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Production of ethanol from lactose in a bioreactor integrated with membrane distillation

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HIGHLIGHTS

- Ethanol production from lactose in bioreactor coupled with DCMD.
- Continuous transfer of ethanol through MD membrane
- Studies on influence of broth composition on ethanol productivity

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ABSTRACT

The fermentation of lactose to ethanol can be performed using yeast species *Saccharomyces cerevisiae*. The strategy involved the fermentation of prehydrolyzed lactose solutions in an enzymatic process using ß-galactosidase. The yeast consumes the resulting mixtures of glucose and galactose producing ethanol.
Ethanol from lactose solutions in a BIOTRON bioreactor integrated with a direct contact membrane distillation system (DCMD) was produced. During MD, the transport of volatile products of fermentation proceeded across the polypropylene, porous membrane submerged in the bioreactor.
It was found that the bioreactor integrated with DCMD can be successfully applied for ethanol production from lactose. A continuous removal of ethanol and other volatile products of fermentation from the broth, through the porous, hydrophobic membrane, resulted in an increase of ethanol productivity from lactose in comparison with the results obtained in a process performed in a classical reactor.

1. Introduction

In recent years, a growing attention has been paid to the conversion of biomass into ethanol, considered as the cleanest liquid fuel alternative to fossil fuels. The production of ethanol from renewable sources such as cellulose-rich organic materials (straw, wood) or agricultural by-products (molasses, whey) has been considered as a promising advance of ethanol fermentation technology [1,2].

Cheese whey is a by-product of dairy industries. It is an aqueous portion that is formed during the coagulation of milk casein in cheese making or in casein manufacturing process. Whey creates an important environmental problem because it is produced in high volumes and due to its high organic matter content (BOD of order 30–50 $g \cdot dm^{-3}$ and COD of 60–80 $g \cdot dm^{-3}$) [3]. On the other hand, whey contains lactose (4.5–5% w/v) which can be used as a substrate for the production of valuable compounds by fermentation [4]. Moreover, a large fraction of whey is dried to cheese whey powders [1], which is mostly used for animal feeding but smaller quantities may be also used in human foods, such as ice-cream, baked goods, cakes, sauces and milk derivatives [4]. Whey proteins have also non-food uses, mainly in cosmetics and pharmaceutical products [5].

There are two main types of whey, according to the procedure used for casein precipitation: acid whey (pH <5), resulting from the production of fresh or soft cheeses (such as cream and cottage cheese), and sweet whey (pH 6–7), resulting from hard (ripened) cheeses. The composition of different types of whey is variable [4].

Alcoholic fermentation is an interesting alternative for the bioremediation of the polluting liquor that remains after the separation of whey proteins. It is a biological process in which sugar such as lactose can be directly used by lactose-consuming microorganisms to produce ethanol and CO_2 or, alternatively, pre-hydrolyzed lactose solutions may be used as a substrate for lactose negative microorganisms [2,3].

The fermentation of whey lactose to ethanol can be also performed using yeasts. Although the yeasts that assimilate lactose aerobically are widespread, those that ferment lactose are rather rare [4], including e.g. *Kluyveromyces lactis*, *Kluyveromyces marxianus*, and *Candida pseudotropicalis*. The most commonly used yeast for lactose fermentation is *Saccharomyces cerevisiae*, which has also attracted much attention in recent years and it is usually the first choice for economic industrial processes involving alcoholic fermentation [3]. This yeast is also generally considered as a safe (GRAS) food additive for human consumption [2]. Since wild *S. cerevisiae* strains are unable to

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metabolize lactose, the initial strategies involved whey fermentation of prehydrolyzed lactose solutions. An enzymatic process using ß-galactosidase was frequently applied to the resulting mixtures of glucose and galactose [3]. *S. cerevisiae* can utilize galactose due to the catabolite repression phenomenon [6], but this strain consumes glucose preferentially to galactose [7]. Therefore, several process parameters must be optimized: substrate concentration, enzyme to substrate ratio, the dosage of the active components (ß-galactosidase to prehydrolyzed lactose ratio) in the enzymatic mixture, and the yeast concentration [2].

Ethanol production by the conventional process of a batch fermentation of sugar with yeast, followed by distillation to recover ethanol is rather uneconomical in comparison with fossil fuels [8]. Membrane technologies as highly selective and energy-saving unit operations have a great potential in the ethanol production [9]. The separation of ethanol from the fermentation broth may be successfully performed using membrane distillation (MD) [10].

