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BIODEGRADATION OF PHENOLS AND CYANIDES USING MEMBRANES OBTAINED BY CHEMICAL IMMOBILIZATION

The purpose of this study was to immobilize a group of enzymes isolated from a bacterial strain *Pseudomonas* adapted to degradation of phenol and cyanide on polyacrylonitrile membranes and to test their efficiency in biodegradation of the above mentioned xenobiotics. Ultrafiltration enzymatic membranes were tested using simulated phenol-cyanide wastewaters which contained 376 mg/dm^3 of phenol and 5 mg/dm^3 of cyanide. Carrying out ultrafiltration at optimal parameters being specified enabled an 89% degradation of phenol and a 65.2% degradation of cyanide after 6 hours.

1. INTRODUCTION

Due to the stricter standards determining a discharge of sewage into waters and soils, there is a need for a constant search for new unconventional technologies of sewage treatment as well as for improvement of existing ones. Wastewaters coming from petrochemical and coke industry and produced in the process of coking and coal liquefaction are extremely troublesome for the natural environment. They comprise a lot of toxic xenobiotics, particularly phenol and its derivatives (pyrocatechol, quinone, pyrogallol) as well as nitrogen derivatives: ammonia, isocyanate and cyanide. Standard methods of their purification are mainly biological and may be successfully applied if the concentration of the xenobiotics does not exceed the acceptable value above which the growth of microorganisms is restrained. Therefore, it is necessary to apply multistage methods of treatment, and ultrafiltration through enzymatic membranes may be one of them. They are characterized by separation properties and catalytic activity. The latter is influenced by the type and amount of biocatalyst and the method of its immobilization.

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Enzyme immobilization offers many advantages such as:

- high resistance of the enzymes to high temperature, inhibitors and chemical agents,
- easy separation of the reaction products from the biocatalyst,
- products of high purity,
- possibility of multiple application of the enzyme,
- low running-costs and reduced enzyme consumption.

However, it has also disadvantages such as reduction in enzyme affinity to the substrate or reduction in the maximum rate of the catalyzed reaction. These phenomena are usually caused by spherical problems – an active centre of protein is not easily accessible to the substrate. However, the selection of the immobilization method usually depends on the necessity of maintaining the highest catalytic activity of the enzyme [1]–[3].

The most important immobilization methods applied in practice are as follows:

- adsorption on the surface of the polymer carrier,
- covalent bonds with the carrier,
- inclusion in the porous structure of the membrane (inclusion inside the polymer),
- copolymerization with the protein carrier,
- gelation [4]–[7].

Synthetic polymers constitute the most numerous group of the carriers in use. Due to specificity of the immobilized enzymes and necessity of carrying out immobilization and catalyzed processes in aqueous environment, they should fulfil a number of conditions. Owing to chemical and physical modification of polymers, it is possible to obtain new polymers with reactive groups of a demanded physical structure. The following three different adaptations of polyacrylonitrile (PAN), functioning as a carrier, are an example of such a modification:

- In the reaction of polyacrylonitrile with dry hydrogen chloride in methanol, an imidoester is obtained which reacts with amino groups of the enzyme contained in cells. However, the method has one disadvantage, i.e. hydrolysis of imidoester to methyl ester which decreases the number of bound cells.
- Reduction of PAN nitrile groups with lithium aluminium hydride in the ether environment. The amino derivative obtained in this way is activated with glutaric aldehyde and bound with active proteins.
- Treatment of PAN with hydrazine hydrate results in the reduction of nitrile groups to primary amines; in this way we obtain a hydrazite which then is modified with glutaric aldehyde.

The purpose of this study was to immobilize chemically the enzymes isolated from bacterial strains *Pseudomonas* on polyacrylonitrile membranes and to determine their effectiveness in phenol and cyanide biodegradation. The mechanism of their biodegradation was described in detail in [8]–[14].

2. EXPERIMENTAL

2.1. APPARATUS

Immobilization was carried out applying a NUCLEPORE apparatus, type S-76-1000 [3]. Ultrafiltration was carried in the cross-flow mode (figure 1) on flat membrane made from polyacrylonitrile with its active surface of 155 cm^2. Initial feed volume equalled 25 dm^3.

