Thermolysine adsorption on membranes at parallel flow of retentate
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Thermolysine adsorption on membranes at parallel flow of retentate

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1. Summary

Enzyme surface concentration and permeate stream obtained in the membrane module with a dynamically formed biocatalyst layer were determined experimentally using two different, polymeric membranes. Correlations were formulated to calculate these values. It was found that their variability range was so big that when controlling the transmembrane pressure and turbulence of a retentate stream, the mass of used biocatalyst in membrane bioreactor could be distributed in a controlled way between both reaction zones (with the native and adsorbed enzyme) in bioreactor.

Keywords: enzymatic membrane bioreactor, enzyme adsorption, dynamic membrane, enzyme surface concentration, parallel flow

2. Introduction

Adsorption is the most common method for enzymes and microorganism cells immobilization (Moueddeb et al., 1996; Fernandez- Lafuente et al., 1998; Jurado et al., 2006). It is relative simple method that does not require a special preparation procedure and the number of potential carriers is huge (Tsai et al., 1998; Junichi et al., 2000; Zhen-Gang et al., 2006). An interesting kind of the carriers there are polymeric membranes which can serve not only to enzyme immobilization but also can separate some reagents (i.e. substrate of reaction) (Gan et al., 2002; Deng et al., 2005). However, a stability of biocatalyst bond on the sorption way is generally low.
In practical solutions of the membrane modules, a circulating stream flows parallel to the membrane surface. This implies the presence of shear stresses which tear off the biocatalyst deposited on the membrane from its surface. It means that under precisely condition, in equilibrium state, the part of enzyme will be immobilized on membrane surface when another one will be suspended in solution in the native form – Fig. 1.

Applied membrane has to separate the enzyme molecules what is easy to receive by the application of membrane with suitable pores side.

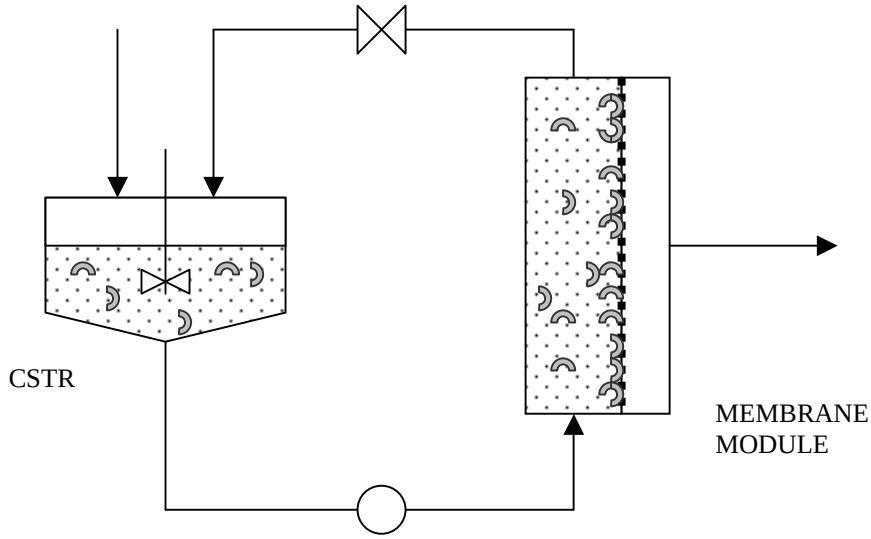

Fig. 1 Distribution of enzyme (marked as) in membrane bioreactor.

The main parameter which determines distribution of the biocatalyst mass in membrane bioreactor between both reaction zones is hydrodynamics of the stream flowing along the membrane surface. It is obvious that the higher is turbulence of the stream flowing past a membrane deposited in the module of given geometry, the smaller mass of the used biocatalyst will be kept on its surface. It is easy to control stream circulation, so distribution of the biocatalyst mass in the both bioreactor zones. It is the important parameter because usually kinetic activity and conformation stability of the native and immobilized enzyme are different. Also with an increase of the amount of immobilized protein permeate flux decreasing.
In this paper thermolysine surface concentration and permeate stream obtained in the membrane module with a dynamically formed biocatalyst layer under different process conditions were determined experimentally using two different, polymeric membranes.

