [17] from r_{p0} to r_{p1} ($=r_{p0} - a$), we can write the Poiseuille equation as

$$J_v = \frac{N\pi r_{p1}^4}{8\eta e}\Delta P = L_{p1}\,\Delta P \tag{2}$$

$$L_{p0} = \frac{N\pi r_{p0}^4}{8\eta e} \tag{3}$$

$$L_{p1} = \frac{N\pi r_{p1}^4}{8\eta e} \tag{4}$$

where a is the pore size reduction due to adsorption, N the number of pores per unit area, e the membrane thickness (m), η the solvent viscosity (Poiseuille) and the reduced pore radius (r_{p1}) may be expressed as

$$r_{p1} = r_{p0}\left(\frac{L_{p1}}{L_{p0}}\right)^{0.25} \tag{5}$$

The permeabilities of the membrane were calculated by deducing the slope of the linear curves obtained by plotting the change of the permeate flux against the average transmembrane pressure during the filtration of prefiltered water.

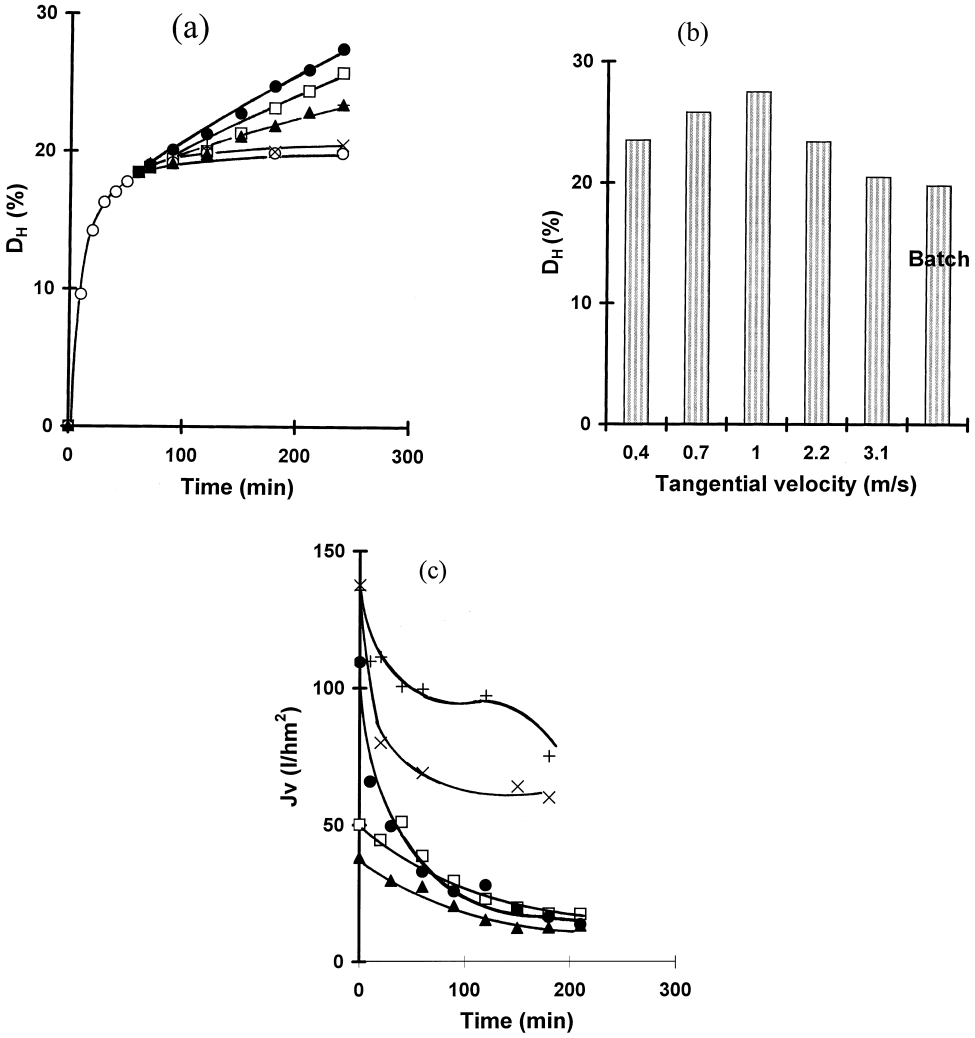

Fig. 5. Hydrolysis of haemoglobin in continuous membrane bioreactor at constant pressure. with Carbosep M14 membrane. $S_0 = 12.5\,g\,dm^{-3}$, pH = 8, $C_E = 4\,g\,dm^{-3}$, $\Delta P = 1.25 \times 10^5$ Pa and $T = 55°C$; (a) Influence of the tangential velocity on the hydrolysis degree. (▲) $U = 0.4\,m\,s^{-1}$, (□) $U = 0.7\,m\,s^{-1}$, (●) $U = 1\,m\,s^{-1}$, (+) $U = 2.2\,m\,s^{-1}$, (×) $U = 3.1\,m\,s^{-1}$ and (○) batch mode, (b) Final degree of hydrolysis against tangential velocity, (c) Variation of permeate flux during the enzymatic hydrolysis at various tangential velocity. (▲) $U = 0.4\,m\,s^{-1}$, (□) $U = 0.7\,m\,s^{-1}$, (●) $U = 1\,m\,s^{-1}$, (+) $U = 2.2\,m\,s^{-1}$ and (×) $U = 3.1\,m\,s^{-1}$.

The calculated value $r_{p1} = 0.05\,\mu m$ for the Carbosep M14 membrane confirmed the effect of adsorption in reducing the effective mineral membrane permeability.

The calculated value from the analysis of filtration data and by Poiseuille equation was found in good quantitative agreement with the pore diameter measured directly by atomic force microscopy for the conditions in which in-pore blocking was dominant during microfiltration of the enzyme yeast alcohol dehydrogenase (YADH) [14].

The important papain retention with the Carbosep M14 membrane although the high value of r_{p1} may be explained by the formation of a dynamic membrane on the mineral membrane (M14) which operated like a second membrane with a porous pore less important than that of the membrane itself. In a previous work [18], similar results were obtained during the treatment of fishery water by ultrafiltration modules equipped with Ceraver membranes. Indeed, for average transmembrane pressures greater than 2×10^5 Pa, high apparent rejection coefficients of the same order were obtained for the membranes with molecular cut-off values that were very different, indicating the formation of dynamic membranes on ultrafiltration membranes.

