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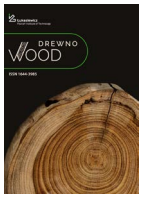

Enzymatic hydrolysis of pretreated lignocellulosic feedstocks improved by membrane separation

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Enzymatic hydrolysis is crucial in processing lignocellulosic biomass into valuable products in biorefineries. Due to the synergistic action of used enzymes the cellulose and hemicelluloses chains are digested into fermentable monosaccharides. It is known that the process efficiency can be improved by the separation of reaction end-products that are cellulase inhibitors. This work investigated the efficiency of enzymatic hydrolysis of corn stover and poplar wood biomass in a stirred dead-end membrane bioreactor, enabling continuous separation of end-products. Four UF membranes with different molecular weight cut-offs were tested, and PES 5 kDa was chosen as the most suitable. To pretreat biomass before hydrolysis, soaking in aqueous ammonia (SAA) and liquid hot water (LHW) methods were compared. The LHW treatment led to relatively high glucose contents (up to 73.7%). In turn, the SAA method led to high xylose contents up to 23.5%. In general, remarkable improvements (up to 72.6%) were observed in monosaccharides contents in hydrolyzates after the membrane bioreactor process. Only in the case of corn stover after SAA pretreatment were the reaction efficiencies in the membrane bioreactor similar to those obtained in batch mode, with an improvement of 4.3%.

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Introduction

Due to sustainability policies, efficient methods are needed to create various valuable products (fuels, medicines, polymers and many others) from renewable raw materials, especially non-food biomass. Therefore, much research has been devoted in the last decades to developing economically feasible technology for processing second-generation feedstocks, including lignocellulosic agricultural waste, wood residues and targeted crops [Bernacki et al. 2023; Lesar et al. 2016; Rathour et al. 2023; Reshmy et al. 2022; Zborowska et al. 2022]. Despite this, the general implementation of lignocellulosic biorefineries is still limited due to the relatively high biomass conversion costs.

Lignocellulosic biomass is a complex, heterogeneous material consisting of carbohydrates (cellulose and hemicelluloses), lignin and other minor components [Isikgor and Becer 2015]. Its conversion into the desired products can involve the fermentation of monosaccharides released from cellulose and hemicelluloses during acid or enzymatic hydrolysis [Antczak et al. 2018;

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El-Zawawy et al. 2011; Kołtuniewicz and Dąbkowska 2016; Krutul et al. 2024]. However, given the high lignin content, cellulose crystallinity, its degree of polymerization, and other factors, lignocellulosic raw materials are not prone to enzymatic breakdown [Zoghlami and Paës 2019]. Thus, it is essential to employ a step involving the pretreatment of lignocellulose before hydrolysis to alter its structure and expose the polysaccharide fractions for easy access to enzymes.

Accordingly, the pretreatment focuses on decreasing hemicelluloses and lignin content in the feedstock while simultaneously increasing available surface area and its porosity, and this may be achieved by different techniques. There are four main categories of pretreatment methods based on the nature of the impact: physical, chemical, physicochemical, and biological techniques [Alvira et al. 2010]. One of the most prevalent physicochemical treatments is the liquid hot water (LHW) method, praised for its relatively low cost, general simplicity and high efficiency [Akus-Szylberg et al. 2020; Antczak et al. 2022, 2023; Li et al. 2014]. It consists of applying hot water under pressure and at high temperature, which prompts autohydrolysis of acetyl groups present in hemicelluloses and their further separation from cellulose. On the other hand, among chemical methods, soaking in aqueous ammonia (SAA) is distinguished as a remarkable delignification process [Akus-Szylberg et al. 2021a, 2021b] in which utilized ammonia may be purified and reused [Gao et al. 2016]. In this pretreatment the biomass is altered either by swelling cellulose or changing its polymorphic form, solubilizing lignin and hemicelluloses, which leads to easier access to the cellulose.

Considering biomass diversity and complexity, it is crucial to designate and apply the most suitable pretreatment method, especially chosen for particular feedstock, to maximize the cellulose's susceptibility to the enzymes engaged in the subsequent process of the lignocellulose hydrolysis. The main objective of the next bioethanol procurement phase is to degrade biopolymers present in the plant-based material and generate fermentable monosaccharide hydrolyzate. The use of enzymes is one of the available hydrolysis methods, of which the main advantages are low by-product formation and energy consumption, mild operating conditions and environmental friendliness, although it also entails high costs [Zheng et al. 2009].