Membrane distillation is a process in which a hot feed solution evaporates through the pores of a hydrophobic membrane. In direct contact membrane distillation (DCMD) the feed is in a direct contact with a hydrophobic porous membrane and the permeate is directly condensed in the cooling stream flowing along the membrane surface. Non-volatile solutes contained in the feed are completely retained by the membrane. The driving force for mass transfer is the difference in vapor pressure between the feed and the permeate side of the membrane. During MD, ethanol vapor is transferred preferentially through the membrane pores because its partial pressure is higher than that of water [10,11]. Thus, the application of a bioreactor coupled with a MD system enables to achieve a distillate enriched with ethanol [9,12,13].

The use of MD for the removal of ethanol and other volatile metabolites from broth will both decrease the inhibitory effect of these compounds on microbial culture and reduce the cost of further separation and concentration of alcohol. The major requirement of MD process is that membranes must not be wetted by separated solutions. However, the products of a fermentation can decrease the surface tension of the feed and may accelerate membrane wettability. During broth separation by the MD process, a significant decrease in the membrane module productivity was observed due to increased membrane wettability. Moreover, a small part of microorganisms may be deposited on the membrane surface and partially block the membrane pores. However, the results obtained in other studies did not confirm any such rapid fouling of membranes caused by broth during MD [13].

The main object in the study was ethanol production from model lactose solutions in a BIOTRON bioreactor integrated with a direct contact membrane distillation system (DCMD). The use of DCMD for the removal of ethanol and other volatile metabolites from the broth during fermentation process was studied. The influence of broth component concentrations (sugar and ethanol) in a bioreactor coupled with membrane distillation (MDBR) on the magnitude of ethanol flux was also determined.

2. Experimental

The experiments were carried out using a BIOTRON bioreactor equipped with a capillary membrane made from polypropylene (Membrana GmbH, Germany). The scheme of the DCMD experimental set-up was shown in Fig. 1 The details of this set-up performance were presented in [12]. The membrane module consisted of two capillary membranes which have lengths that amounted to 112 mm. These membranes had pore sizes with a nominal and maximum diameter of 0.2 μm and 0.6 μm, respectively, and porosity of 70%.

The outside side and lumen side diameter of the capillary membranes were 2.6 and 1.8 mm, respectively. The effective area of mass transfer amounted to 0.0183 m^2. The membranes (without shell)

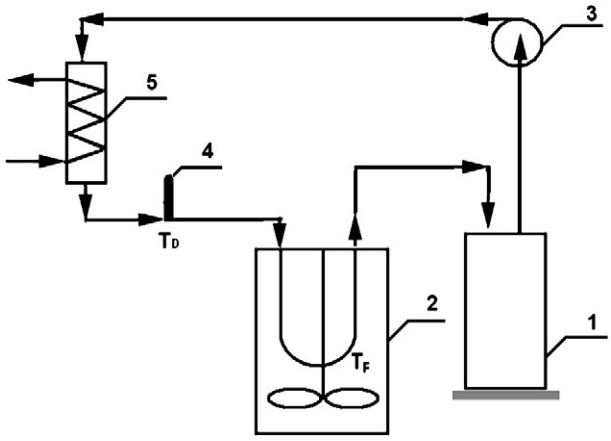

Fig. 1. Scheme of the experimental apparatus. 1— distillate tank, 2— bioreactor with capillary module, 3— pump, 4— thermometer (T_D-temperature of the distillate), 5— cooling system.

were directly immersed in a fermentation broth in the bioreactor tank. The bioreactor was equipped with a high speed agitator. The membranes were located around the agitator in the way protecting them against mechanical damage. The peristaltic pump supplied the distillate from the distillate tank, through a cooling system to the lumen side of the capillary membranes. Ethanol produced from lactose and the water vapor diffuse through the air filling membranes pores and then condense directly in the cold distillate stream (direct contact MD).

The ethanol flux, J_E, was calculated from the material balance of ethanol in the distillate performed every 24 h taking into account the changes in the mass and the ethanol concentration in the distillate:

$$J_E = \frac{C_{t+1}\cdot m_{t+1} - C_t \cdot m_t}{d\cdot A\cdot 1000\cdot t}\left(kg\cdot m^{-2}\cdot 24\cdot h^{-1}\right) \quad (1)$$

where:

C_{t+1}	distillate concentrations at time $t+1$ [$g\cdot dm^{-3}$]
C_t	distillate concentrations at time t [$g\cdot dm^{-3}$]
m_{t+1}	mass of the distillate at time $t+1$ [g]
m_t	mass of the distillate at time t [g]
d	density [$g\cdot dm^{-3}$]
A	membrane area outside capillaries [m^2]
t	time [h]