3.3. Influence of hydrodynamic parameters on the degree of hydrolysis (D_H)

The influence of tangential velocity on the degree of hydrolysis of the continuous bioreactor membrane was studied. The results obtained with the Carbosep M14 membrane are shown in Fig. 5a. It appeared that a high degree of hydrolysis was obtained with tangential velocities ranging from 0.7 to $1\,m\,s^{-1}$. Above these values, one can observe a decrease of

the degree of hydrolysis with a tangential velocity increase. This result may be explained by the fact that at higher values of U (3.1 m s^{-1}) the papain enzymes were submitted to an important shear stress inducing a decrease of D_H of about 8% than values obtained at $U = 1\,m\,s^{-1}$. Values of shear stress in the range of 8 MPa were attained during the experiments at highest U. Indeed, in solutions of biological origins, like enzyme solutions, the effect of the condition formed on the polarization layer (shear stress [6,15] and possible variation in ionic strength and pH) results in denaturation of biological compounds [19]. It is important to note that the degrees of hydrolysis obtained in continuous membrane bioreactor at various tangential velocities were more important than those obtained in a batch bioreactor (Fig. 5b).

Measurements of the permeate flux (J_v) during the experiment (Fig. 5c) permitted to deduce first a fast flux declining rate for all tangential velocities. Secondly, the permeate flux reached a steady-state value after an equilibrium time of 150 min expected for $U = 2.2\,m\,s^{-1}$. It is also observed that the permeate flux at the tangential velocity $U = 3.1\,m\,s^{-1}$ decreased more than the permeate flux at $U = 2.2\,m\,s^{-1}$. This result indicates that denaturation of enzymes and proteins occurred at $U = 3.1\,m\,s^{-1}$which modify the enzyme and protein structure. This result is in good agreement with the results obtained for the study of the influence of U on the degree of hydrolysis, and hence, provides further confirmation for the explanation of the enzyme denaturation at high U. Optimum tangential velocity about $0.7\,m\,s^{-1}$ was retained for the Carbosep M14 microfiltration membrane.