3. Materials and methods

The following materials were used in the experiments:
- thermolysine [EC 3.4.24.27.], protease from *Bacillus thermoproteolyticus rokko* (Sigma, USA);
- module containing 10 capillary membranes from polysulphone 1700 NT LCD, (IBIB, Warszawa), capillary inner diameter 0.575 mm, length 100 mm; cut-off 24

kDA and module containing 8 capillary membranes from polyetherosulphone E6020P (IBIB, Warszawa), capillary inner diameter 0.670 mm, length 100 mm; cut-off 20 kDA
- pressure pump (ColeParmer, USA);
- Helios γ spectrophotometer (ThermoSpectronic, England).

Sorption of the selected enzyme (thermolysine) was carried out on the surface (A=10 cm^2) of capillary membranes made from polysulfone and polyetherosulphone at the temperature 50ºC. Prior to measurements, the membrane module was rinsed each time with deionized water and next with 0.1 M phosphate buffer, pH 7.0. In this buffer also a solution of thermolysine was prepared at the concentration in the range 0.08 - 0.82 g l^{-1}. The degree of enzyme immobilisation as a function of retentate flow corresponding to Re number ranging from 33 to 2464 and transmembrane pressure ranging from 0.030 to 0.152 MPa was investigated.
50 ml solution with determined thermolysine concentration was circulating in the system. After taking samples for analysis (after 15, 30, 45 and 60 min) the tested solution was completed each time with thermolysine solution at a volume and concentration corresponding to the sample. Practically, steady state was obtained in every case after 60 min. The adsorbed enzyme mass was determined from the enzyme mass balance basing on Lowry's test (Lowry et al., 1951) ($A(750nm)= 0.1149 \cdot c^2$ [g l^{-1}] + 0.1385 · c [g l^{-1}]) from the initial samples and samples taken after 1 h of the sorption process at given process parameters.

In between consecutive experimental series the adsorbed enzyme was removed from the module by prolonged (30 h) alternate elution with fresh portions of 3% NaOH solution, water and 80 % ethanol at high turbulence of the retentate stream.

4. Results and discussion

Influence of turbulence of the retentate stream (Re), pressure difference and the native enzyme concentration in the system on enzyme surface concentration and permeate stream was tested.

4.1. Enzyme surface concentration

Two different polymeric membranes made from polysulphone and polyetherosulphone were tested. Cut-off of these membranes was similar (respectively, 24 and 20 kDa) and in both cases no enzyme molecules were found in permeate.
As results from experiments the surface concentration of the biocatalyst changed the most with the change of turbulence of the retentate stream – Fig. 2. This function for both membranes is linear. Linear dependence was found also with the pressure difference influence – Fig. 3.

