Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.0709

69 (2017) 35–42
March

Application of membrane filtration for integrated production of ethanol and carboxylic acids from pre-treated straw

Mariusz Kuglarz, Klaudiusz Grübel*

Faculty of Materials, Civil and Environmental Engineering, Institute of Environmental Protection and Engineering, University of Bielsko-Biala, Willowa 2, 43-309 Bielsko-Biala, Poland, Tel. +48 338279181; Fax: +48 338279101; emails: mkuglarz@ath.bielsko.pl (M. Kuglarz); kgrubel@ath.bielsko.pl (K. Grübel)

Received 23 June 2016; Accepted 25 July 2016

ABSTRACT

Rape straw was treated by two methods (dilute-H_2SO_4 as the most commonly used for this type of feedstock and glycerol-based as an alternative method), before its application as feedstock for bioethanol production. Fermentation broth was treated by means of membrane processes (nanofiltration [NF] and reverse osmosis [RO]) with the main aim to minimize ethanol rejection and maximize sugars rejection. Both processes (RO; SW30XLE membrane; NF; DK membrane) ensured complete recovery of ethanol in the permeate. While filtration by SW30XLE membrane allowed to achieve the highest xylose retention (>99%), resulting in high xylose concentration (42 g/L) compared with its content in the residue after ethanol production (6.5 g/L). Using DK membrane, about 60% of xylose was rejected, which allowed concentrating the xylose to the level of 17.5 g/L. Retentate after membrane filtration was tested as the feedstock for succinic acid production. Succinic yield amounted to 70%, which resulted in 80% of xylose utilization.

Keywords: Ethanol separation; Xylose separation; Membrane filtration; Stillage; Rapeseed straw; Waste glycerol

1. Introduction

Bio-ethanol produced on large-scale from lignocellulosic biomass can be blended with petrol and used in flexible fuel vehicles or even used as 100% ethanol in dedicated vehicles. EU member states commitments require increasing areas of land for growing energy crops to achieve 10% share of biofuels used in transportation in 2020 [1]. First generation bioethanol based on starch plants used primary for food production is not sustainable and provokes social objections in many parts of the world. Therefore, second generation cellulose-based bioethanol from agricultural residues or dedicated crop plantations is a more promising approach. Generally, second-generation ethanol production includes at least the following stages: pre-treatment, saccharification and fermentation. The first stage, i.e., pre-treatment is the most important as the effective pre-treatment can overcome limitations of enzyme accessibility [2]. Thus, effective pre-treatment is required to loosen the lignocellulosic structure prior to further processing, i.e., enzymatic hydrolysis and ethanol fermentation. Biomass pre-treatment methods can be classified as chemical, mechanical and biological. Current biomass pre-treatments used are most likely to be chemical or thermo-chemical, due to shorter processing times, higher fermentable sugar yields, and lower energy requirements [3]. Acid-based thermal pre-treatment is very effective in hemicellulose solubilization; whereas, this method produces some inhibitors (e.g., furfural,

*Corresponding author.

Presented at the EDS conference on Desalination for the Environment: Clean Water and Energy, Rome, Italy, 22–26 May 2016.

hydroxymethylfurfural) and is not effective in biomass delignification, and usage of large amounts of acid can cause reactor corrosion [4,5]. Disadvantages of dilute-acid methods can partially be solved by the application of recently emerging pre-treatments, e.g., glycerol-based methods. Glycerol, by-product of biodiesel production, can be purified and used in pharmaceutical, cosmetic and food industry. However, extensive biodiesel production has already resulted in price decrease [6]. Consequently, the development of new usages is required for dealing with the glycerol surplus. Glycerol can be considered as a good agent for lignocellulosic materials treatment, if the appropriate fractionation conditions are established. The hemicellulose is soluble in the aqueous phase, the lignin is soluble in the organic phase, and the cellulose remains in solid fraction. The available studies revealed that industrial glycerol pre-treatment of wheat straw led to a high recovery of cellulose (>95%), removal of lignin (>70%) – from wheat straw [7]. Also, this type of pre-treatment improved the yield of enzymatic hydrolysis. In such processes, lignin and hemicellulose are removed and thus, pore volumes and hydrolytic enzyme accessibility are improved [8]. However, research connected with broader biomass types is necessary. According to our knowledge, there are no reports comparing the glycerol-based method with dilute-acid pre-treatment used for straw biomass processing, especially before combined ethanol and succinic acid production.

Ethanol is most frequently produced using ordinary baker's yeast, *Saccharomyces cerevisiae*, due to the simplicity of the conversion and high process efficiency. However, *Saccharomyces cerevisiae* can effectively convert glucose to ethanol with high efficiency, and the ethanol fermentation of C5 sugars from hemi-cellulosic fraction still remains a challenge, due to low conversions yields [9]. One of the current challenges of bio-ethanol production from lignocellulosic biomass is an effective utilization of hemicellulose-derived sugars into valuable products, e.g., succinic acid, using *Actinobacillus succinogenes.* This strain is considered as one of the most promising for the industrial production of succinic acid. Moreover, succinic acid production via fermentation consumes CO_2, which can significantly improve the sustainability of the combined biorefinery (bioethanol + succinic acid conversion) process [10]. Succinic acid, also known as amber acid or butanedioic acid, has been recognized as one of the top 12 platform chemicals, i.e., compounds that can be utilized to synthesize valuable commodities, i.e., biodegradable polymers, fine chemicals, green solvents, pharmaceuticals etc. [11].