To avoid denaturation of biological compounds, tangential velocity was limited to $U = 2.2\,m\,s^{-1}$ for the study of the influence of U on the degree of hydrolysis with the Carbosep M5 ultrafiltration membrane. The results obtained are presented in Fig. 6. These results are in agreement with those obtained with the Carbosep M14 membrane indicating that the high degrees of hydrolysis were obtained for values of U around $0.7\,m\,s^{-1}$. This result is not surprising, since we know that the microfiltration membrane (M14), after adsorption of haemoglobin proteins onto the membrane and the formation of a dynamic membrane on mineral membrane surface, allowed to give a new molecular cut-off near the molecular cut-off of Carbosep M5 membrane.

The effect of transmembrane pressure on the degree of hydrolysis was studied at a tangential velocity $U = 0.7\,m\,s^{-1}$ (Fig. 6a). The transmembrane pressures $\Delta P = 1.6\ 10^5$ Pa and $\Delta P = 1.25 \times 10^5$ Pa gave close degrees of hydrolysis. Nevertheless, greater degrees of hydrolysis were obtained for the higher transmembrane pressure $\Delta P = 1.6 \times 10^5$ Pa.

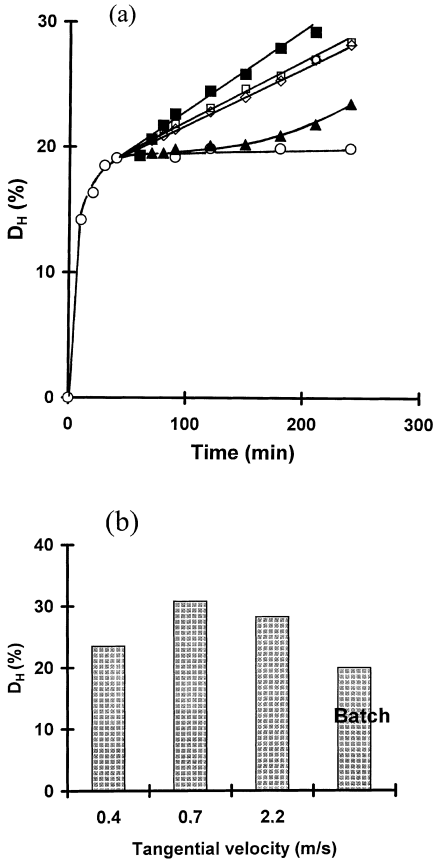

Fig. 6. Hydrolysis of haemoglobin in continuous membrane bioreactor at constant pressure. with Carbosep M14 membrane. $S_0 = 12.5\,g\,dm^{-3}$, pH = 8, $C_E = 4\,g\,dm^{-3}$, $\Delta P = 1.25 \times 10^5$ Pa and $T = 55°C$; (a) Influence of the tangential velocity on the hydrolysis degree. (▲) $U = 0.4\,m\,s^{-1}$, (□) $U = 0.7\,m\,s^{-1}$, (■) $U = 0.7\,m\,s^{-1}$ at $\Delta P = 1.6\ 10^5$ Pa, (◇) $U = 2.2\,m\,s^{-1}$, and (○) batch mode, (b) Final degree of hydrolysis against tangential velocity.

3.4. Effect of the substrate mass to enzyme mass ratio (R) on the performance of the CMB

Until now, the ratio used is the optimum ratio $R = 3\,g\,g^{-1}$ determined in the batch mode. The study of the R parameter with enzymatic hydrolysis in CMB permitted, firstly, to determine the continuous bioreactor kinetics. The data obtained for various R are shown in Fig. 7a. An apparent steady state was reached within 80 min for $R = 1\,g\,g^{-1}$ and it took longer times at moderate R. For higher R, the steady state was not obtained.

The double-inverse relationship according to Lineweaver–Burk's formula provides the kinetic constants K_m and V_m, presented in Table 1, obtained for the CMB (Fig. 7b).

For the batch mode, the experimental results agree with the Lineweaver–Burk's formula for the low substrate concentrations (i.e. high $1/S_0$). At substrate concentration greater than $12\,g\,dm^{-3}$, inhibitory effects were encountered.