It has been found that some sugars (glucose and cellobiose) inhibit cellulases [Hsieh et al. 2014; Smith et al. 2010], adversely affecting the yield of the enzymatic process. Therefore, removing them from the reaction mixture during hydrolysis is highly advantageous. Membrane separation has shown promising results in this regard. Various membrane bioreactor configurations, including a reaction vessel integrated with a membrane operating in a dead-end mode, e.g. [Gan et al. 2002; Zhang et al. 2011] or a reactor coupled to an external module with cross-flow, e.g. [Yang et al. 2006, 2009] have been proposed to increase the enzymatic hydrolysis efficiency of cellulosic biomass. Usually, ultrafiltration (UF) membranes, nonpermeable for applied cellulases and unreacted substrate particles, are used. In this case, the low-molecular-weight hydrolysis end-products pass the membrane and thus are separated from the reaction mixture in the permeate stream. The retentate remaining in the reactor contains the enzymes, which can be reused in the subsequent hydrolysis, making the process more cost-feasible. A literature review concludes that the improvement of the process achieved by applying membrane separation depends on many factors, including used biomass type and enzymatic preparation. For example, [Zhang et al. 2011] used a flat sheet polyethersulfone UF membrane with 10 kDa cut-off for corn stover hydrolysis. Due to the continuous removal of inhibitors from the reaction space, they observed the enhancement of substrate conversion by 5% compared with the batch reactor. However, a slight protein content decrease in the bioreactor was observed during three hours, indicating that low-molecular-weight enzymes could gradually pass the membrane. In another study [Yang et al. 2009] a hollow fibre UF membrane bioreactor was used to perform enzymatic hydrolysis of corn stalks. In comparison to the batch process, the hydrolysis yield was enhanced by about 206% in this case, depending on the process conditions.

This study investigated the efficiency of enzymatic hydrolysis of corn stover and poplar wood biomass in a UF membrane bioreactor with continuous separation of end-products. To pretreat biomass before hydrolysis, soaking in aqueous ammonia (SAA) and liquid hot water (LHW) methods were applied, and their effects were compared. Four UF membranes with different molecular weight cut-offs (MWCOs) were tested, and the one most suitable was chosen. The activity of the enzymes retained by the membrane was taken into account. Among the studies of lignocellulosic feedstock hydrolysis performed so far in membrane bioreactors, UF membrane with a MWCO of 10 kDa has generally been applied, whereas the molecular masses of cellulolytic enzymes are higher (25–150 kDa) [Jung et al. 2019]. However, enzymatic preparations may contain other components necessary for enzymatic action. Therefore, the impact of ultrafiltration on cellulase activity should be considered when testing the separation capacity of membranes. Besides, cellulose or non-wood biomass has so far been generally applied as a substrate. It is evident that different compositions and structures of biomass influence its hydrolysis yield. Thus, the efficiency of the process carried out in a membrane

bioreactor should be analyzed separately for each biomass characteristic. According to our knowledge, this is the first publication comparing the performance of membrane bioreactors for hydrolysis of various lignocellulosic feedstocks differing in susceptibility to enzymatic digestion.

Materials and methods

1. Lignocellulosic feedstocks

This research was performed on two lignocellulosic materials: corn stover obtained from Polish commercial fields, and stem-wood of poplar (fast-growing *P. trichocarpa* Torr. & A. Gray ex Hook, age: 7 years), which was harvested at the end of winter from an experimental field in Wolica owned by the Warsaw University of Life Sciences, Department of Plant Genetics, Breeding and Biotechnology. The poplar wood material was dried in air and debarked. Both raw plant-based materials were milled, and a fraction with dimensions of 0.43–1.02 mm was used for further analysis.

2. Reagents and enzymes

In the enzymatic hydrolysis process, Cellic® CTec2 (Novozymes, Bagsvaerd, Denmark, mixture of hemicellulases, cellulases and β-glucosidases with a density of 1.203 g/cm^3) was used. The other chemicals were analytically pure and were purchased from Merck Life Science Sp.z.o.o. (Poznań, Poland).

3. Experimental set-up

The experiments were performed in the set-up shown in Fig. 1. It comprised a laboratory-scale UF membrane bioreactor (Amicon Stirred Cell 0.2 dm^3, Millipore, USA) connected to a reservoir tank (Amicon Cell Reservoir 0.8 dm^3, Millipore, USA). The bioreactor was equipped with an appropriate flat-sheet membrane (Microdyn Nadir, Wiesbaden, Germany). The membrane was rinsed with distilled water for 15 min and placed at the bottom of the bioreactor with the active layer upward. A magnetic plate stirrer (M21, CAT, Germany) was used to provide mixing of the feed, while an external water jacket was used to thermostat the bioreactor vessel. Nitrogen gas was supplied to the reservoir tank to maintain constant pressure in the system, ensuring the permeate flow.