Nowadays, membrane processes are being used in fermentation technologies (methane or ethanol production), especially for wastewater purification, water reuse or compounds recovery. In case of methane production, membrane processes were successfully tested for the treatment of post-digestion liquors, rich in organic matter, phosphorus and nitrogen. The treatment of such liquors with a struvite precipitation/ammonia stripping (as initial steps) and a subsequent reverse osmosis (RO) process turned out to be effective and allowed to decrease contaminants' concentration below discharge limits [12–14]. Different biological and physico-chemical processes have been applied together with membrane filtration for the treatment of residue after distillation. Among the various processes that can be used for final treatment of residue after ethanol production (stillage), nanofiltration (NF) and RO appear to be the most suitable to produce high-quality water or even meet the effluent discharge standards [15,16]. Another application of membranes techniques constitutes the recovery of valuable compounds, such as lignin and hemicellulose. For example, ultrafiltration is most commonly used for the recovery, fractionation and purification of hemicellulose from hydrolysates. However, effective separation of dissolved sugars (i.e., xylose and arabinose) present in fermentation broth after using ordinary baker's yeast *Saccharomyces cerevisiae*, still remains a challenge. Taking into account molecular weights of xylose/arabinose (150 g/mol) and ethanol (46 g/mol), the separation of ethanol from other components should be possible using membrane processes via molecular sieving. However, the effect of other components and impurities present in broth after fermentation on the separation process should also be taken into account.

The study presents biofuel (cellulosic ethanol) and biochemical (succinic acid) production from rape straw, integrated with membrane filtration of the fermentation broth. Rapeseed straw is a processing residue generated by the bio-oil industry. It is an abundant and low-cost lignocellulosic material in many European and Asian countries. Its excessive amounts are usually disposed of by combustion. In the present study, rape straw was treated by two methods (dilute-H_2SO_4 as the most commonly used for this type of feedstock and glycerol-based as an alternative method), before its application as feedstock for bioethanol production. Our goal of membrane filtration was to minimize ethanol rejection, while maximizing sugar rejection. The retentate achieved after membrane filtration was used as feedstock for bio-succinic acid production. While the present filtration experiment do not represent the full range of possible conditions and materials, the main goal of this part of study was to demonstrate proof-of-concept and show the usage of membrane processes in integrated bioethanol and succinic acid production from rape straw.

2. Materials and methods

2.1. Feedstock

The straw used in this study originated from rape cultivated for energy purposes. The farm is located in the Silesian province, south of Poland. Straw samples were collected uniformly from the surface of approximately 2 ha. Dry straw was chopped using a shredder (4–5 cm length) and ground to particles of 3 mm, using a cutting mill. The dry matter (DM) content was 93%–94%. The main components of the rape straw in this study were cellulose (38.3% ± 1.6%), hemicellulose (20.4% ± 1.5%) and lignin (21.7% ± 1.6%) (Table 2).

2.2. Biomass pre-treatment and hydrolysis

Biomass of straw was pre-treated with acid-diluted method and glycerol-based method. After pre-treatment, the

slurry was separated, using a commercial filtration Buchner unit, into solid (water insoluble fraction, WIS) and liquid fraction. Solid fractions recovered after pretreatment were directly used as feedstock for enzymatic hydrolysis. Each pre-treatment was conducted at solid content of 10% (w/v) and repeated four times. In case of dilute acid method, the process was based on temperature (180°C) and H_2SO_4 addition (1% w/v). While glycerol method involved the addition of glycerol fraction instead of water, sulfuric acid was not used in glycerol-based method. Glycerol fraction was obtained from local company, producing biodiesel from rapeseed. Chemical composition of glycerol fraction was as follows: glycerol 86.5%; inorganic components: 2.55%, methanol: <0.02%; non-glycerol organic substances: <2.0%. Density of the glycerol fraction amounted to 1235 g/L at 20°C; pH 6.3–6.4. In both cases, mixtures were steam treated in a batch reactor at 180°C for 15 min.

2.3. Enzymatic hydrolysis and ethanol fermentation

Untreated biomass (for comparison) and solid fractions recovered after pre-treatment tested (acid-based or glycerol method) were used as feedstock for enzymatic hydrolysis. The process was conducted at a solid loading of 5% in a 50 mM sodium citrate buffer, pH 4.8. The process was conducted at 50°C for 48 h. The following enzymes were used for enzymatic hydrolysis: Celluclast 1.5 L® (Celluclast, 20 FPU/g glucan) derived from *Trichoderma reesei* and Novozyme 188 (15 IU/g glucan) from *Aspergillus niger*. Ethanol was produced from enzymatically hydrolyzed straw, supplemented with the following amounts of minerals (g/L): $(NH_4)_2SO_4$, (3.75); K_2HPO_4, (2.11); $MgSO_4 \cdot 7H_2O$, (0.375) and $CaCl_2 \cdot 2H_2O$, (0.5).

