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ORIGINAL PAPER

Ethanol production in a bioreactor with an integrated membrane distillation module‡

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Ethanol production in a bioreactor with integrated membrane distillation (MD) module has been investigated. A hydrophobic capillary polypropylene membrane (Accurel PP V8/2 HF), with an external/internal diameter ratio, d_{out}/d_{in} = 8.6 mm/5.5 mm and pore size 0.2 μm, was used in these studies. The products (mainly ethanol and acetic acid) formed during the fermentation of sugar with *Saccharomyces cerevisiae* inhibited the process. These products were selectively removed from the fermentation broth by the MD process, which increased the efficiency of the conversion of sugar to alcohol from 0.45 g to 0.5 g EtOH per g of fermented sucrose. The bioreactor efficiency also increased by almost 30 %. Separation of alcohol by the MD generates a higher yield of ethanol in the permeate than in the broth. The enrichment coefficient amounted to 4–8, and depended on the ethanol concentration in the broth. The separated solutions did not wet the membrane in use for 2500 h of the MD experiments and the retention of inorganic solutes was close to 100 %.

Keywords: bioreactor, membrane distillation, bioethanol

Introduction

Ethanol is regarded as a source of energy and valuable fuel, and it is also an important material in the food and chemical industries (Demirbas, 2007). Ethanol fermentation from renewable feedstock using *Saccharomyces cerevisiae* is a product-inhibited process. The inhibition begins when alcohol concentration exceeds 30 g dm^{-3} and it is very significant for ethanol concentration over 80 g dm^{-3} (Bai et al., 2008). Therefore, fermentation is traditionally conducted in batch reactors, resulting in a solution containing 5–12 % of ethanol. Due to bioethanol's low concentration, its recovery from the culture broth by distillation is seen to be a cost-intensive operation. Moreover, a major problem in commercial ethanol production is the excessive amount of waste water discharged from the distillation column. Several methods have been proposed to minimise the product inhibition by the continuous removal of ethanol from the fermentation broth. These new technologies are frequently both more environmentally friendly and cheaper than the traditional ones (Bai et al., 2008; Cardona & Sanchéz, 2007; Kargupta et al., 1998).

Continuous fermentation is more attractive than the batch process due to its higher productivity, better process control and improved yields (Choi et al., 2009; Kargupta et al., 1998; Park et al., 1999). The challenge is how to effectively remove the yeast-produced metabolites from the broth. A selective separation of ethanol only has the effect of increasing the concentration of other metabolites and substrates that have not been used. It creates an unfavourable reaction environment for yeast and, as a result, suddenly decreases

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the number of yeast cells and fermentation efficiency after a few dozen hours (Gyamerah & Glover, 1996; Maiorella et al., 1984). In order to decrease the concentration of metabolites, a proportion of the broth is removed from the bioreactor (Escobar et al., 2001; Kaseno et al., 1998).

Several investigations have shown that the application of membrane processes reduced many technological problems, such as the negative influence of fermentation conditions on yeast, increased productivity, and reduced production costs (Escobar et al., 2001; Kargupta et al., 1998; Maiorella et al., 1984). Membrane distillation can be successfully applied to effect ethanol removal from the fermenting broth (Gryta et al., 2000; Gryta, 2001; Kaseno et al., 1998).

The MD is an evaporation process of volatile feed components through air-filled pores of a hydrophobic membrane (non-wetted). An MD membrane separates two aqueous solutions differing in temperature and composition. The driving force for the mass transfer through the membrane pores is the difference in vapour pressure on both sides of the membrane, which depends on the temperature and the solution composition in the layers adjacent to the membrane. By distilling a water–ethanol solution by the MD, a flow of both ethanol and water vapour through the membrane is achieved. However, at a given temperature, the volatility of ethanol is higher so the distillate obtained would be enriched in ethanol. For diluted solutions of alcohol (up to 10 %), it is possible to obtain a distillate with a concentration of ethanol 3–8 times higher than that in the feed (Gryta, 2001; Kaseno et al., 1998).