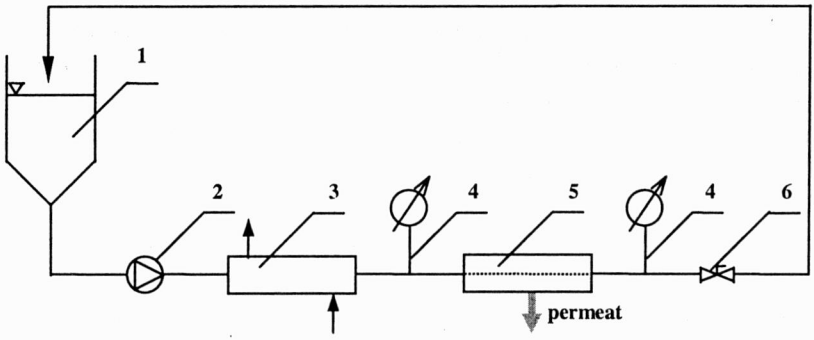

Fig. 1. A schematic diagram of the cross-flow mode: 1 – feed tank, 2 – pump, 3 – heat exchanger, 4 – pressure gauge, 5 – membrane module, 6 – pressure valve

2.2. ISOLATION OF THE ENZYMES FOR IMMOBILIZATION

In order to isolate a fraction of enzymes which degrade phenol and cyanide, bacterial strains of *Pseudomonas* isolated from a mixed population of microorganisms of activated sludge obtained by means of transformation of organic sludges after methane fermentation were used. The sludge came from the Municipal Sewage Treatment Plant in Częstochowa. The microorganisms were adapted to new conditions by enriching their culture with the increasing doses of wastewater produced in the Coking Plant in Zdzieszowice.

The following strains were predominant in the population:

- *Pseudomonas putida*,
- *Micrococcus sedentarius*,
- *Bacillus cereus*,
- *Staphylococcus sciuri*.

All the strains used in the research were adapted to the degradation of 400 mg/dm^3 of phenol and 10 mg/dm^3 of cyanide.

Enzymatic fractions were isolated according to Hagemann's method [15]. Activity of the catechol dioxygenase was assayed in the isolates in order to determine meta-

bolic pathways of phenol degradation by the strains examined. The isolated enzymes degraded the above-mentioned xenobiotic according to ortho- and meta-pathways.

2.3. PREPARATION OF NEUTRAL ULTRAFILTRATION MEMBRANES

The phase inversion method which is based on casting a film from a polymer solution in a solvent, and then on gelating it in a non-solvent (water) was used to prepare the membranes. They were formed from a non-matted fibre of PAN produced by Artificial Fibre Plant Chemitex-Anilana in Łódź and "Mavilon" PAN (imported from Hungary). The casting solution contained 12% wt of PAN (Łódź) and 17.5% and 18% wt of "Mavilon" in dimethyl formamide. The membranes thus obtained constituted a support for enzyme immobilization. Due to low mechanical strength of the membranes made from PAN "Mavilon", the most important part of the research was carried out on the flat PAN-12 membranes.

2.4. PREPARATION OF ENZYMATIC MEMBRANES

Immobilization of enzymes was carried out on a neutral ultrafiltration membrane modified chemically to obtain a stable, chemical cross-linking of the proteins on its surface. 500 cm^3 of a 25% hydrazine hydrate solution ($H_2NNH_2 \cdot H_2O$) was filtered through the membrane at a pressure of 0.5×10^5 Pa, then the membrane was washed with distilled water (5×1000 cm^3) and 500 cm^3 of a 5% glutaric aldehyde solution was filtered through it.

Immobilization of active proteins on the supports prepared in this way consists in a repeated filtration of an enzyme aqueous solution (concentration of 124 mg of protein/dm^3) through the modified neutral membrane at a pressure of 0.5×10^5 Pa until the permeate obtained is protein-free. Then, the immobilized membrane is washed with physiological salt in order to remove enzymes from its surface which are not bound permanently with the membrane [15].