The stock culture of *S. cerevisiae* was first subcultured on Difco™ Yeast Mold (YM) Agar. For preparation of inocula, cells were added to 200 mL liquid YM media and incubated at 30°C for 24 h. For ethanol production, *S. cerevisiae* (5% (v/v)) was added as an inoculum. The fermentation was performed at 35°C for 48 h in 300 ml Pyrex flasks equipped with air locks. Samples of 1 ml were taken after 0, 3, 6, 12, 24, 36 and 48 h. Samples were centrifuged at 10.000 g for 10 min, and then the supernatants were filtered via 0.2 µm pore size filters – before sugars and ethanol determination.

2.4. Succinic acid production procedure

Retentates containing concentrated xylose, after effective membrane filtration, was used as feedstock for succinic acid production. Before using as feedstock for succinic acid production, pH value of retentate was adjusted to about 6.5. *A. succinogenes* 130Z (DSM 22257) was obtained from Leibniz-Institute DSMZ–German Collection of Microorganisms and Cell Cultures. Seed culture medium composition and details about succinic production tests were previously described [17,18]. Succinic acid tests were performed in 200 ml sealed anaerobic bottles with 100 ml working volume and 75:25 (% vol.) ratio of feedstock to experimental medium. Sugars (glucose, xylose) and acids (succinic, acetic, formic) were analyzed at the beginning of the process and after 48 h.

2.5. Membrane materials and filtration procedure

Osmonics device (type GH-100–400) was used for membrane filtrations. The device worked in the dead-end mode, on flat sheet membranes with active surface of 36.3 cm^2. The processes were conducted under trans-membrane pressure of 2 MPa; rotary velocity of the stirrer was maintained at the level of 200 rpm/min. The pH value of the liquors treated amounted to 4.5 and pH values were not corrected before membrane processes. Fig. 1 shows the concept of bioethanol/succinic acid production integrated with membrane processes. Characteristics of the membranes used are presented in Table 1.

2.6. Analytical methods

Total solids (TS), volatile solids (VS), ash, and pH were determined according to standards methods [19]. The concentrations of sugar monomers (glucose, xylose, arabinose), organic acids (succinic-, lactic-, formic- and acetic

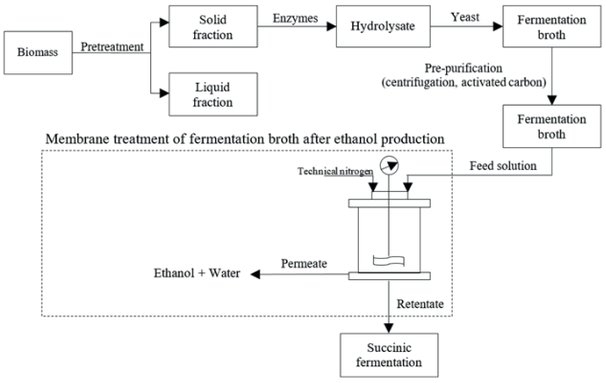

Fig. 1. Concept of bioethanol and succinic acid production – integrated with membrane separation of xylose after ethanol fermentation.

Table 1
Characteristics of the membranes used

Symbol	DK	SW30XLE
Type	Nanofiltration	Reverse osmosis
Manufacturer	GE	Dow
Material[a]	Polyamide skin layer	Polyamide
Cut-off[a], Da	150–300	100
Retention coefficient NaCl[b], %	40	99.5
Retention coefficient $MgSO_4$[b], %	98	99.5
Contact angle[c], degrees	37	45
pH range[a]	2–10	2–11

[a]Data provided by manufacturer.
[b]Established experimentally for 1 g/L solution of NaCl and $MgSO_4$ under working pressure of ΔP = 2 MPa.
[c]Determined using a goniometer.

acid), ethanol, inhibitors (furfural and 5-hydroxymethyl-2-furaldehyde HMF) were determined by using high performance liquid chromatography HPLC (Agilent 1260) equipped with a BioRad Aminex HPX-87H column at 63°C, refractive index detector (RID 1362A) and ultraviolet (UV) detector using 4 mM H_2SO_4 as eluent at 0.6 ml/min flow rate. Compositional analysis of the cellulosic feedstock used in this study was based on a two-step acid hydrolysis as described in our previous study [18]. All chemicals used in this study were of analytical grade (Sigma Aldrich ApS). Results are presented as average values (n = 4) with standard deviations (±).

2.7. Calculations

2.7.1. Pre-treatment and enzymatic hydrolysis

The effectiveness of the pre-treatment methods was based on WIS (fraction insoluble solids) recovery, i.e., (WIS/initial weight of material used for pre-treatment). Cellulose and hemicellulose recovery were calculated as the amount of cellulose and hemicellulose recovered in solid fractions after pre-treatment and expressed as percentage related to cellulose and hemicellulose content in biomass before pre-treatment. Glucose ($Y_{Gluc.}$) and xylose ($Y_{Xyl.}$) yields of enzymatic hydrolysis were based on glucose and xylose released and these yields were expressed as initial percentage of glucose or xylose used for enzymatic hydrolysis. Formulas were presented in our previous study [20].

2.7.2. Ethanol and succinic acid fermentation

Ethanol yield (Y_{EtOH}) (Eq. (1)) was based on the assumption that all glucose found in the feedstock could be converted into ethanol with a yield of 0.51 g EtOH/g of glucose, using *S. cerevisiae.*

$$Y_{EtOH}(\%) = \frac{EtOH_{Prod.}(g)}{EtOH_{Theoret.}(g)} \cdot 100 \quad (1)$$

where $EtOH_{Prod.}$ is the highest ethanol amount obtained in fermentation process (g); $EtOH_{Theoret.}$ is theoretical ethanol production based on stoichiometric conversion of glucose to ethanol, i.e., 0.51 g-EtOH/g-glucose.