The components of a fermentation broth can quickly foul the surface of membranes (fouling). Fouling is one of the main reasons limiting the industrial application of membrane processes. In the case of the MD, the presence of alcohol poses an additional threat as it may accelerate the membrane wettability. During separation of the broth by the MD process, a significant decrease in membrane module productivity was observed due to increased membrane wettability (Udriot et al., 1989). However, results obtained in other studies did not confirm any such rapid fouling of membranes caused by broth during the MD (Gryta, 2001; Kaseno et al., 1998). In addition to the fermentation conditions, the results obtained in the studies were most probably influenced by the type of membranes used. Therefore, further research is needed into the resistance of membranes used for fermentation broth separation in the MD process.

The study presents the results of long-term investigations into fermentation linked with the MD process. The performance of hydrophobic polypropylene capillary membranes was evaluated when volatile metabolites were released from the bioreactor. The influence of such broth components as sugar, yeast, and alcohol on the magnitude of ethanol flux was determined.

Experimental

The fermentation process was carried out in the laboratory installation described previously (Gryta et al., 2000). The installation was made from elements and materials produced for constructing industrial installations. The reactor vessel (5 dm^3), the valves, and the pipeline were made from acid-resistant steel (ASI 316L). The content of the bioreactor was stirred using an external circulation system in which the liquid was pumped by a GN-G35JF59ETZ pump with an impeller (Micropump, USA). A water cooler and an electric heater were mounted in the circulation system, controlled by a RE26 regulator (Lumel, Poland). The regulation systems used in the installation stabilised both the fluid flow-rate ($\pm$ 5 dm^3 h^{-1}) and its temperature ($\pm$ 1.5 K).

For the fermentation studies with the MD, the membrane module was connected to the external circulation system of the bioreactor. A polypropylene capillary membrane (Accurel PP V8/2 HF, Membrana GmbH, Germany) was assembled inside the MD module. The length and external and internal diameters of the capillary amounted to 215 mm, 8.6 mm, and 5.5 mm, respectively. The membrane had pore sizes with a nominal and maximum diameter of 0.2 μm and 0.6 μm, respectively, and porosity of 73 %. The effective membrane area amounted to 0.037 m^2. The broth and distillate streams flowed co-currently from the bottom to the upper part of the MD module. The broth flowed alongside the membrane surface (inside the capillary bore), whereas the distillate flowed on the shell side. The inlet temperatures of the streams of the broth and distillate were kept at 310 K and 293 K, respectively.

The fermentation solution was prepared by dissolving 100 g of sucrose into thrice boiled tap water. The tap water used had the following average ionic composition AIC/(mg dm^{-3}): K^+ 7, Na^+ 29, Mg^{2+} 18, Ca^{2+} 65, Br^- 0.15, SO_4^{2-} 100, NO_3^- 1.3, Cl^- 55 (ion chromatographic analysis). The tap water was considered to be an adequate source of mineral salts for microorganisms for the 100 h period of fermentation (Gryta, 2001). A commercially available Gamma HEFE yeast (*Saccharomyces cerevisiae*, AB Enzymes, Germany) was used as the microorganism in the amount of 5 g dm^{-3}. The dry yeast was rehydrated for 30 min, while the broth was agitated periodically. Next, the broth (2 dm^3) was poured into the MD bioreactor. The fermentation process was carried out in the continuous mode for 5 days. After each series of experiments, the installation was rinsed with water several times and then with a solution of NaOH (1 mass %), the residue of which was removed by rinsing the installation 2–3 times with distilled water.

Samples of the broth (15 mL) were collected every 24 h. A sample was first centrifuged (2000 min^{-1}, centrifuge MPW-350R, Med-Instruments), and then

filtered (membrane filter 0.45 μm, Millipore). The content of sugar and alcohol in the solution was determined on the basis of an analysis of the total organic carbon (TOC-Analyser multi N/C, Analytic Jena). For this analysis, a filtered sample was divided into two portions. One portion was allowed to evaporate to obtain an approximately 50 % reduction in the sample volume and later 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 sucrose. For this assumption, as was shown earlier on the basis of chromatographic analyses, the determination error of ethanol and sucrose concentration did not exceed 3–5 % (Barancewicz et al., 2009).