Table 1
Kinetic parameters for the batch reactor and the continuous-membrane bioreactor

Mode	K_m (g dm^{-3})	V_m (g dm^{-3} min^{-1})
Continuous mode	12.7	1.1
Batch mode	40	8.3

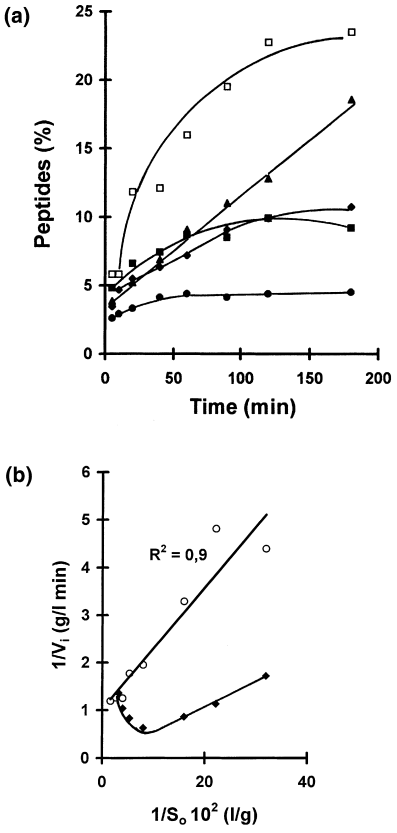

Fig. 7. Determination of the kinetics parameters of the enzymatic hydrolysis of the haemoglobin in CMB-arbosep M14 membrane. pH = 8, $C_E = 4\,g\,dm^{-3}$, $\Delta P = 1.25 \times 10^5$ Pa, $U = 0.7\,m\,s^{-1}$ and $T = 55°C$; (a) Variation of peptides concentration $[(S - S_0)/S]$ during the experiment. (●) $R = 1\ g\,g^{-1}$ enzyme, (■) $R = 3\ g\,g^{-1}$ enzyme, (◆) $R = 6.25\ g\,g^{-1}$ enzyme , (▲) $R = 9.25\,g\,g^{-1}$ enzyme and (□) $R = 15.5\,g\,g^{-1}$ enzyme, (b) Lineweaver–Burke plot for papaine -haemoglobine hydrolysis in a CMB and batch bioreactor. (○) CMB reactor and (◆) batch bioreactor.

K_m and V_m with the CMB were three and eight times lower than with the batch bioreactor. This result might indicate a lower affinity of the papain enzyme for the haemoglobin substrate probably due to the blocking of the biocatalysts at the membrane surface or in the membrane pore and thermal inactivation [5,13,14]. Bressolier et al [6] indicated a decrease of K_m and V_m values for plasma protein hydrolysis by alcalase in a continuous membrane reactor. It has also appeared that inhibitory effects was not encountered in CMB although substrate concentrations attained $60\,g\,dm^{-3}$ (or $R = 15\,g\,g^{-1}$ enzyme) while the batch bioreactor they were limited to the ratio $R = 3\,g\,g^{-1}$ enzyme (or $S_0 = 12\,g\,dm^{-3}$).

3.5. Modeling of the continuous membrane bioreactor

At first, it was observed that the CMB operated like an ideal continuous stirred tank reactor, as deduced from the tracer injection and perfect fit of C_t/C_0 data with the theoretical curve (data not shown). In such a reactor, for conversion of a single substrate in an uninhibited irreversible reaction, performance relationship, Eq. (6), according to Lee and Ryu [20], can be obtained after combination of the substrate mass balance and the Michaelis–Menten expression of initial reaction rate:

$$\frac{V_m \tau}{K_m} = \frac{X S_0}{K_m} + \frac{X}{1 - X} \tag{6}$$

where: $X = (S_0 - S/S_0)$

The experimental values of the fractional conversion X against $(V_m\tau/K_m)$ for various substrate concentrations are shown in Fig. 8. In the same figure, the theoretical courses of the fractional conversion calculated by means of Eq. (6) are presented. The curves obtained indicate that the theoretical curves fit the experimental X values satisfactorily for the various substrate concentrations ranging from 3 to $40\,g\,dm^{-3}$. The kinetic model based on the Michaelis–Menten expression of the initial reaction rate and the substrate mass balance may be successfully utilized for the enzymatic hydrolysis of haemoglobin by papain enzyme in a continuous membrane bioreactor.