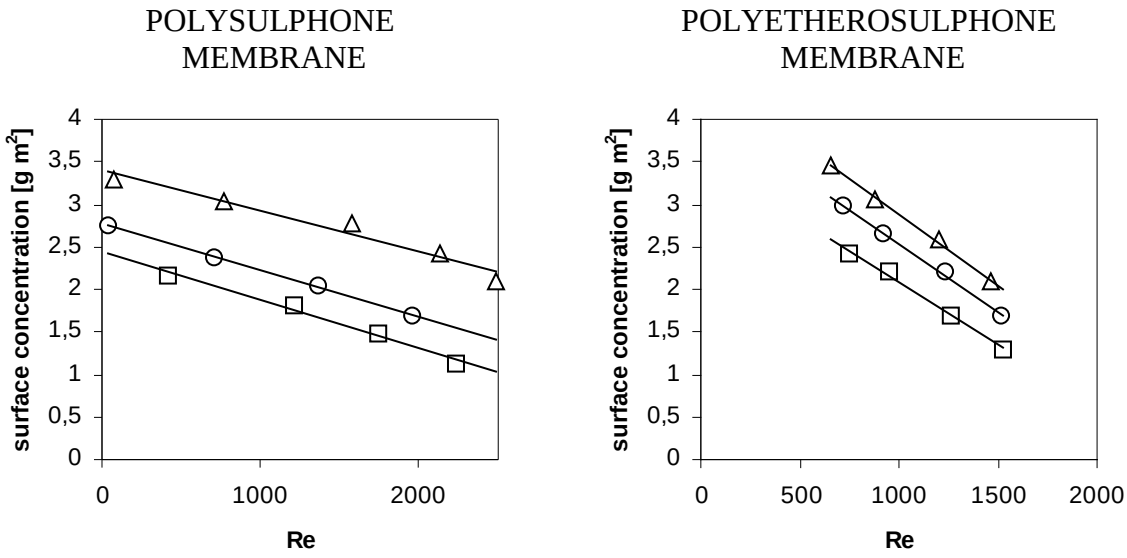

Fig. 2 Influence of turbulence of the retentate stream on thermolysine surface concentration. Thermolysine concentration in solution was 0.210 g l^{-1} and pressure difference in the case of polysulphone membrane (Δ – 0.152 MPa, o- 0.071 MPa, □ - 0.035 MPa) and (Δ – 0.130 MPa, o- 0.090 MPa, □ - 0.060 MPa) in the case of polyetherosulphone membrane.

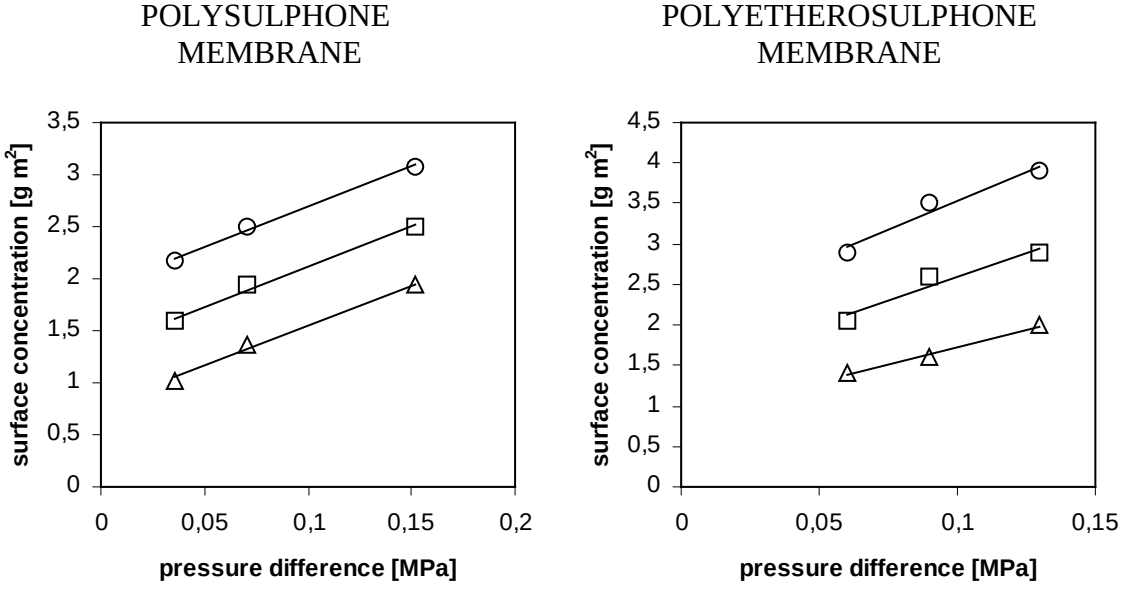

Fig. 3 Influence of pressure difference on thermolysine surface concentration. Thermolysine concentration in solution was 0.210 g l^{-1} and Re No. of retentate stream turbulence in the case of polysulphone membrane (Δ – 2500, o- 1500, □ - 500) and (Δ – 1500, o- 1000, □ - 500) in the case of polyetherosulphone membrane.