The concentrations of anions and cations were measured using the ion chromatographic method with conductivity detector (850 Professional IC, Metrohm Herisau, Switzerland). The separation of anions was achieved on 1.7 mm × 3.5 mm Metrosep RP guard column in series with a 250 mm × 4.0 mm Metrohm A Supp5-250 analytical column. A 150 mm × 4.0 mm Metrosep C2-150 analytical column was used for separation of cations.

The electrical conductivity of the solutions examined was measured by a 6P Ultrameter (Myron L Company, USA). The morphology and composition of the fouling layer was studied using scanning electron microscopy (SEM) coupled with energy dispersion spectrometry (EDS).

Results and discussion

Materials used in the construction of industrial installations are subject to corrosion and moderate dissolution, which may influence the rate of growth of microorganisms and lead to the formation of a scaling layer on the membrane surfaces (Gryta, 2007). The laboratory installation was made from the same materials as were used in the design of large-scale industrial installations. Those elements that came into contact with the broth (e.g. reactor vessel, pipelines) were made from acid-resistant steel. This type of steel contains heavy metals the presence of which can have a negative influence on the yeast growth. For this reason, at the first stage of the investigations, the process of sugar fermentation in the bioreactor was repeated several times. The results of the experiments in some series of measurements show discrepancies in the course of the concentration changes in sugar and ethanol. However, a similar discrepancy was obtained for the fermentation conducted for comparison purposes in a thermostated glass vessel (Fig. 1). Therefore, a conclusion can be drawn that the ASI 316L steel used for the installation construction did not affect the course of fermentation, and the differences in

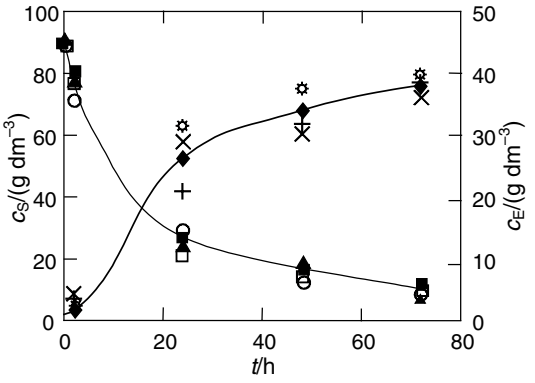

Fig. 1. Variations in sugar and ethanol concentrations in the broth (c_S, c_E) during the fermentation process. Average value of four fermentation series in steel bioreactor: sugar (■) and ethanol (⧫); three fermentation series in glass reactor: ethanol (+), (×), (☼) and sugar (▲), (□), (○).

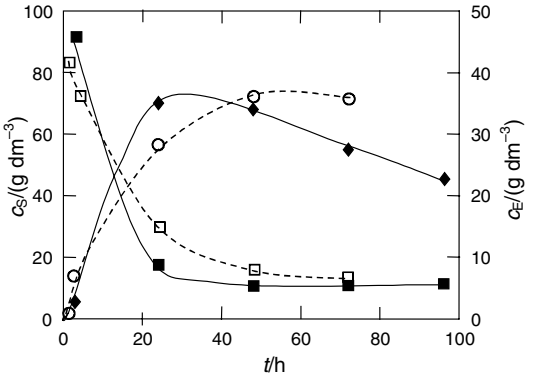

Fig. 2. Variations in sugar and ethanol concentrations in the broth (c_S, c_E) during the fermentation process with MD: sugar (■) and ethanol (⧫); without MD: sugar (□) and ethanol (○).

the rate of yeast growth in the early stages of fermentation (lag-phase) were probably responsible for the concentration changes recorded.

Most of the fermentation experiments presented here were conducted at a constant rate of rotation of the pump impeller (approximately 700 min^{-1}). A rotating impeller is capable of tearing yeast cells apart, which reduces the concentration of active yeast. In order to assess the intensity of the process, fermentation tests were carried out in which the speed of rotation was increased up to 1600 min^{-1}. However, the results of ethanol and sugar concentrations thus obtained were similar to those presented in Fig. 1, which were obtained at 700 min^{-1}, suggesting that even if the rotating impeller destroyed some yeast cells, it had no decisive influence on the results of the experiments performed.