Activity of the membrane was determined by filtering the model wastewater of phenol and cyanide concentrations of 9.4×10^{-2} g/dm^3 and 6.5×10^{-2} g/dm^3, respectively, at a temperature of 298 K for 10 minutes and measuring the concentrations the xenobiotics degraded during that time.

2.5. METHODS OF TESTING NEUTRAL AND ENZYMATIC ULTRAFILTRATION MEMBRANES

Transport properties of the membranes were determined for transmembrane pressures in the range of 1.0×10^5–3.0×10^5 Pa, at a constant velocity of the filtered medium flowing over their surface. Therefore, the membranes were tested with distilled water and a dependence of the volumetric water flux on pressure was determined. The

process was carried out in such a way as to collect 50% of the feed at a temperature of 298 K. The volumetric water flux J_w was calculated from the formula:

$$J_w = V_w/S \times t \ [\mathrm{m^3/m^2 \cdot s}]. \tag{1}$$

The separation properties of the membranes were determined by testing them 1) with a dextran solution of nominal molecular mass of 200 000 and the concentration of 5 g/dm^3, at a pressure of 1.0×10^5 Pa, collecting 10% of the feed [16], and 2) with model wastewater of phenol and cyanide concentrations of 0.376 g/dm^3 and 0.01 g/dm^3, respectively, applying a variable transmembrane pressure in the range from 1.0×10^5 Pa to 3.0×10^5 Pa. Each time, a constant temperature of the process (298 K) and a constant linear velocity of the flowing solution (2 m/s and 3 m/s) over the membrane's surface were maintained. A volumetric permeate flux (J_v) was determined for each test and the concentrations of phenol and cyanide were determined (by colorimetric methods [16], [17]) in the permeate and retentate which enabled calculating the concentrations of both xenobiotics:

$$J_v = V_v/S \times t \ [\mathrm{m^3/m^2 \cdot s}]. \tag{2}$$

When the membranes were examined with a dextran solution, a distribution of the molar masses of dextran was determined in the feed and permeate by means of gel permeation chromatography [18].

A biodegradation degree of phenol and cyanide (B_d) was calculated additionally for the membrane with immobilized enzymes:

$$B_d = 1 - (C_p \times V_p + C_r \times V_r)/C_f \times V_f. \tag{3}$$

3. RESULTS AND DISCUSSION

3.1. CHARACTERISTICS OF THE NEUTRAL ULTRAFILTRATION MEMBRANES

Figure 2 presents the characteristics of the neutral PAN-12 membrane. An increase in the transmembrane pressure caused an increase in the volumetric water flux. An increase in the pressure from 1.0×10^5 to 3.0×10^5 Pa brought about an increase in J_w by 60.1%. The dependence $J_w = f(\Delta P)$ was characteristic of a power function described by the following formula:

$$J_w = 1.31 \times 10^{-9} \times (\Delta P)^{0.83}. \tag{4}$$

Transport properties of the neutral membrane were determined by testing the membrane with model phenol–cyanide wastewater as well and establishing the dependence of the volumetric permeate flux on the transmembrane pressure (figure 2). It was described by the following formula:

$$J_v = 1.65 \times 10^{-9} \times (\Delta P)^{0.81}. \tag{5}$$

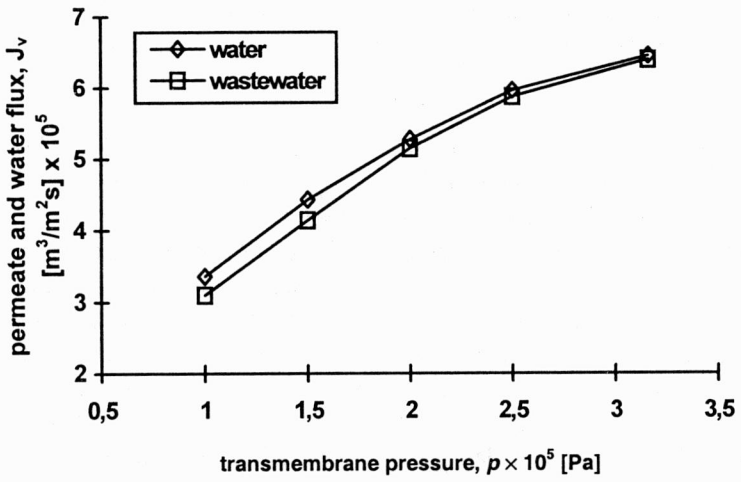

Fig. 2. Dependence of the volumetric permeate flux and the water flux on the transmembrane pressure for the neutral PAN-12 membrane