Succinic acid yield was expressed as the amount of succinic acid produced (g) from 1 g of xylose present in the hydrolysate, and was expressed as a percentage.

2.7.3. Effectiveness of membrane filtration

Rejections of sugars and ethanol were based on their concentration in the retentate/permeate compared with initial concentration of the analyzed compound in the feedstock before filtration; whereas, the efficiency of the membrane processes was determined according to the following Eq. (2):

$$J_v = \frac{V}{F \cdot t}, \text{L}/\text{m}^2 \cdot \text{h} \quad (2)$$

where J_v is volumetric permeate flux, L/m²·h; V is permeate collected after the particular period of time, L; F is active membrane surface, m²; and t is filtration time, h.

3. Results and discussion

3.1. Biomass pre-treatment

Both pre-treatment methods (dilute-acid and glycerol-based) had a significant effect on biomass composition, i.e., cellulose, hemicellulose and lignin content (Table 2). Cellulose content increased by 43% and 62% after acid- and glycerol-based method, respectively. These pre-treatment methods allowed retaining >98% of initial

Table 2
Influence of dilute-acid and glycerol-based pretreatment on composition of rape straw biomass (average values n = 4, ± standard deviations)

Pretreatment	Solid fraction recovered					
	Glucan, % DM	Cellulose rec.[b], %	Xylan, % DM	Hemic. rec.[b], %	Lignin, % DM	WIS, %
Untreated[a]	38.3 ± 1.6	–	20.4 ± 1.5	–	21.7 ± 1.6	–
1% H_2SO_4, 180°C	54.8 ± 1.8	97.9 ± 1.2	4.5 ± 0.4	15.3 ± 2.1	31.5 ± 2.0	69 ± 3
Glycerol-based 180°C	61.9 ± 1.2	98.3 ± 1.1	17.9 ± 0.8	53.8 ± 3.8	9.5 ± 1.0	61 ± 2
Pretreatment	Liquid fraction after pretreatment					
	Glucose, g/L	Xylose, g/L	Arabinose, g/L	Hemic. rec.[c], %	Soluble lignin, g/L	Recovery, %
1% H_2SO_4, 180°C	0.83 ± 0.2	15.3 ± 1.5	1.5 ± 0.5	43.4 ± 4.8	n.d.	67 ± 5
Glycerol-based 180°C	n.d.	14.9 ± 1.3	1.6 ± 0.4	20.9 ± 1.9	55.4 ± 3.2	33 ± 2

Note: n.d. – not detected or below detection limit; rec. – recovery.
[a]Initial biomass without acid-based or glycerol-based pretreatment.
[b]Cellulose and hemicellulose recovered in solid fractions after pretreatment and expressed as percentage related to cellulose and hemicellulose content in untreated biomass.
[c]Xylose and arabinose released into liquid fraction and expressed as a percentage of initial hemicellulose in untreated biomass,
WIS – water insoluble fraction, represents the recovered solids mass (dry basis) after pretreatment.

glucose (untreated biomass) in solid fractions and there were only insignificant amounts of glucose (<1 g/L, Table 2) released in liquid fractions and only recorded after dilute acid method. Results obtained in this study clearly showed that applied conditions of straw pretreatment did not cause a significant hydrolysis of cellulose structure. Inappropriate pretreatment conditions, e.g., too high acid concentration or temperature range used, can cause polysaccharides degradation and generate high amounts of degradation products, e.g., carboxylic acids and furan derivatives [20]. The dilute acid method turned out to be very effective in hemicellulose fraction solubilization (Table 2). It allowed decreasing its content from about 20.5% to 4.5%. In this case, about 43% of hemicellulose was solubilized (15.3 g of xylose/L, 1.5 g of arabinose/L, liquid fraction, Table 2); whereas, the rest was lost during the process, which is strictly connected with dilute-acid method conducted at high temperature [20,21]. Similar concentrations of xylose and arabinose released into liquid fraction were recorded in case of glycerol-based pretreatment. However, due to lower recovery of liquid fraction, this method allowed to solubilize only about 20% of hemicellulose fraction (Table 2), leaving still about 50% in solid fraction and the lost accounted for only 25% of initial hemicellulose present in untreated biomass. This can be considered as the advantage of glycerol-based method compared with thermal dilute acid method.

Dilute-acid method did not allow decreasing the lignin content in solid biomass. In fact, lignin content in biomass composition increased significantly as a result of hemicellulose removal (Table 2). Thermal pre-treatment, facilitated by sulfuric acid, could also generate pseudo-lignin and thus increase the measured Klason lignin content. Similar observations have been made in previous studies [20]. Glycerol-based method allowed to decrease the insoluble lignin content in solid fraction to <10% of DM (Table 2). Biomass delignification is one of the main advantages of glycerol used as solvent for biomass pre-treatment. For example, Sun and Chen [7] showed that >70% of lignin fraction was removed from wheat straw through glycerol autocatalytic organosolv process. What is more, the organic solvents can be recovered and recycled, while recovered lignin depending on its purity can be burned or used for production of desirable chemicals, i.e., resins, lignin binders, lignin-carbon fibers etc. [22].