In our study the distillate constitutes a sum of water initially contained in the MD system in a cool loop together with the vapor of water and ethanol transferred through the membrane during MD. Moreover, taking into account the permeate flux and the changes in ethanol concentration in the distillate, the ethanol concentration in the permeate (the portion of the ethanol and water vapor of the feed transferred through the membrane), ΔC_p, was calculated from the equation:

$$\Delta C_P = \frac{C_{t+1}\cdot m_{t+1} - C_t\cdot m_t}{m_{t+1} - m_t}\left(g\cdot dm^{-3}\right) \quad (2)$$

where:

C_{t+1}	distillate concentrations at time $t+1$ [$g\cdot dm^{-3}$]
C_t	distillate concentrations at time t [$g\cdot dm^{-3}$]
m_{t+1}	mass of the distillate at time $t+1$ [g]
m_t	mass of the distillate at time t [g]

The studies of fermentation were performed using the aqueous solutions of lactose at the following concentrations: 50, 100 and

200 g·dm^{-3}. Before the fermentation, lactose was subjected to the hydrolysis with the use of *Aspergillus oryzae* β-galactosidase enzyme. The previous studies demonstrated that the ratio of enzyme to lactose equal 1 mg enzyme per 1.0 g lactose was favorable. The hydrolysis process proceeded for 24 h at 283 K. Subsequently, the hydrolyzed solutions were subjected to the fermentation process in a BIOTRON bioreactor integrated with the DCMD. A commercially available dry Gamma Hefe yeast (*S. cerevisiae*, AB Enzymes, Germany) was used. The ratio of lactose to yeast was 15 g·g^{-1}. Yeast rehydration was performed for 30 min using small amounts of the lactose solution. The mixture was periodically agitated.

A periodical dosing of the prehydrolyzed sugar solution after each 24 h (the mass which was supplied was equal to the mass transferred from the broth to the distillate during fermentation in the MDBR) allowed stabilization of the yeast productivity of the bioreactor.

The productivity (P) is a measure of the efficiency of ethanol production in the MDBR calculated on a unit of lactose mass per time according the equation:

$$P = \frac{\Delta m_P + \left(C_{b(t+1)} \cdot m_{b(t+1)} - C_{b(t)} \cdot m_{b(t)}\right)}{m \cdot t} \left(g \cdot dm^{-3} \cdot h^{-1}\right) \quad (3)$$

where:

Δm_p the ethanol mass in the permeate [g]
$C_{b(t+1)}$ sugar concentrations at time $t+1$ [g·dm^{-3}]
$C_{b(t)}$ sugar concentrations at time t [g·dm^{-3}]
$m_{b(t+1)}$ mass of the broth at time $t+1$ [g]
$m_{b(t)}$ mass of the broth at time t [g]
m initial mass of the broth[g]
t time [h]

The fermentation experiments were carried out at the feed (broth) temperature of 310 K. The bioreactor was equipped with a thermostat and temperature sensor which measured broth temperature continuously. The inlet temperature of the cold distillate was kept at 293 K for all the experiments. The initial mass of fermentation broth used as the feed was 2800 g. The cold system was initially supplied by 600 g of distilled water. The fermentation process combined with DCMD was run for 96 h with a continuous separation of ethanol by the capillary membrane and thus the process was repeated seven times. The aim of this procedure was the assessment of the impact of the fouling degree on the membranes performance. After each experiment, the feed was replaced by distilled water and the MD installation was rinsed for 1 h. The influences of broth composition, on the fermentation process were studied. Moreover, the effect of the fermentation conditions on the ethanol flux through the pores of capillary membrane in the membrane distillation process was investigated.

Samples of the broth (25 cm^3) and distillate were collected every 24 h. The sample of broth was first centrifuged at 9000 rpm (Centrifuge MPW-350R, Med-Instruments), and then filtered (membrane filter 0.45 μm, Millipore). The content of alcohol both in the feed (broth) and distillate was determined on the basis of the total organic carbon analysis using TOC-Analyzer multi N/C, Analytic Jena. For this analysis, filtered sample (10 cm^3) was divided into two the same portions (5 cm^3). One portion was allowed to evaporate ethanol and other volatile metabolites to obtain an approximately 50% reduction in the sample volume and the other portion was diluted in the same manner as the sample that was not evaporated. The ethanol concentration in the broth was calculated based on the TOC difference between the evaporated and non evaporated samples. This method assumes that the main components of the broth are ethanol and sugar. For this assumption, the error of ethanol concentration determination did not exceed 3–5% [14]. As was shown in the article cited, the validation of this method by chromatographic method was confirmed.