On the basis of the results obtained we can conclude that, similarly to water filtration, the volumetric permeate flux increases with increasing transmembrane pressure; however it is lower by 1–6% in comparison with the water flux obtained for the pressures examined. The increase in the pressure from 1.0×10^5 to 3.0×10^5 Pa affected the increase in J_v by 51.6%.

3.2. CHARACTERISTICS OF THE ENZYMATIC MEMBRANES

Enzymatic activities of the proteins immobilized on the membrane are presented in the table.

Table

Enzymatic activity of the immobilized membrane

Protein mass [mg]	Protein activity AC [mg of degraded xenobiotic/min.]	
	AC_{PH}	AC_{CN}
124	0.0132	0.0075

The enzymatic membranes were tested with a dextran solution (its nominal molecular mass reaches 200000, and the concentration – 5 g/dm^3) in order to determine the changes of their separation properties in relation to the neutral membrane used as support. Figure 3 illustrates differential curves of the dextran molar masses in the feeds and permeates. It follows from the curves that the separation properties of the

enzymatic membrane are to a large extent determined by the layer of the enzyme immobilized on the neutral support in the form of a secondary membrane.

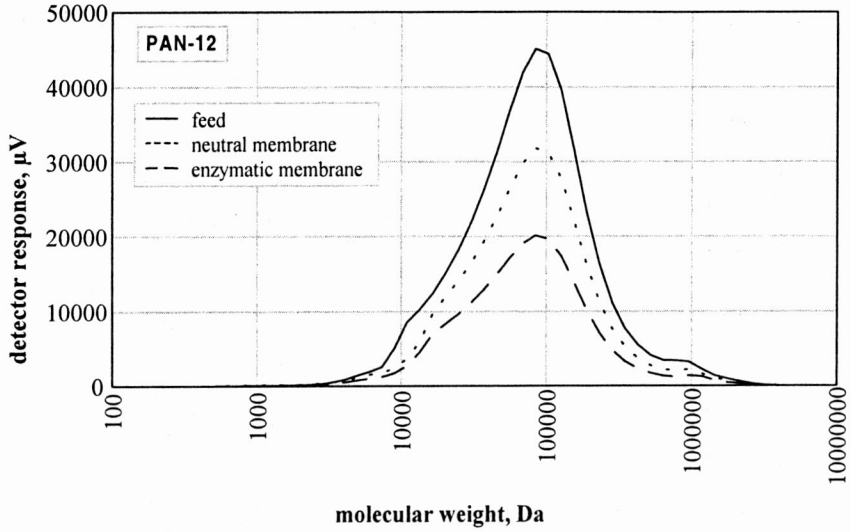

Fig. 3. Differential curves of dextrans' molar masses in the feeds and permeates for the neutral PAN-12 and enzymatic PAN-12E membranes

In order to determine the transport–separation properties of the enzymatic membrane, it was, similarly to the neutral one, tested with distilled water and model solution of phenol–cyanide wastewater.