3.2. Enzymatic hydrolysis and ethanol fermentation

Influence of dilute-acid and glycerol-based pre-treatment methods – on the effectiveness of enzymatic hydrolysis, using the commercial celluclast and β-glucosidase mixtures, was analyzed. The untreated biomass turned out to be difficult to hydrolyze (glucose yield: 30%, data not shown), which is thought to be connected with specific cell-wall structure of lignocellulosic feedstocks. This is commonly observed in case of different lignocellulosic materials. Both applied methods influenced the enzymatic yields in a positive way. Significantly higher yields of enzymatic hydrolysis (glucose: 93%; xylose: 80%, calculation based on Fig. 2) were achieved after glycerol-based pre-treatment compared with the sample pretreated by dilute-acid method (glucose: 60%; xylose: 75%, calculations based on Fig. 2). Enhanced

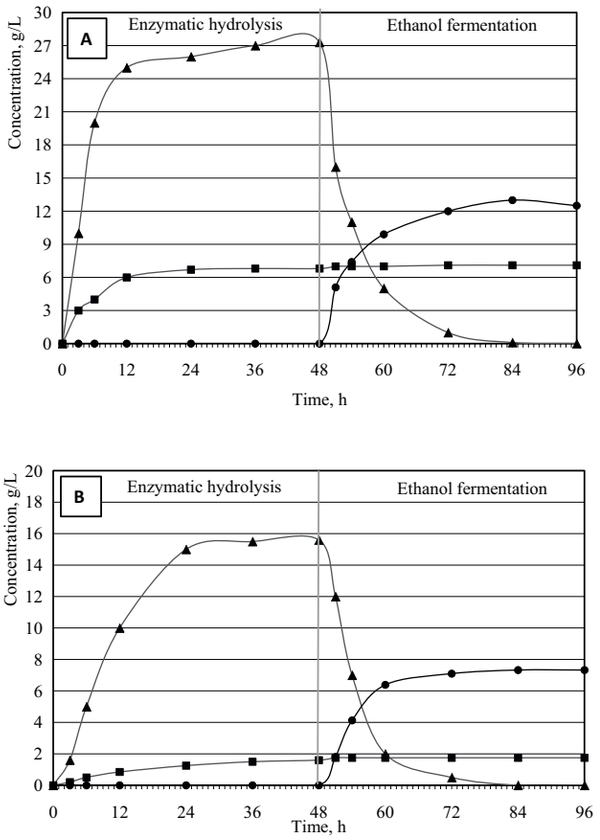

Fig. 2. Enzymatic hydrolysis and ethanol fermentation course: (a) biomass pre-treated by steam with 1.0% H_2SO_4; (b) biomass pre-treated by glycerol-based method; filled triangles represent glucose; filled squares represent xylose; filled circles represent ethanol.

course of enzymatic hydrolysis of biomass pre-treated by glycerol-based method is probably a result of high degree of biomass delignification (Table 2). It is commonly known that lignin content is one of the main factors influencing the effectiveness of enzymatic hydrolysis [4].

In both cases, bioethanol production started immediately without any lag phase (Fig. 2). The ethanol production proceeded until the glucose was completely consumed. Applied native strain of *S. cerevisiae* (non-engineered) was not able to use xylose (Fig. 2). A slight increase of xylose concentration was noticed during ethanol production, which was probably connected with the presence of enzymes used during previous stage of treatment. The type of biomass pre-treatment did not significantly impact on effectiveness of enzymatic hydrolysis. In both cases (dilute-acid, glycerol-based), ethanol yield amounted to 90%–92% (0.46–0.47 g-EtOH/g glucose released during enzymatic hydrolysis) (based on results presented on Fig. 2).

3.3. Fermentation broth separation

Ethanol production generates large amounts of stillage as a byproduct, which can be used as animal feed. Another

option to increase the economic profit of bio-ethanol production is an effective utilization of hemicellulose-derived sugars into valuable products, e.g., succinic acid. In the present study, ethanol and xylose separation was performed for broth originated from biomass pre-treated by glycerol-based method, – which was selected as the most optimal based on the results presented (Table 2, Fig. 2). In both filtration cases (DK and SW30XLE membrane), ethanol was not rejected by membranes. Ethanol diffusivity ($12{\cdot}10^{-6}$ cm^2/s) is significantly larger than xylose diffusivity ($7.69{\cdot}10^{-6}$ cm^2/s) [23], what can account for the complete permeation of EtOH during our experiments (Fig. 3). In particular, RO separating mechanisms in RO are based on solute diffusion across a membrane and sterically hindering the solute transport through pores [24]. Thus, complete recovery of ethanol in permeate was not unexpected taking into account a very low molecular weight of ethanol (46.07 g/mol). What is more, the pK_a of sugars is greater than 12 [23], thus xylose was in uncharged state during separation and the sieving mechanism might have played a significant role in separation of ethanol/xylose mixtures. However, a slight rejection of ethanol was noticed at the beginning of the filtration experiment (up to 1 h) (Fig. 3), which was unexpected and might have been connected with membrane hydrophilic properties. Membranes used were hydrophilic and ethanol concentration after fermentation low, so the membrane firstly permeated water before establishing stable transport conditions.