The amount of sugars (mixture of lactose, glucose and galactose) was determined by the Bertrand method [15] which is based on the reducing ability of various compounds. The reduction reaction was carried out in the alkaline environment while reducing sugars can easily reduce oxide copper II into oxide copper I ($Cu^{+2} \rightarrow Cu^{+1}$). The concentrations of individual sugar after enzymatic hydrolysis and residual sugar in the broth during fermentation were not determined.

The FT-IR method was used for analysis of deposits which were formed on the membrane surface both on the internal and external side as a result of the broth separation in the MD process. The studies were performed with four membrane samples collected from dried module after 860 h of broth separation using MD. The samples of fouling membrane were cut along and both layers were analyzed. The clean membrane samples and dried protein standard were used in the studies. A Thermo Scientific Nicolet 380 FT-IR Spectrometer equipped with a special adapter to investigate a thin layer surface was used to determine the FT-IR spectra.

3. Results and discussion

The fermentation processes in a BIOTRON bioreactor integrated with the direct contact membrane distillation system (MDBR) were performed. The DCMD process was used to separate and remove the volatile compounds produced in the bioreactor during the fermentation of prehydrolyzed lactose solutions with the contribution of the yeast species *S. cerevisiae*. Both the water vapor and ethanol vapor were transferred through pores of the polypropylene capillary membrane and then condensed in a cold distillate. Whereas, all sugars including non-hydrolyzed lactose, galactose and glucose as well as cells of yeast were totally retained in the broth. The efficiency of ethanol production during the fermentation process in a MDBR was directly dependent on the initial sugar (and respectively β-galactosidase and yeast) concentration in the broth (feed). As fermentation process proceeds the successive doses of sugar were converted to ethanol. The addition of sugar solution into the broth maintained continuity of the fermentation process. The highest ethanol concentration in the broth was obtained when the initial concentration of lactose was 200 g·dm^{-3} (Fig. 2). Therefore, the ethanol concentration in the broth enhances the driving force for MD (difference in the ethanol vapor pressure on both sides of the membrane) and the ethanol concentration in the distillate increased. In the case of continuous separation and removal of ethanol in the MDBR system, the broth concentration comprises an important parameter affecting the magnitude of permeate flux. As can be seen the ethanol concentration in the feed increased gradually during the first 24 h of the fermentation in a MDBR when the initial concentration of lactose in broth solution was respectively 50 and 100 g·dm^{-3}. However, for the initial lactose concentration equal to 200 g·dm^{-3}, the maximum ethanol concentration was achieved after 48 h of fermentation process. At the end of fermentation the ethanol concentration in a broth decreased markedly in all processes.

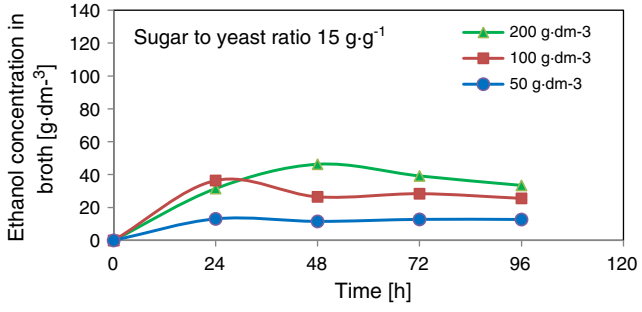

Fig. 2. Changes in ethanol concentration in the broth as a function of time during the fermentation in a MDBR. Feed temperature: 310 K.

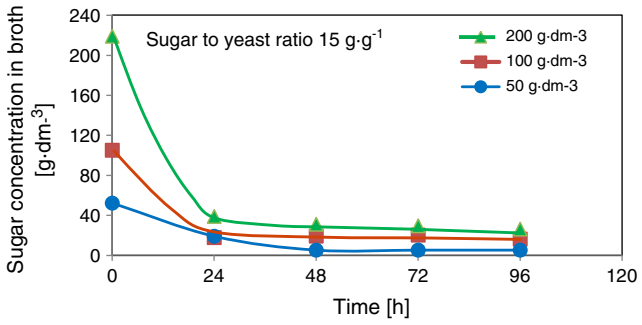

Fig. 3. Changes in sugar concentration in the broth as a function of time during the fermentation in a MDBR. Feed temperature: 310 K.