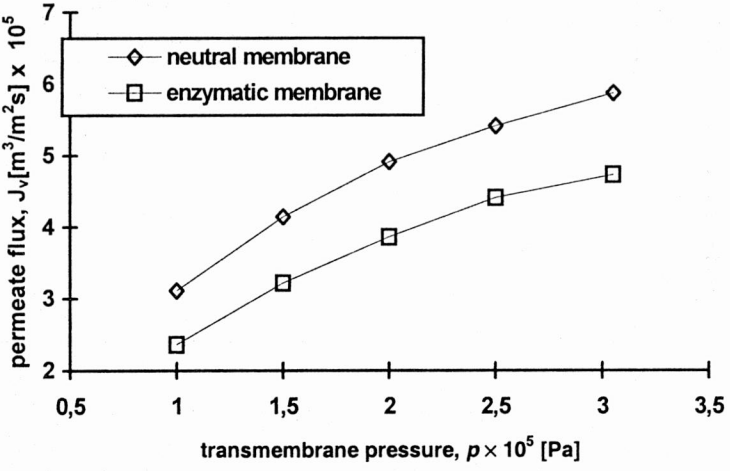

Fig. 4. Volumetric permeate flux versus transmembrane pressure for the neutral PAN-12 and the enzymatic PAN-12E membranes

Figure 4 presents a comparison of the volume water fluxes for the neutral and enzymatic membranes as a function of the transmembrane pressure. In the case of the enzymatic membrane as well as the neutral one, an increase in pressure causes an increase in the volume water flux and the dependence $J_w = f(\Delta P)$ is also characteristic of a power function.

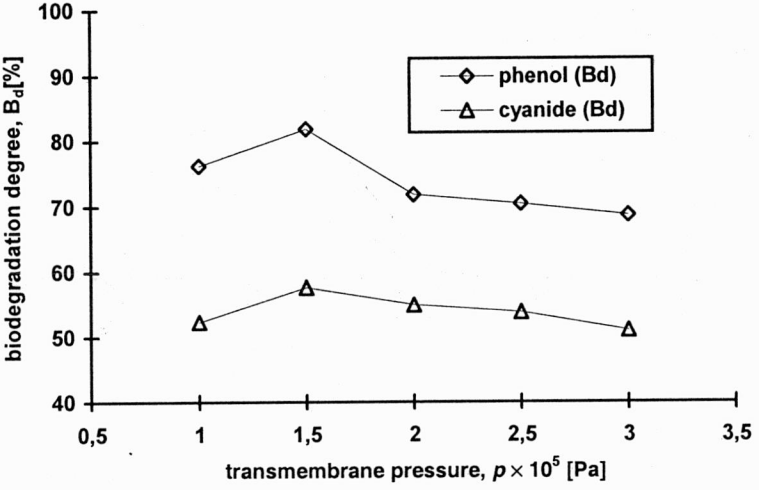

Fig. 5. Biodegradation degree of phenol versus transmembrane pressure for enzymatic membrane PAN-12E at linear velocity u = 3 m/s

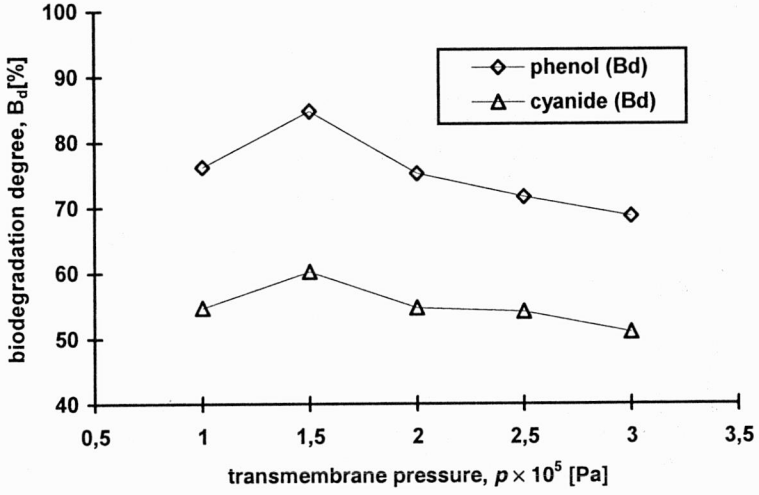

Fig. 6. Biodegradation degree of cyanide versus transmembrane pressure for enzymatic membrane PAN-12E at linear velocity u = 2 m/s

Ultrafiltration of model wastewater solution was conducted at a transmembrane pressure ranging from 1.0×10^5 to 3.0×10^5 Pa and a constant linear velocity (for each series) of the flowing medium. Then the biodegradation of the xenobiotics was estimated. The values obtained for particular pressures and linear velocities are shown in figures 5 and 6.