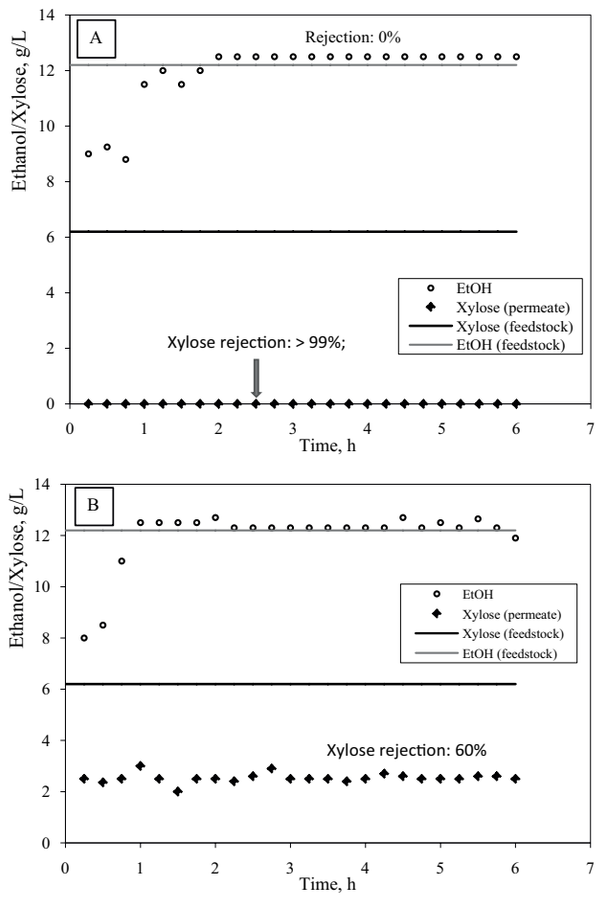

Fig. 3. Concentration of xylose and ethanol in permeate and water flux values: (a) filtration with 100 Da membrane and (b) filtration with 150–300 Da membrane.

Besides ensuring a low ethanol rejection, the second aspect of membrane filtrations performed was to reject (concentrate) xylose, – which can be effectively used in further experiments for biochemical production. Using DK membrane, classified as NF membrane, 60% of xylose was rejected, which allowed reaching more than 2.5 higher concentrated xylose solution compared with feed solution (Table 3); whereas, using SW30XLE membrane, classified as RO membrane, almost all xylose present in the solution (99.2%–99.8%) was rejected. In this case, xylose was above 6 times more concentrated compared with its content in the residue after ethanol production (Table 3) and reached the value of 42 g/L. These are very promising results, taking into account biological conversion of xylose into succinic acid in the next step of the experiment. Sugar concentration by means of membrane techniques, before fermentation processes, has previously been shown, e.g., from sweet sorghum juice [24]. According to our knowledge, the current study presents for the first time the application of NF/RO processes, in integrated production of ethanol and bio-succinic acid from rape straw hydrolysate. While the permeate consisted of water and ethanol (Fig. 3, complete permeation of ethanol and complete rejection of xylose), ethanol can be further recovered by conventional processes, e.g., distillation or pervaporation. An increasing progress in developing effective and energy-efficient cases of ethanol dehydration, using pervaporation, has been reported in the literature [25,26]. The recovered water can be integrated into ethanol/succinic acid production and used for feedstock dilution before pre-treatment. Thus, reducing the volume of water used in lignocellulosic biomass processing.

The values of volumetric permeate flux was also taken into account. The initial value of both volumetric fluxes reached the value of 37 L/m^2·h (NF) and 27 L/m^2·h (RO). A greater value observed in case of NF membrane compared with RO is strictly connected with membrane characteristics, which was also confirmed by larger water fluxes (NF) (Fig. 4). The values stabilized at 31–32 L/m^2·h and 14–16 L/m^2·h after 0.5 h of NF and 1 h of RO respectively. These values constitute about 60%–62% of water flux recorded for NF process and between 34% and 39% of water flux achieved for RO. A significant decrease of flux during filtration was also previously observed during treatment of distillery spent wash [16]. Taking into account the fact that post-digestion wastes contain minor amounts of inorganic salts added for microbial growth promotion during fermentation as well as various organic compounds (e.g. amino acids, peptides etc.) coming from yeast extract used as nitrogen source for fermentation [27], such liquors are considered as difficult to treat. Membranes were washed at the end of experiment and their permeability measured again. In our case, about 10% (DK, 150–300 Da) and 30% (SW30XLE, 100 Da) decrease in water permeability was observed. Further research should focus on additional pre-treatment of such residues in order to increase the efficiency of membrane treatment, as it was successfully shown in case of liquors after AD for methane production [12,14].