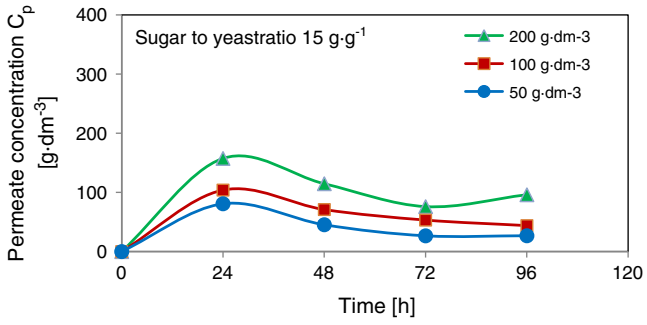

Fig. 5. Changes in ethanol concentration in the permeate as a function of time during the fermentation in a MDBR. Feed temperature: 310 K.

The sugar concentration during the fermentation also decreased rapidly after the first 24 h of process operation. Thus, the fermentation productivity was reduced. Sugar is a substrate for the alcohol production, therefore its lowest concentration in the broth affected the lowest ethanol concentration. The changes in sugar concentration in the broth during the fermentation are presented in Fig. 3. A significant increase in the rate of sugar consumption in the broth was observed during the entire fermentation course in the MD system. The broth was supplemented by small dosages of sugar solutions during the process. As a result, a constant volume of the feed was maintained during the fermentation by yeast.

In DCMD, for broth solution used as a feed, the permeate flux is a sum of water and ethanol flux and other volatile components transferred in a vapor form through the hydrophobic membrane. The results shown in Fig. 4 indicated that the permeate flux across the membrane initially decreased during the course of MD and was then stabilized during the experiments of fermentation in the MDBR. The highest decline of the permeate flux was observed during the fermentation process when the initial lactose concentration was 200 $g \cdot dm^{-3}$. After that process the permeate flux was practically maintained on the constant level during fermentation processes and varied from 16.4 to 13.3 $kg \cdot m^{-2} \cdot 24 \cdot h^{-1}$. Fig. 4 presents changes in the permeate flux during the consecutive fermentations as a function of the elapsed time of the membrane performance. After the fermentation of lactose solution with the initial concentration of 200 $g \cdot dm^{-3}$, the initial permeate flux have not been attained (after 384 h) despite of the membrane washing. It was probably caused by the adsorption of proteins on the membrane surface. Thus the permeate flux was a little lower than that in previous experiments. At the beginning of each fermentation process, when the concentrations of sugar in the broth was high, it was found that the permeate flux was markedly lower. However, the sugar concentrations in the broth for initial values of 50 $g \cdot dm^{-3}$ and 100 $g \cdot dm^{-3}$ after 24 h of the fermentation process (Fig. 3) were similar and amounted to 10–20 $g \cdot dm^{-3}$ and about 20 $g \cdot dm^{-3}$, respectively. Thus, the permeate flux as a function of the initial lactose concentration was not varied after 408 h in Fig. 4. Moreover, a comparison of the permeate flux for fermentation broth with the permeate flux amounted to 22.7 to 17.8 $kg \cdot m^{-2} \cdot 24 \cdot h^{-1}$ for the distilled water used as a feed after fermentation, demonstrated that a thin layer of yeast protein was deposited on the membrane surface causing its fouling. It was found that under process conditions, the fouling was on the same level for subsequent experiments. Probably CO_2 produced during the fermentation allow to remove an excess of deposited yeast [10]. The permeate flux for water increased after the membrane cleaning, what indicated on the partial removal of deposits responsible for fouling.

In MD, all volatile components of the feed are transferred through a non-wetted membrane to the distillate (comprising water and ethanol) composition of which depends on both the volatility of these components and the feed concentration. The changes in ethanol concentration produced in the broth during the fermentation in the MDBR are shown in Fig. 2. The ethanol concentration in the permeate corresponds to the changes of ethanol content in the feed. According to broth-vapor equilibrium, the permeate was enriched in ethanol during the fermentation especially when the concentration of ethanol in the broth was increased. The highest ethanol concentration in the permeate, equal to 157.38 $g \cdot dm^{-3}$ was achieved for the broth with initial lactose concentration amounted to 200 $g \cdot dm^{-3}$. The ethanol concentration both in the feed (Fig. 2) and permeate (Fig. 5) increased considerably during the first 24 h for the fermentation in a MDBR independently on the sugar concentration in the feed and was significantly higher for higher initial concentration of lactose in a broth solution. At a lower ethanol concentration (resulting from a lower lactose concentration), the driving force for ethanol transfer was decreased and resultant ethanol concentration in the permeate (Fig. 5) was lower.