In the next stage of the fermentation experiments, the evaporation of volatile compounds from the broth in the MD process was used. In the initial stage of fer-

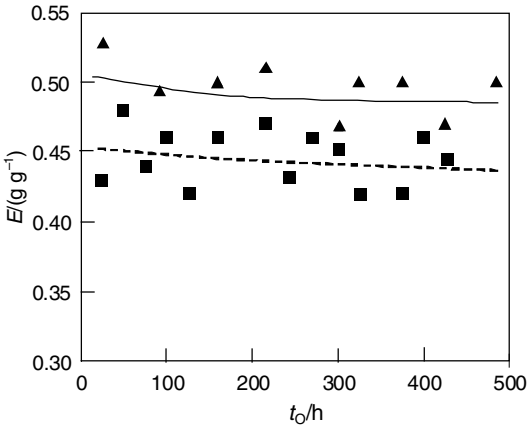

Fig. 3. Efficiency (E, g of ethanol per g of sugar) of batch fermentation (consecutive 400 h of bioreactor operation time) with MD (▲) and without MD (■).

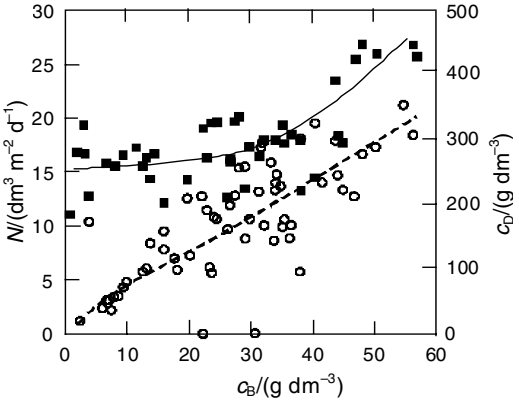

Fig. 5. Batch fermentation with MD process: The influence of ethanol concentration in the broth (c_B) on the distillate flux (N) (■) and on the ethanol concentration in distillate (c_D) (○).

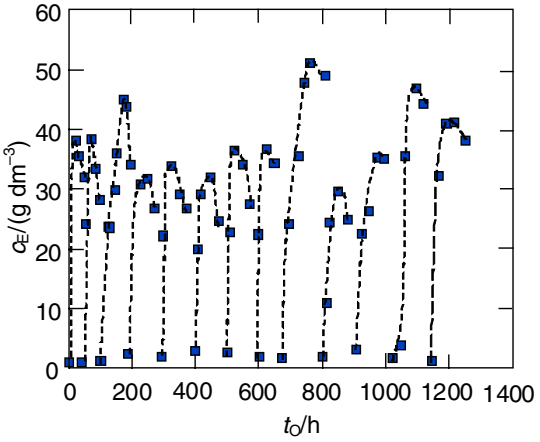

Fig. 4. Batch fermentation with MD process: The changes in ethanol concentration in the broth (c_E) during operation time (t_O).

mentation with the MD, the rate of sugar to ethanol conversion increased slightly and its concentration in the broth decreased due to steady evaporation of ethanol through the membranes (Fig. 2).

The calculations showed that application of the MD process increased the efficiency of fermentation from 0.45 g to 0.5 g of ethanol per gram of sucrose (Fig. 3).

In addition, productivity of the bioreactor with the MD connected increased by almost 30 %. The results obtained are consistent with the earlier reports, which highlighted the benefits of combining fermentation with the MD process (Gryta, 2001; Gryta et al., 2000).

The long-term experiments confirmed the consistently high efficiency of the MD process used for the separation of ethanol from the broth during the batch fermentation. Due to the steady evaporation of ethanol through the membranes, its concentration decreased when most of the sugar was fermented (Fig. 4).