3.3. THE INFLUENCE OF PROCESS PARAMETERS ON THE EFFECTIVENESS OF PHENOL AND CYANIDE BIODEGRADATION

Ultrafiltration efficiency is greatly affected by the following parameters: transmembrane pressure, linear velocity of the medium flowing over the membrane's surface and duration of the process. The research was aimed at determining the influence of the above-mentioned parameters on the biodegradation degree of phenol and cyanide present in the model wasterwater and on the efficiency of the process.

3.3.1. TRANSMEMBRANE PRESSURE AND LINEAR VELOCITY

A dependence of the volumetric permeate flux on the transmembrane pressure is represented by equations (6) and (7). It was found that an increase in the pressure causes an increase in the volumetric permeate flux and the equations which describe this correlation take the form of a power function:

$$J_v = 2.14 \times 10^{-3} \times (\Delta P)^{0.63} \text{ (for the linear velocity, 3 m/s)}, \quad (6)$$

$$J_v = 1.88 \times 10^{-3} \times (\Delta P)^{0.51} \text{ (for the linear velocity, 2 m/s)}. \quad (7)$$

An increase in the pressure from 1.0×10^5 to 3.0×10^5 Pa brought about and increase in the permeate flux by 67.2% for $u = 3$ m/s and $u = 2$ m/s.

An analysis of the influence of pressure on phenol biodegradation (figures 5 and 6) showed that increased pressure promoted biodegradation. Within the pressure range from 1.0×10^5 to 1.5×10^5 Pa the biodegradation of this xenobiotic at $u = 3$ m/s and $u = 2$ m/s increased by 5.5% and 6.7%, respectively. However, at the pressure values exceeding 2.0×10^5 Pa, the biodegradation efficiency decreases at both linear velocities.

In the case of cyanide, biodegradation degree under the same process conditions increased by 5.3% at $u = 3$ m/s and by 8.4% at $u = 2$ m/s. There was a decrease in both values when the pressure exceeded 2.0×10^5 Pa. Therefore, 1.5×10^5 Pa is the most favourable transmembrane pressure for the biodegradation process, and 2 m/s is the most favourable linear velocity.

3.3.2. DURATION OF THE PROCESS

Figures 7 and 8 present the efficiency of phenol and cyanide biodegradation versus its duration. Lenghtening the time of the contact between xenobiotics and the membrane caused an increase in their biodegradation degree. The process was carried

out with linear velocity of 2 m/s and 3 m/s at the transmembrane pressure of 1.5×10^5 Pa. Then the biodegradation degrees B_d of both xenobiotics were determined every 30 minutes. Their values increased with the elapsing time (for both linear velocities) and after 360 minutes reached:

- at the linear velocity $s = 3$ m/s: for phenol, $B_d = 84.72\%$ and for cyanide, $B_d = 56.2\%$;
- at the linear velocity $s = 2$ m/s: for phenol, $B_d = 89.2\%$ and for cyanide, $B_d = 65.2\%$.

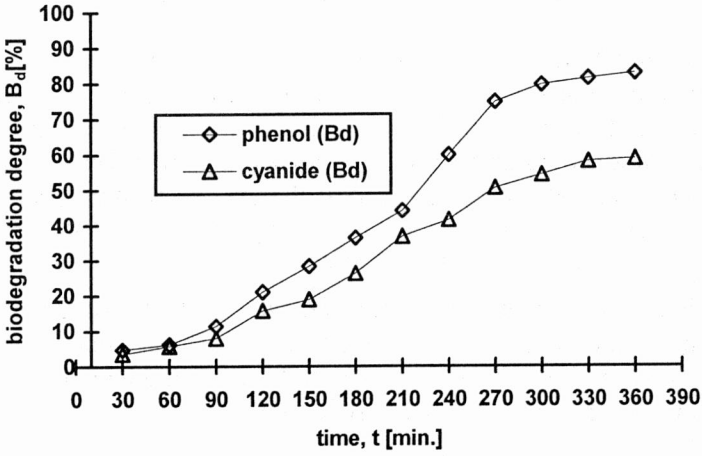

Fig. 7. Biodegradation degree of phenol versus time of biodegradation for the enzymatic membrane PAN-12E at linear velocity $u = 3$ m/s