Fig. 6 presents the changes in the ethanol concentration in the distillate as a function of fermentation time. The changes in the ethanol concentration were the highest for the first 24 h. In the consecutive hours of the process, the changes became smaller due to a lower ethanol concentration in the permeate (Fig. 5). It is worthy to note, that the driving force for ethanol transfer in the process decreased and the

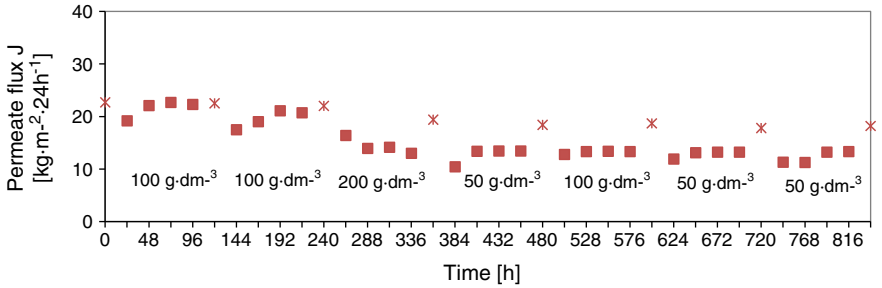

Fig. 4. Changes in permeate flux as a function of time during the fermentation in a MDBR. Feed temperature: 310 K. *permeate flux for distilled water used as a feed performed after the fermentation in the MDBR.

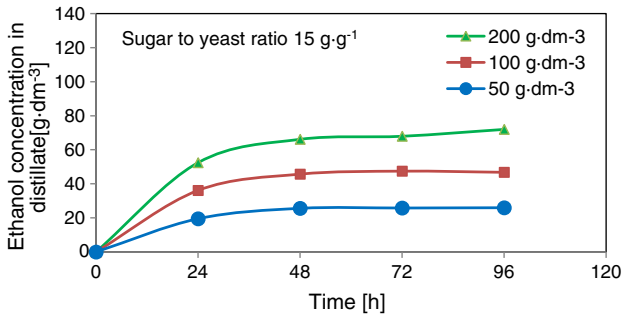

Fig. 6. Changes in ethanol concentration in the distillate as a function of time during the fermentation in a MDBR. Feed temperature: 310 K.

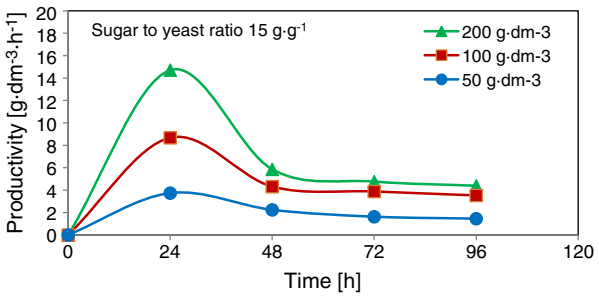

Fig. 9. Changes in productivity in time during the fermentation in a MDBR. Feed temperature: 310 K.

changes in the ethanol flux were smaller (Fig. 7). It should be also noticed that the ethanol concentration in the distillate was 1.4 times higher than that in the broth during the first 48 h of fermentation for the case when the initial sugar concentration was equal to 200 $g \cdot dm^{-3}$ and the maximum concentration was achieved.

In an accordance with the changes of ethanol concentration in the broth, the highest value of the ethanol flux was obtained at the first 24 h of the process and after that time the flux slightly decreased. A comparison of Figs. 4 and 7 presenting the permeate flux and ethanol flux indicates, that the rate of water transfer was much higher than that of ethanol. It was associated with differences in water and ethanol partial pressure in the equilibrium with broth under variable operation conditions.