The changes in ethanol concentration in the broth had no significant influence on the rate of permeate flux, but they did affect the concentration of the distillate obtained (Fig. 5). The amount of separated ethanol increased when its concentration in the broth also increased and, after several hours of fermentation, it was possible to obtain a distillate containing more than 200 g of ethanol per dm^3. For the results presented here, the enrichment factor obtained amounted to 4–10. The enrichment of the distillate in ethanol is an important benefit of the MD process because, besides increasing the fermentation efficiency (separation of volatile metabolites from the broth), a concentrated solution of ethanol devoid of yeast and salts is also obtained. In the case of dilute solutions, an increase in distillate concentration, e.g. in the range of 50–100 g of ethanol per dm^3 requires the evaporation of 500 dm^3 of water from 1 m^3 of the feeding solution. Due to this, pre-concentration of the ethanol solution by the MD process affords significant energy saving during further alcohol pre-concentration by traditional distillation.

In order to limit the amount of waste water generated during ethanol production, it is desirable to increase the final concentration of alcohol through the fermentation of concentrated solutions of sugar. This, however, leads to a high inhibition of the product formation. The problem may be resolved by using a membrane bioreactor with a continuous dosage of sugar. The results obtained for continuous fermentation linked with the MD process are presented in Fig. 6. A continuous dosage of sugar solution into the bioreactor allowed stabilisation of the alcohol concentration at 40 g dm^{-3} and, as a result, increase of the permeate flux up to 4.5 dm^3 m^{-2} d^{-1}. During sugar fermentation, ethanol and other by-products such as acetaldehyde, formic acid, lactic acid, acetic acid, 1-propanol, 2-methyl-1-butanol, and 2,3-butanediol are

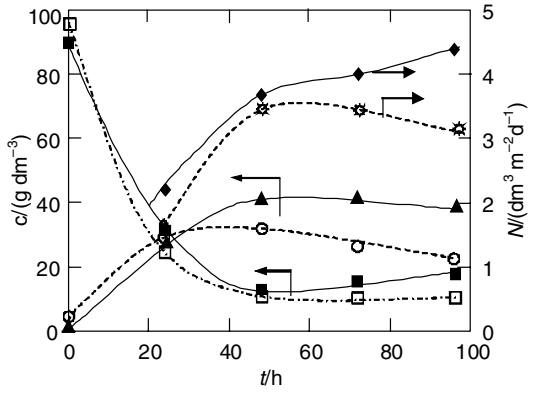

Fig. 6. Changes in broth composition (c) and permeate flux (N) over the fermentation time (t): with continuous sugar dosage: sugar concentration (■), ethanol concentration (▲), permeate flux (♦); without continuous sugar dosage: sugar concentration (□), ethanol concentration (○), permeate flux (☼).

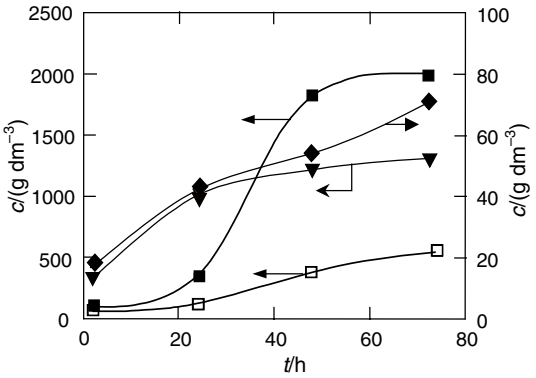

Fig. 7. Changes in organic acids concentration (c) in the broth and MD distillate over the fermentation time (t). Broth: acetic acid (■), succinic acid (▼), formic acid (♦); distillate: acetic acid (□).

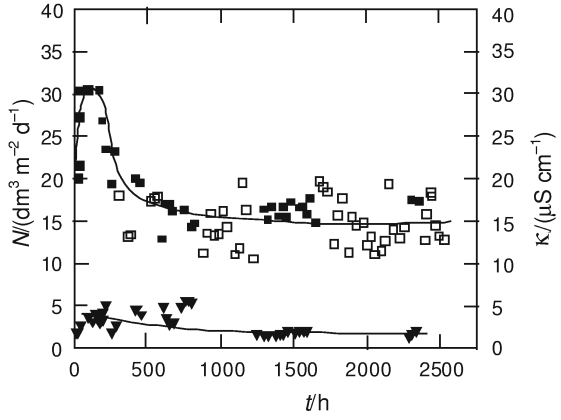

Fig. 8. Changes in the permeate flux (N) and distillate electrical conductivity (κ) during the MD module operation time (t): permeate flux (■), electrical conductivity (▼); feed: distilled water with NaCl (■), broth (□).