No models describing relationships between surface concentration of a catalyst bound with the membrane and process parameters of membrane separation are known. Thus, basing on the analysis of relations obtained experimentally, the following correlation was proposed:

$$\frac{x}{c} = Z_1 \Delta P^{Z_2} - Z_3 \, Re \qquad (1)$$

where: x- enzyme surface concentration (g m^{-2}), c – enzyme concentration in solution (g l^{-1}), ΔP – pressure difference (MPa), Re – Reynolds No.of retentate stream, Z_1, Z_2, Z_3– constants.

For the tested systems the obtained constants are presented in Table 1.

Table 1: The constant values of Eq. (1) for thermolysine immobilized on the surface of polysulfone and polyetherosulfone membrane.

membrane	Z_1	Z_2	Z_3
polysulfone	127.0	1.0	0.0033
polietherosulfone	55.0	1.0	0.0073

Mean error of this correlation base for each membrane on 64 experimental points is 13 and 11.4 % for the polysulfone and polyetherosulfone membrane.
For both tested membranes the constant Z_1 is equal to 1.0, hence considered dependence (x/c) is linear in relation both to pressure difference and to Reynolds No. of retentate stream. As results from values of constant Z_1 and Z_3 , in the case of the enzyme deposited on the surface of the polysulfone membrane influence of the retentate turbulence (Re) is two times lower than in the case of the enzyme deposited on the surface of the polietrosulfone membrane and influence of the pressure difference is opposite.

4.2. Permeate flux

The qualitative effect of solution concentration and its turbulence on the stream of obtained permeate is known in the literature (Koltuniewicz et al., 1995). The lower is the solution concentration and the higher is turbulence of the stream flowing past the membrane, the closer is the permeate stream value to the value obtained for a pure solvent. According to the known models, transmembrane mass transport for small pressure difference of the permeate stream depends linearly on ΔP. For high values of ΔP its effect on the value of obtained permeate stream disappears. The range of parameters interesting for the considered bioreactor is between these border states. For this range of changing parameters it is difficult to describe relations between process parameter using the known models – one should refer to experimental determination.
Figures 4-6 show an example of experimentally obtained influence of Reynolds No. of retentate stream, surface concentration and pressure difference on permeate flux.