The changes in the total ethanol concentration (recalculated as a sum of the ethanol content in the broth and distillate) determined during the fermentation as a function of process time are shown in Fig. 8. The tendencies of these changes indicate that, the ethanol concentration increased and reached maximum values after the first 24 h of the fermentation process. Similar results were found during the fermentation without DCMD (results not published here). The total ethanol concentration was 1.5, 2.3 and 2.4 times higher at 24 h when the initial concentration of broth solution was 50, 100 and 200 $g \cdot dm^{-3}$, respectively, in comparison with the classical fermentation (without DCMD). The total ethanol concentration values were dependent on the initial sugar concentration in the broth. When the sugar concentration was higher, the ethanol concentration was also higher and thus the maximum productivity was achieved. The removal of volatile fermentation products by MD allows to maintain a high productivity of the bioreactor at the first 24 h during the fermentation in a MDBR for all the initial broth compositions (Fig. 9). The productivity of 1.34, 3.04 and 5.75 $g \cdot dm^{-3} \cdot h^{-1}$ was obtained when the fermentation was carried out for the broth concentration of 50, 100 and 200 $g \cdot dm^{-3}$, respectively. After that, the productivity of the fermentation was gradually decreased. In comparison with literature data [16] for both the membrane distillation and pervaporation, the values of productivity depend on the process conditions and the microorganism used in the fermentation process. For example, the highest ethanol productivity from lactose was 1.5–2 $g \cdot dm^{-3} \cdot h^{-1}$ in micro-aerated fermentations (without membrane separation) [17]. In the case of fermentation combined with the MD the production rate was in the range of 2.5–4 $g \cdot dm^{-3} \cdot h^{-1}$ whereas in the classical batch fermentation from saccharose this rate was lower and amounted to 0.8–2 $g \cdot dm^{-3} \cdot h^{-1}$[13]. The productivity of ethanol obtained in pervaporation process was also lower than that in MD and was in the range of 1.58 to 2.48 $g \cdot dm^{-3} \cdot h^{-1}$ [18]. The experimental results presented in Figs. 9 and 10 demonstrated, that the fermentation combined with the membrane distillation process proceeded considerably faster and with a higher productivity compared to the fermentation carried out without DCMD despite a similar ethanol concentration in a MDBR. The productivity was 3, 4 and 8 times higher at 24 h when the initial concentration of broth solution was 50, 100 and 200 $g \cdot dm^{-3}$, respectively in comparison with the results obtained in the classical fermentation (without DCMD) (Fig. 10).

The composition of fermentation broth can be negatively affected on the performance of a hydrophobic membrane. However, the conducted long-term investigations demonstrated the high durability of

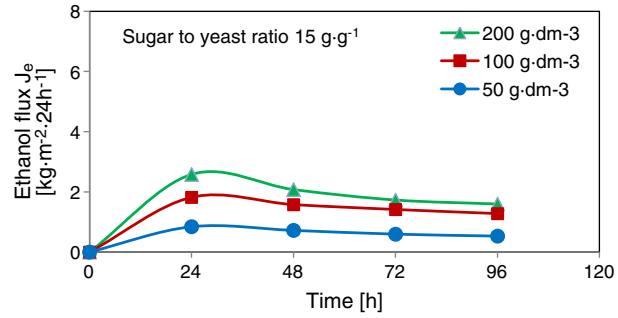

Fig. 7. Changes in ethanol flux as a function of time during the fermentation in a MDBR. Feed temperature: 310 K.

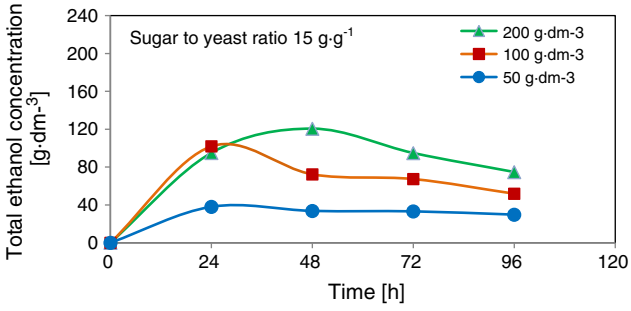

Fig. 8. Changes in total ethanol concentration as a function of time during the fermentation in a MDBR. Feed temperature: 310 K.

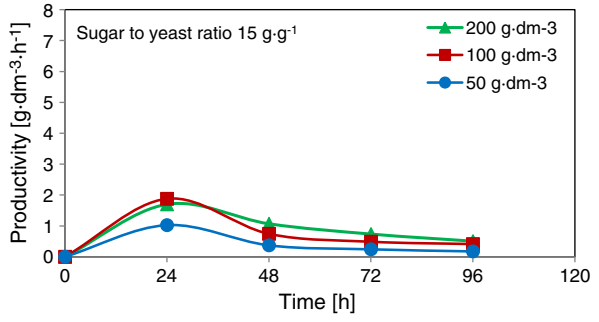

Fig. 10. Changes in productivity in time during the fermentation in a classical reactor. Feed temperature: 310 K.