formed (Gryta, 2001). Most of these compounds are volatile so they can be separated by the MD process. However, the MD process does not separate non-volatile metabolites and their concentration increases steadily. Fig. 7 presents changes in the concentration of organic acids in the broth, the amounts of which increased when both the fermentation time and the amount of sugar used in the experiment were increased. The data show that, where continuous fermentation is concerned, it is necessary to limit the increase in non-volatile metabolites, e.g. through a partial broth removal (bleeding) in addition to the MD process.

The MD module assembled in the installation was in operation, with just a few weeks' suspension, for almost one year. The degree of wettability of the membranes used in the study was assessed by periodic examination of changes in the maximum flux (distilled water with 10 g of NaCl per dm^3 as a feed). The values measured of permeate flux obtained for water and broth are presented in Fig. 8. The efficiency of the MD module decreased significantly during the first 500 h of its operation, which was also observed in the case of other hydrophobic membranes (Gryta & Barancewicz, 2010). The MD process efficiency was stable for the subsequent 2000 h. Despite the presence of salt in the feed (electrical conductivity of 5000 μS cm^{-1}), the electrical conductivity of the distillate obtained did not exceed 5 μS cm^{-1}. Such a low value for the distillate's electrical conductivity indicated that, despite being in contact with water and fermentation broth for almost a year, the membrane did not become wetted.

The Accurel PP V8 HF membranes have a spongy structure and their pore size is below 1 μm. The SEM investigations revealed that the surface area of the membrane walls also contains larger pores, several micrometers in diameter (Fig. 9). These large pores can become wetted, resulting in an increase in temperature polarisation. As a result, a decrease is observed in the MD module efficiency.

The decrease in efficiency observed in Fig. 8 may also be associated with biofouling of the membrane. However, the SEM investigations showed that, despite long-term use of the MD module for broth separation, no yeast cells were observed on the membrane surfaces (Fig. 9D). Therefore, it may be concluded that biofouling will not occur in the MD modules applied to ethanol separation from the fermentation broth. This result is consistent with previous observations (Gryta, 2001).

After drying the membrane which was extracted from the module, gas permeability investigations were conducted. The changes in the air permeability of the membrane extracted from the bioreactor were similar to the changes in permeability observed in a new membrane. This confirms the conclusion that a small amount of precipitate visible on the membrane surface