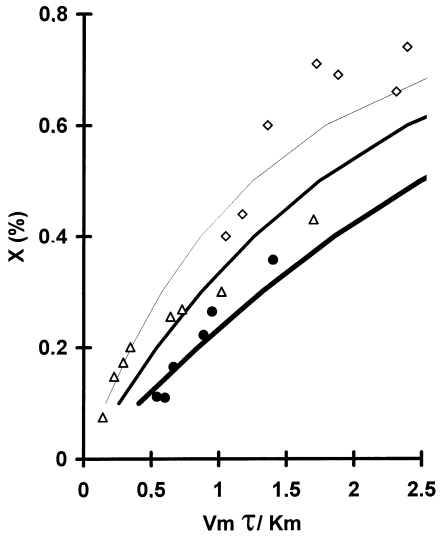

Fig. 8. Course of fractional conversion (X) against $V_m\tau/K_m$. Experimental data (●) $S_0 = 37.5\,g\,dm^{-3}$, (△) $S_0 = 18.8\,g\,dm^{-3}$ and (◇) $S_0 = 4.5\,g\,dm^{-3}$. Theoretical curve (▬) $S_0 = 37.5\,g\,dm^{-3}$, (▬) $S_0 = 18.8\,g\,dm^{-3}$and (—) $S_0 = 4.5\,g\,dm^{-3}$.

4. Conclusion

Enzymatic hydrolysis of haemoglobin in a continuous membrane bioreacteur, which was equipped with either a microfiltration membrane (CARBOSEP M14) or an ultrafiltration membrane (CARBOSEP M5), presented practically similar performances. The molecular cut-off of the virgin membranes appeared to be not important since the porosity of the membrane was modified by the adsorption of protein or enzyme and by the formation

of a dynamic membrane on the mineral membranes. At high tangential velocity ($U = 3\,m\,s^{-1}$) enzyme and protein denaturation occurred. Practically similar optimum conditions were also found for the CMB independently of the membrane.

It is also observed that the inhibitory effects were not encountered in CMB with substrate concentrations five times greater than the concentrations obtained in batch bioreactor.

A simple theoretical kinetic model was successfully applied for the enzymatic hydrolysis of the haemoglobin in the continuous membrane bioreactor.

5. Nomenclature

a pore size reduction due to adsorption (m)
C_E enzyme concentration ($g\,dm^{-3}$)
C_0 NaCl concentration of the bioreactor feed ($g\,dm^{-3}$)
C_t NaCl concentration of the permeate at time t ($g\,dm^{-3}$)
CMB continuous membrane reactor
D_H degree of hydrolysis (dimensionless)
e membrane thickness (m)
g gravity constant ($m\,s^{-2}$)
J_v permeate flux ($l\,h^{-1}\,m^{-2}$)
K_m Michaelis–Menten constant ($g\,dm^{-3}$)
L_{p0} initial membrane permeability ($l\,h^{-1}\,m^{-2}\,Pa^{-1}$)
L_{p1} membrane permeability after ultrafiltration operation ($l\,h^{-1}\,m^{-2}\,Pa^{-1}$)
M_p mass of proteins (N. 6.25)
N number of pores per unit area (pores m^{-2})
N_B normality of the base ($mole\,l^{-1}$)
N_p number of peptides bonds ($equivalent g^{-1}$ protein)
P_0 outlet membrane pressure (Pa)
P_1 inlet to membrane pressure (Pa)
R substrate mass to enzyme mass ratio ($g\,g^{-1}$)
r_{p0} pore radius (m)
r_{p1} reduced pore radius (m)
S substrate concentration at time t and with steady state regime ($g\,dm^{-3}$)
S_0 initial substrate concentration ($g\,dm^{-3}$)
T temperature (°C)
t experimental time (s)
t_f time necessary to hydrolysis the required amount of substrate (s)
U tangential velocity ($m\,s^{-1}$)
V reactor volume (l)
V_B volume of base consumed (l)
V_m maximum enzymatic reaction rate ($g\,dm^{-3}\,min^{-1}$)
X fractional conversion (dimensionless)
ΔP average transmembrane pressure (Pa)
η solvent viscosity (Poiseuille)
α degree of dissociation of NH_4^+ (dimensionless)
τ reactor space time (s)