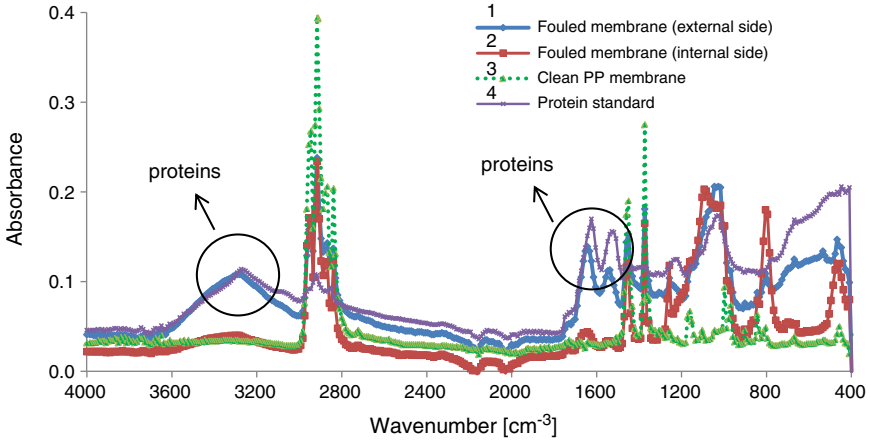

Fig. 11. FT-IR spectra of clean polypropylene membrane, fouled membrane after the fermentation in a MDBR and dry protein standard.

used polypropylene membranes under the conditions existing in the bioreactor. The studies of fermentation integrated with MD were carried out for 860 h and the MD module efficiency (value of permeate flux) was maintained at the same level (Fig. 2).

During the separation of broth by the DCMD process its components were partially adsorbed on the surface of the hydrophobic membrane. It was suggested that a small part of yeast proteins can penetrate the membrane pores or deposit on the membrane surface resulting in a partial wetting of MD membrane during the fermentation in a MDBR. The FT-IR method was used to determine the composition of the deposit precipitated from the feed as well as deposit accumulated on the membrane surface on the internal and/or external side. The studies were performed with four membrane samples collected from the module. The FT-IR spectra of clean and fouled polypropylene membranes are shown in Fig. 11. The spectrum of dried protein standard is also shown in this figure. The fingerprint region of protein IR spectrum is the region from 1800 to 800 cm^{-1}. In this range the bonds forming the amide group (C═O, N–H, and C–N) exhibit absorption. Only the spectrum of the internal side of the fouled membrane prepared from PP exhibits IR peaks, i.e. a broad band in the region of 3700–3000 and 1800–1500 cm^{-1}, which are absent for a clean membrane. These peaks are characteristic for proteins [19]. The two most important vibrational modes of amides are the amide I vibration, caused primarily by the stretching of the C═O bonds, and the amide II vibration, caused by deformation of the N–H bonds and stretching of the C–N bonds. The amide I vibration is measured in the range from 1700 to 1600 cm^{-1} and the amide II region from 1600 to 1500 cm^{-1}. The exact frequencies at which these bonds absorb depend on the secondary structure of the proteins or peptides [20]. A very broad peak in the region between 3700 and 3000 cm^{-1} indicates on the presence of exchangeable protons, typically for amine, amide or carboxylic acid groups present in the proteins. The FT-IR analysis confirmed the presence of proteins only on the membrane surface. The proteins did not penetrate the membrane structure in the examined module, therefore their presence on the internal side was not observed (Fig. 11). It can be seen in Fig. 10 that the intensity of the peaks characteristic for polypropylene (PP) undergoes a significant reduction in the case of fouled membranes, due to the accumulation of foulants on the membrane surface with foulants. The spectrum of internal side of fouled membrane exhibits peaks with a high intensity characteristic for PP, and a lower intensity derived from protein. In the case of the membrane being more fouled, the above mentioned relationship is reversed. The results confirm that the fermentation solutions cause only a slight biofouling during the fermentation in the MDBR. Moreover, the polypropylene membranes used in the study were not wetted by the broth solutions. The module was replaced after 860 h of broth separation in the MDBR by a new membrane module because of the mechanical damage was observed.

4. Conclusions

It was found that the fermentation of lactose into ethanol in a bioreactor coupled with DCMD can be performed successfully. The polypropylene membranes used in the study were not wetted by the solutions of ethanol and the broth. A continuous removal of ethanol and other volatile products of fermentation during the process carried out in the MDBR resulted in an increase of the productivity of ethanol from lactose in comparison with results obtained for fermentation performed in the classical reactor.

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