Table 3
Characteristics of feedstock for ethanol and succinic acid production, fermentation broth after ethanol and succinic acid production as well as fractions recovered during broth membrane filtration (average values $n = 4$, ± standard deviations)

Compound	Feedskock for EtOH	EtOH broth	Retentate after RO	Retentate after NF	Feedstock for S.A.	Succinic broth
Glucose, g/L	27.3 ± 1.8	–	–	–	–	–
Xylose, g/L	6.8 ± 0.8	6.5 ± 0.7	42.5 ± 2.5	17.5 ± 1.5	31.9 ± 1.8[b]	5.25 ± 0.8
EtOH, g/L	–	12.5 ± 1.0	12.4 ± 0.8[a]	12.4 ± 0.7[a]	–	–
Succinic acid, g/L	–	–	–	–	–	18.7 ± 1.0

[a]100% negative retention of ethanol was received, ethanol concentration in retentate was connected with dead-end filtrations to about 90% of feed solution.
[b]Ratio of feedstock to experimental medium 75:25% vol, EtOH – ethanol production, S.A. – succinic acid.

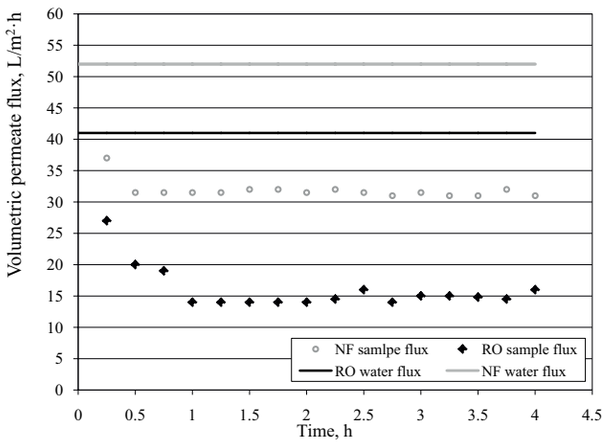

Fig. 4. Volumetric flux values of permeate during fermentation broth filtrations and tested membranes water fluxes.

Membrane fouling is considered as the main disadvantage of membrane application, as it leads to shorter lifetime of filtration materials. What is more, amounts of water/chemicals used during cleaning mainly depend on the degree of membrane fouling.

3.4.Succinic acid production

The retentate after filtrations by SW30XLE membrane (100 Da), which ensured complete recovery of ethanol in the permeate and the highest xylose concentration in the retentate (Table 3), was tested as the feedstock for succinic acid production. Succinic acid production started immediately without any lag phase and the succinic yield amounted to 70% (calculation based on results presented in Table 3). About 80% of xylose was consumed during the experiment, leaving about 20% of initial xylose in the fermentation broth. It has been previously reported that succinic yields based on xylose are significantly lower compared with yields originated from glucose [20,28]. Almost complete sugar utilization can be achieved in more controlled bioreactors, ensuring stable pH condition and mixing [28]. The aim of this part of the study was to demonstrate proof-of-concept by showing the succinic acid production from retentate after membrane filtration. As the residue after ethanol production contains some nutrients and minerals used in ethanol fermentation step as well as proteins, lipids and volatile fatty acids (VFAs) derived from yeast metabolism etc. [16], further research should also focus on nutrients supply optimisation.

4. Conclusions

The concept of ethanol/succinic acid production from rape straw pre-treated by glycerol-based method and integrated with membrane separation of xylose, was presented. Both analyzed membrane processes (NF and RO) ensured complete recovery of ethanol in the permeate, which can be further recovered by existing methods, e.g., distillation or pervaporation; whereas the recovered water can be used for feedstock dilution before pre-treatment, and thus reduce the volume of water used in lignocellulosic biomass processing. RO filtration of fermentation broth ensured the highest retention (>99%) of xylose, resulting in high xylose concentration (42 g/L) as compared with its content in the residue after ethanol production. Taking into the fact that significant decreases of permeate flux was observed during membrane filtrations, further research should focus on fermentation broth pre-treatment to enhance the efficiency of membrane treatment. Moreover, presented filtration experiments do not represent the full range of possible filtration conditions/materials and the results presented have to be verified in a larger scale. Xylose concentrated in retentate was successfully converted into bio-succinic acid (average succinic yield of 70%), which is considered as one of the top 12 building-block chemicals of the future.

Acknowledgment

This work was supported by the University of Bielsko-Biala (Poland) within internal funds for development of young scientists as well as EU project POKL04.01.02-00-196/09-00 "Construction – A Bridge Between Tradition and Modernity in the region". The research was also conducted during 3 months internship (M. Kuglarz, University of Bielsko-Biala, Poland) in the Silesian Technical University (Faculty of Energy and Environmental Engineering, Institute of Water and Wastewater Engineering, Environmental Chemistry and Membrane Processes Research Group).

References

[1] Directive 2009/28/EC of the European Parliament and of the Council of 23 April 2009 on the Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC.

[2] F. Talebnia, D. Karakashev, I. Angelidaki, Production of bioethanol from wheat straw: an overview on pretreatment, hydrolysis and fermentation, Bioresour. Technol., 101 (2010) 4744–4753.

[3] B.F. Chong, M.D. Harrison, I.M. O'Hara, Stability of endoglucanases from mesophilic fungus and thermophilic bacterium in acidified polyols, Enzyme Microb. Tech., 61–62 (2014) 55–60.

[4] A.T.W.M. Hendriks, G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, Bioresour. Technol., 100 (2009) 8–10.

[5] Y.B. Huang, Y. Fu, Hydrolysis of cellulose to glucose by solid acid catalysts, Green Chem., 6 (2013) 1095–1111.

[6] C. Martín, J. Puls, B. Saake, A. Schreiber, Effect of glycerol pretreatment on component recovery and enzymatic hydrolysis of sugarcane bagasse, Cellulose Chem. Technol., 7–8 (2011) 487–494.