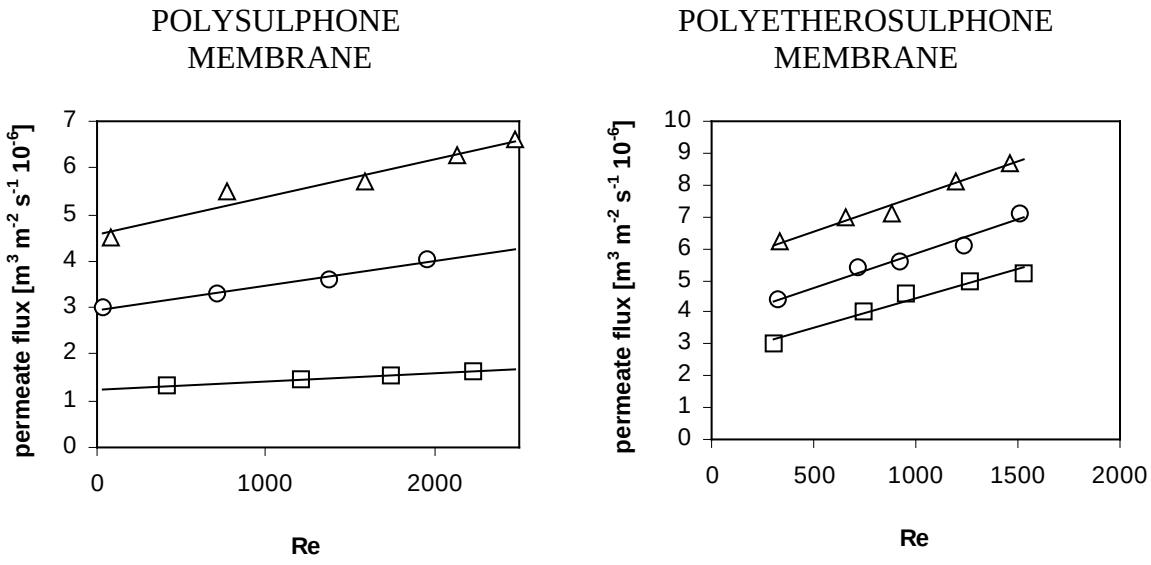

Fig. 4 Influence of Reynolds No. on permeate flux for polysulfone and polyetherosulfone membrane. Thermolysine concentration in solution was 0.210 g l^{-1} and pressure difference in the case of polysulphone membrane (Δ – 0.152 MPa, o- 0.071 MPa, □ - 0.035 MPa) and (Δ – 0.130 MPa, o- 0.090 MPa, □ - 0.060 MPa) in the case of polyetherosulphone membrane.

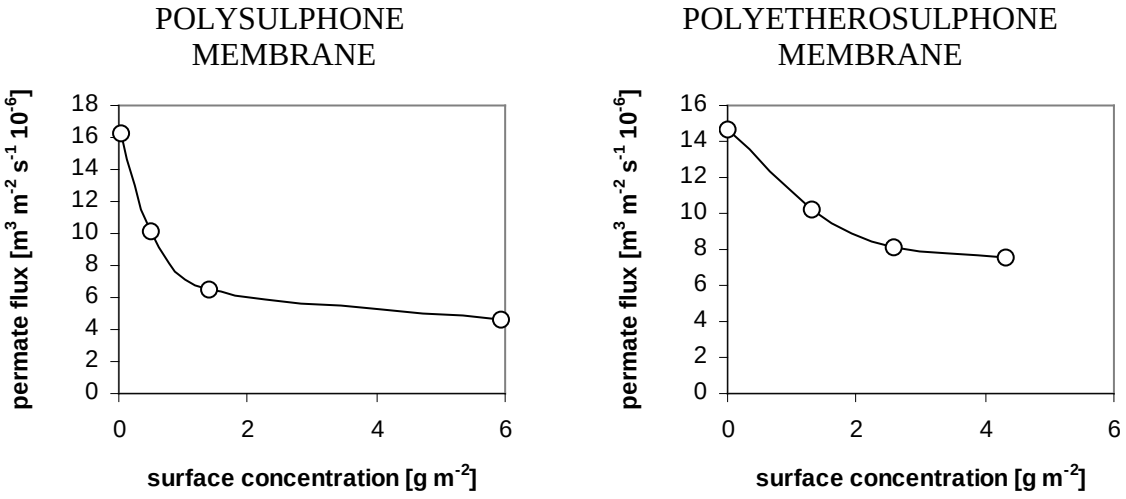

Fig. 5 An example of influence of thermolysine surface concentration on permeate flux. The process conditions were ΔP=0.152 MPa, Re=2482 and ΔP=0.130 MPa, Re=1236, respectively for polysulfone and polyetherosulfone membrane.