References

[1] J. Karam, J.A. Nicell, Potential applications of enzymes in waste treatment, J. Chem. Tech. Biotechnol. 69 (1997) 141–153.
[2] V. Venugopal, M.D. Alur, D.P. Neckar, Solubilization of fish proteins using immobilized microbial cells, Biotechnol. Bioeng. 33 (1989) 1098–1103.
[3] P.G. Dalev, Utilisation of waste feathers from poultry slaughter for production of protein concentrate, Bioresource Technol. 48 (1994) 265–267.
[4] M. Cheryan, M.A. Mehaia. Membrane bioreactors, In Membrane separations in biotechnology, W.C. Mc Gregor (Ed.), Marcel Dekker, New York, 1986 pp. 255–301.
[5] A. Mannheim, M. Cheryan, Continuous hydrolysis of milk protein in a membrane reactor, J. Food Sci. 55 (2) (1990) 381–390.
[6] O. Gaouar, C. Aymard, N. Zakhia, G.M. Rios, Enzymatic hydrolysis of Cassava starch into maltose syrup in continuous membrane reactor, J. Chem. Tech. Biotechnol. 69 (1997) 367–375.
[7] Ph. Bressollier, J.M. Petit, R. Julien, Enzyme hydrolysis of plasma proteins in a CSTR ultrafiltration reactor: performances and modeling, Biotechnol. Bioeng. 31 (1988) 650–658.
[8] J.M. Piot, D. Guillochon, D. Leconte, D. Thomas, Application of ultrafiltration to the preparation of defined hydrolysates of bovine hemoglobin, J. Chem. Tech. Biotechnol. 42 (1988) 147–156.
[9] A. Kondo, K. Imura, K. Nakama, K. Higashitani, Preparation of immobilized papain using thermosensitive latex particles, J. Ferment. Bioeng. 78 (3) (1994) 241–245.
[10] N. Cempel, J.M. Piot, D. Guillochon, Preparation of photodynamic hydrolysates from bovine haemoglobin, J. Agric. Food Chem. 42 (1994) 2059–2063.
[11] APHA, AWWA, WEF, Standard Methods for the Examination of Water and Wastewater, 19th edn., American Public Health association, Washington, D.C 1995.
[12] J. Adler-Nissen, Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzene sulfonic acid, J. Agric. Food Chem. 27 (6) (1979) 1256–1262.
[13] H. Mokrane, Valorization of haemoglobin by enzymatic hydrolysis. Master Thesis, Ecole Nationale Polytechnique, Algiers. 1997.
[14] W.R. Bowen, N.J. Hall, Properties of microfiltration membranes: mechanisms of flux loss in the recovery of an enzyme, Biotechnol. Bioeng. 46 (1995) 28–35.
[15] S.E. Charm, B.L. Wong, Shear effects on enzymes, Enzyme Microb. Technol. 3 (1983) 1112–1120.
[16] D. Belhocine, H. Grib, D. Abdesmed, Y. Comeau, N. Mameri, Optimization of plasma proteins concentration by ultrafiltration, J. Membrane Sci. 142 (1998) 159–171.
[17] L.J. Zeman, R. Wales, Steric rejection of polymeric solute by membranes with uniform pore size distribution, Sep. Sci. Technol. 16 (1985) 275–283.
[18] N. Mameri, D. Abdesmed, D. Belhocine, H. Lounici, C. Gavach, J. Sandeaux, R Sandeaux, Treatment of fishery washing water by ultrafiltration, J. Chem. Biotechnol. 67 (1996) 169–175.
[19] P. Aptel and M. Clifton, Ultrafiltration, in: Reidel publishing Company (Ed.), Synthetic Membranes: Science, Engineering and Applications, Nato ASI Series C. Holland, 1986, pp. 249–305.
[20] S.B. Lee, D.D.Y. Ryu, New method of kinetic constants in enzyme reactor systems by transformation of variables. Biotechnol. Bioeng. 21 (12) (1979) 2329–2336.