[7] F.B. Sun, H.Z. Chen, Evaluation of enzymatic hydrolysis of wheat straw pretreated by atmospheric glycerol autocatalysis, J. Chem. Technol. Biotechnol., 82 (2007) 1039–1044.

[8] X.B. Zhao, K.K. Cheng, D.H. Liu, Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis, Appl. Microbiol. Biotechnol., 82 (2009) 815–827.

[9] W. Kricka, J. Fitzpatrick, U. Bond, Challenges for the production of bioethanol from biomass using recombinant yeasts, Adv. Appl. Microbial., 92 (2015) 89–125.

[10] K.K. Cheng, X.B. Zhao, J. Zeng, J.A. Zhang, Biotechnological production of succinic acid: current state and perspectives, Biofuels, Bioprod. Biorefin., 6 (2012) 302–318.

[11] H. Song, S.Y. Lee, Production of succinic acid by bacterial fermentation, Enzyme Microb. Technol., 39 (2006) 352–361.

[12] J. Bohdziewicz, M. Kuglarz, Treatment of post-digestion liquors with the application of struvite precipitation and reverse osmosis, Desal. Wat. Treat., 51 (2013) 366–373.

[13] M. Kuglarz, J. Bohdziewicz, Pre-treatment of co-digestion effluents before reverse osmosis (RO) application, Desal. Wat. Treat., 51 (2013) 4872–4880.

[14] M. Kuglarz, K. Grübel, J. Bohdziewicz, Chemical precipitation and ammonia air stripping as effective pre-treatment methods before membrane filtration of co-digestion effluents, Desal. Wat. Treat., 55 (2015) 1672–1682.

[15] D. Ryan, A. Gadd, J. Kavanagh, G.W. Barton, Integrated biorefinery wastewater design, Chem. Eng. Res. Des., 87 (2009), 1261–1268.

[16] J.M. Prodanović, V.M. Vasic, Application of membrane processes for distillery wastewater purification – a review, Desal. Wat. Treat., 51 (2013) 3325–3334.

[17] I.B. Gunnarsson, M. Kuglarz, D. Karakashev, I. Angelidaki, Thermochemical pretreatments for enhancing succinic acid production from industrial hemp (*Cannabis sativa L.*), Bioresour. Technol., 182 (2015) 58–66.

[18] M. Kuglarz, M. Alvarado-Morales, D. Karakashev, I. Angelidaki, Integrated production of cellulosic bioethanol and succinic acid from industrial hemp in a biorefinery concept, Bioresour. Technol., 200 (2016) 639–647.

[19] APHA, Standard Methods for the Examination of Water and Wastewater, 21st ed, American Public Health Association Fed., Washington DC, USA, 2005.

[20] M. Kuglarz, I.B. Gunnarsson, S.E. Svensson, T. Prade, E. Johansson, I. Angelidaki, Ethanol production from industrial hemp: effect of combined dilute acid/steam pretreatment and economic aspects, Bioresour. Technol., 163 (2014) 236–243.

[21] Z. Barta, J.M. Oliva, I. Ballesteros, D. Dienes, M. Ballesteros, K. Réczey, Refining hemp hurds into fermentable sugars or ethanol, Chem. Biochem. Eng. Q., 24 (2010) 331–339.

[22] P. Varanasi, P. Singh, M. Auer, P.D. Adams, B.A. Simmons, S. Singh, Survey of renewable chemicals produced from lignocellulosic biomass during ionic liquid pretreatment, Biotechnol. Biofuels, 6 (2013) 14.

[23] B. Xiong, T.L. Richard, M. Kumar, Integrated acidogenic digestion and carboxylic acid separation by nanofiltration membranes for the lignocellulosic carboxylate platform, J. Membr. Sci., 489 (2015) 275–283.

[24] K. Sasaki, Y. Tsuge, D. Sasaki, H. Teramura, S. Wakai, H. Kawaguchi, T. Sazuka, C. Ogino, A. Kondo, Increased ethanol production from sweet sorghum juice concentrated by a membrane separation process, Bioresour. Technol., 169 (2014) 821–825.

[25] A.C.D. Salazar, M.A.G. Garcia, J. Fontalvo, M. Jedrzejczyk, J.M. Rynkowski, I. Dobrosz-Gomez, Ethanol dehydration by pervaporation using microporous silica membranes, Desal. Wat. Treat., 51 (2013) 2368–2376.

[26] P. Kaewkannetra, N. Chutinate, S. Moonamart, T. Kamsan, T.Y. Chiu, Experimental study and cost evaluation for ethanol separation from fermentation broth using pervaporation, Desal. Wat. Treat., 41 (2012) 88–94.

[27] Y.H. Cho, H.D. Lee, H.B. Park, Integrated membrane processes for separation and purification of organic acid from a biomass fermentation process, Ind. Eng. Chem. Res., 51 (2012) 1020710219.

[28] K.-Q. Chen, J.A. Li, J.F. Ma, M. Jiang, P. Wei, Z.-M. Liu, H.J. Ying, Succinic acid production by *Actinobacillus succinogenes* using hydrolysates of spent yeast cells and corn fiber, Bioresour. Technol., 102 (2011) 1704–1708.