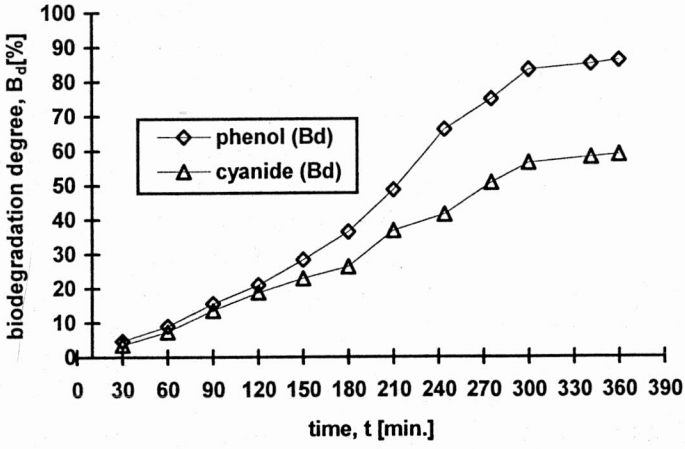

Fig. 8. Biodegradation degree of cyanide versus time of biodegradation for the enzymatic membrane at linear velocity $u = 2$ m/s

4. CONCLUSIONS

1. There is a possibility of applying ultrafiltration to phenol and cyanide biodegradation. To that end the membranes with immobilized enzymes from the *Pseudomonas* group and operating in the cross-flow mode are used.

2. Active proteins can be effectively immobilized using the method based on chemical immobilization of enzymes on the membrane made from polyacrylonitrile.

3. The most favourable parameters of ultrafiltration biodegradation of the xenobiotics examined are:

- transmembrane pressure: 1.5×10^5 Pa,
- linear velocity of the flowing medium: 2 m/s.

4. Ultrafiltration carried out at optimum operation parameters enables an 89% and 62.5% removal of phenol and cyanide, respectively, after 6 hours.

5. The activity of the enzymes immobilized on the membrane did not change during the process (360 minutes).

6. Low concentrations of phenol and cyanide in the permeate amounting to 4.89 mg/dm^3 and 1.6 mg/dm^3, respectively, enable their discharge into a biological sewage treatment plant without dilution with municipal sewage.

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NOTATION

AC – total protein activity; cm^3/min,
B_d – biodegradation degree; %,
C_n – xenobiotic concentration in the feed; g/dm^3,
C_p – xenobiotic concentration in the permeate; g/dm^3,
C_r – xenobiotic concentration in the retentate; g/dm^3,
J_v – volumetric permeate flux; m^3/m^{2}s,
J_w – volumetric water flux; m^3/m^{2}s,
P – difference in pressure on both sides of the membrane; Pa,
R – retention coefficient; %,
S – area of the membrane surface; m^2,
t – duration of the process; s,
V_f – volume of the feed; m^3,
V_p – volume of the permeate; m^3,
V_r – volume of the retentate; m^3,

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BIODEGRADACJA FENOLI I CYJANKÓW ZA POMOCĄ MEMBRAN ENZYMATYCZNYCH OTRZYMYWANYCH METODĄ IMMOBILIZACJI CHEMICZNEJ

Celem prowadzonych badań była immobilizacja wybranych enzymów wyizolowanych ze szczepu *Pseudomonas* sp. i adaptowanych do rozkładu fenoli i cyjanku na membranach poliakrylonitrylowych oraz określenie ich efektywności w procesie ultrafiltracyjnej biodegradacji obu ksenobiotyków. Ultrafiltracyjne membrany enzymatyczne testowano, używając symulowanych ścieków fenolowo-cyjankowych, które zawierały 376 mg/dm^3 fenolu oraz 5 mg/dm^3 cyjanku. Zastosowanie wyznaczonych optymalnych warunków ultrafiltracji pozwoliło usunąć 89% fenolu oraz 65,2% cyjanku w ciągu sześciu godzin.