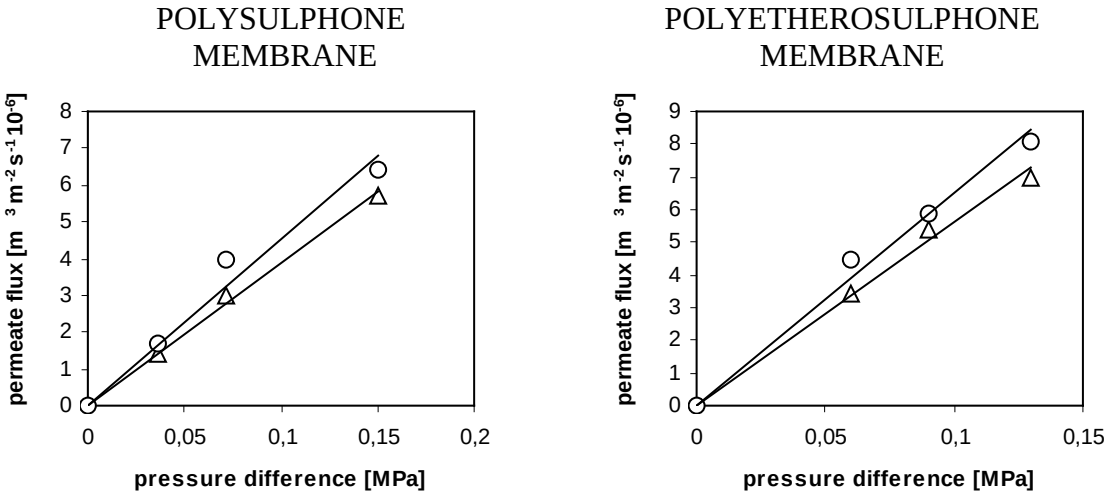

Fig. 6 Influence of pressure difference on permeate flux for polysulfone and polyetherosulfone membrane at thermolysine concentration in solution 0.210 g l^{-1} and different Re No. of retentate stream, in the case of polysulphone membrane (Δ – 1000, o- 2000) and (Δ – 713, o- 1236) in the case of polyetherosulphone membrane.

Correlation describing influence of tested parameters on permeate flux (J) was proposed:

$$J = \frac{\Delta P}{R_m + R_x} = \frac{\Delta P \cdot 10^{-2}}{R_m + b \cdot x} \qquad (2)$$

where: J- permeate flux (m^3 m^{-2} s^{-1}), R_m, R_x- resistance of membrane and enzyme layer (N s^{-1}), b- constant, x- surface enzyme concentration (g m^{-2})

Presented correlation is an implicit function according to pressure difference which one influences also on the value of surface enzyme concentration – Eq. 1. Influence of the Reynolds No. of retentate stream is included also in value of "x".

For the tested systems the obtained constants of Eq. (2) are presented in Table 2.

Table 2: The constant values of Eq. (2) for thermolysine immobilized on the surface of polysulfone and polyetherosulfone membrane.

membrane	R_m	b
polysulfone	179.0	37.0
polieterosulfone	64.0	32.2

Mean error of this correlation base for each membrane on 64 experimental points is 18.1 and 14.4 % for the polysulfone and polyetherosulfone membrane.

As results from above values of constants, polyeterosulfone membrane causes 2.8 times lower resistance than the tested polysulfone membrane and what was expected the resistance of membrane layer (constant b) in both cases is similar.
Turbulence of the retentate stream and difference pressure can be applied with good accuracy to control the amount of the catalyst mass immobilized on the membrane surface. In the performed experiments, the surface concentration of the biocatalyst and the permeate flux changed several times. These values are so significant that they form a set which can be successfully used to control the bioreactor operation. However the value of parameters of Eq. (1) and (2) have to be checked experimentally for given biocatalyst and polymeric membrane.

5. Final conclusion

As it was presented in the paper it is possible to of control with process parameters the distribution of biocatalyst mass between two reaction zones in membrane bioreactor when biocatalyst is dynamically immobilized on the membrane surface.
In the tested system turbulence of the retentate stream and difference pressure had linear influence on the relation amount of the catalyst mass immobilized on the membrane surface to its concentration in solution. However this influence is different for different kinds of polymeric membrane.
Independent on the membrane material the resistance of the cake of immobilized enzyme is the same hence on the value of permeate flux has influence applied pressure difference, the polymeric membrane resistance and the amount of the immobilized enzyme.

Acknowledgments

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