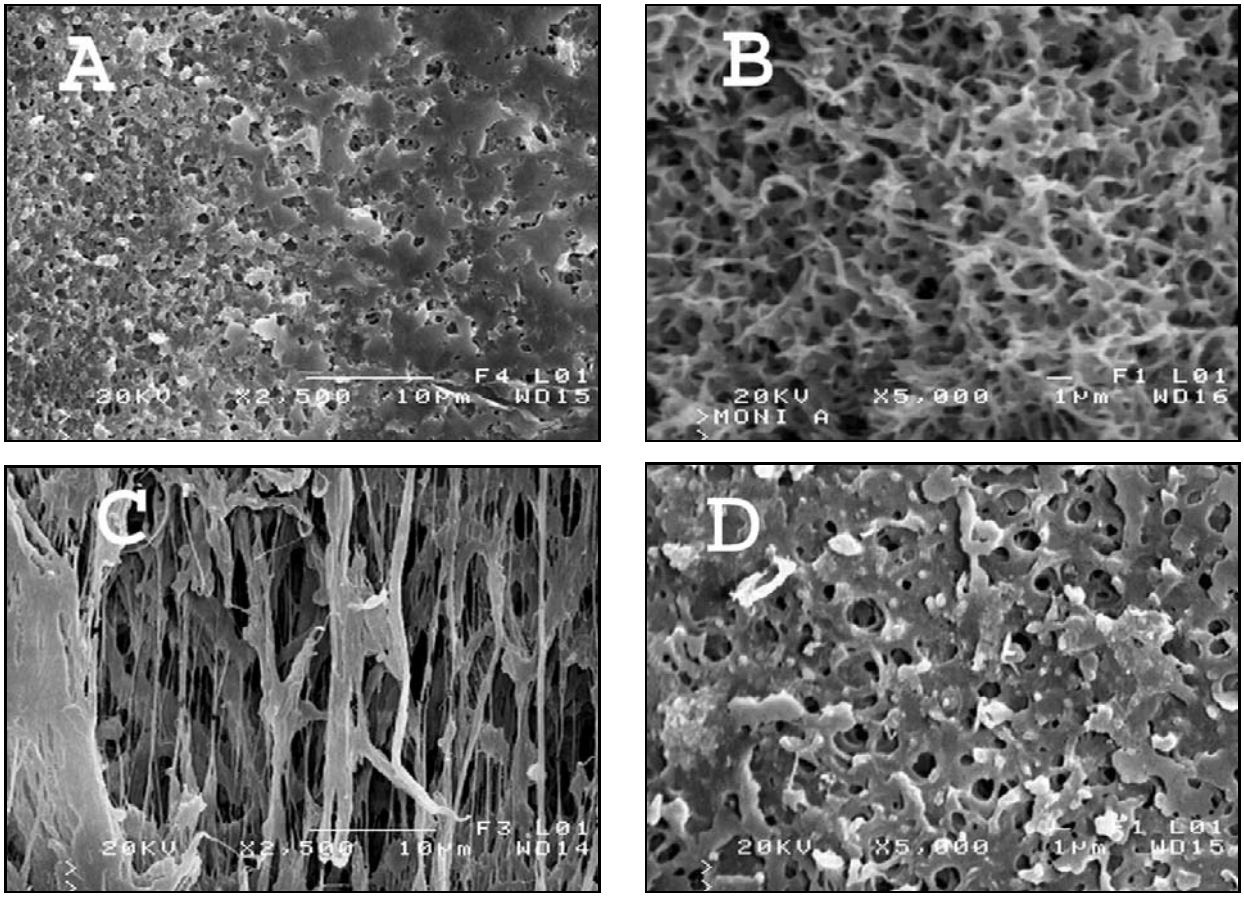

Fig. 9. SEM images of polypropylene capillary Accurel PP V8/2 HF membrane: internal surface (A), cross-section (B), external surface (C), and internal surface after one year of operation in MD bioreactor (D).

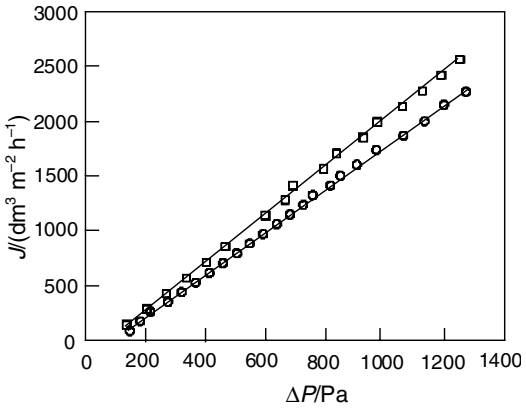

Fig. 10. Influence of pressure difference (ΔP) on air permeability (J) of Accurel PP V8/2 HF membrane: new membrane (□) and membrane after one year of operation in MD bioreactor (○).

(Fig. 9D) did not hinder its permeability (Fig. 10). The result obtained also indicates that the decrease observed in the MD process efficiency (Fig. 8) was largely due to a certain proportion of the larger pores on the membranes surface becoming wetted.

Conclusions

The results confirmed that a combining the fermentation with the MD process effectively separated ethanol and other volatile metabolites from the broth. As a result, efficiency in the sugar to ethanol conversion was increased, as was the productivity of the bioreactor. No intensive biofouling development was detected and the polypropylene capillary membranes used maintained their high efficiency of separation throughout the period of the MD experiments.

No decline in permeability of the polypropylene membranes used was observed over the course of the fermentation experiments. This indicates that the presence of cells in the broth did not lead to their deposition on the membrane surface. The SEM observations of the membrane samples confirmed that the surface of membranes was clean and practically free of yeast cells.

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