4. Membranes and their characteristics

Four flat-sheet UF membranes (Microdyn Nadir, Wiesbaden, Germany) with different nominal molecular weight cut-offs (5, 10, 20 and 30 kDa) and a diameter of 63.5 mm (a surface area of 31.67 × 10^2 mm^2) were applied. All were made of polyethersulfone (PES) and had asymmetric structures, containing a thinner dense layer (active layer) responsible for the membrane separation capacity and a thicker porous support [Li and Walz 2014]. The membrane with a 30 kDa cut-off was made of PESH, i.e. polyethersulfone modified for enhanced hydrophilic properties to ensure better resistance to fouling.

Before hydrolysis, experiments were performed using a cylinder and stopwatch to determine each membrane's water permeability (L_v [dm^3·m^{-2}·h^{-1}·bar^{-1}]) by measuring distilled water flow rates at three different transmembrane pressures of 1.0, 1.5, and 2.0 bar. The average data obtained for each pressure was used to calculate L_v values, using equation (3). Besides, the membrane's retention coefficients (R) for enzymatic proteins included in the used Cellic® CTec2 preparation were determined to choose the best membrane for hydrolysis. To find out the R value, ultrafiltration of 5%$_{w/w}$ solution of Cellic® CTec2 in citric buffer was performed at 1 bar in an Amicon Stirred Cell thermostated at 48 °C with the feed stirred at 500 rpm for 60 min. The feed volume was kept constant by a continuous supply of the buffer to the reactor from the reservoir tank. The protein concentrations were determined in permeate, applying the procedure described in section 7.1. Samples from the feed before and after ultrafiltration were used to analyze its cellulolytic activity, as described in section 7.1.

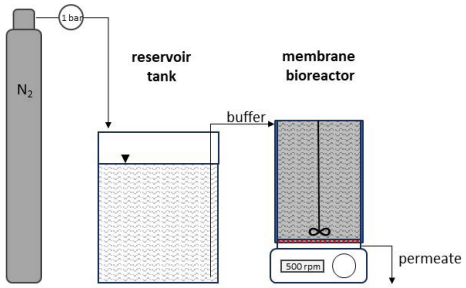

Fig. 1. Membrane bioreactor set-up

5. Biomass pretreatment

LHW pretreatment: in the process a raw material (with 7% moisture content; 20 g of absolutely dry biomass) was soaked in distilled water at 75 °C for 20 min with using a magnetic stirrer to the swell material and remove remaining air. Next, it was quantitatively transferred to a stainless steel reactor (volume 250 cm^3) with the use of water to maintain a solid-to-liquid ratio of 1:12.5. Then the pretreatment was carried out at 190 °C for 20 minutes, and finally the material was cooled rapidly to end the reaction.

SAA pretreatment: a raw material (with 6% moisture content; 20 g of absolutely dry biomass) was transferred to a stainless steel reactor and with a respective amount of 15% (w/w) ammonia solution to maintain a solid-to-liquid ratio of 1:12.5. Then the process was carried out at 90 °C for 20 h, and finally the material was cooled rapidly to end the reaction.

After both the LHW and the SAA pretreatments were performed, solid and liquid fractions were separated by Büchner funnel filtration, and solid fractions were washed with distilled water until the pH reached 7. Two processes were carried out for each method and conditions, and the two obtained solid fractions were mixed. Both neutralized solid fractions obtained from the LHW and the SAA methods were stored at 6 °C until further analysis.

6. Enzymatic hydrolysis in membrane bioreactor

The enzymatic hydrolysis was performed in a bioreactor applying membrane separation of end-products. To prepare the hydrolysis reaction mixture, specimens of wet pretreated lignocellulosic feedstock (corn stover and poplar wood) with known moisture content (4.5 g of absolutely dried material) were placed in a bioreactor thermostated at 48 °C with 9.976 cm^3 of the 25% v/v Cellic® CTec 2 enzyme solution (0.1 g of enzyme per 0.1 g of cellulose), 150 cm^3 of citric acid buffer (pH of 4.8) and 3 cm^3 of a 2% sodium azide solution. The feed was stirred at 500 rpm to guarantee its homogeneity and to minimize filter cake formation and concentration polarization. The citric acid buffer was also included in the reservoir tank. A pressure in the system of 1 bar was generated by supplying nitrogen gas, and then the bioreactor was filled with the buffer from the tank reservoir to a volume of 305 cm^3. At the same time, permeate was forced to flow. It was collected in a separate container during the whole time of hydrolysis. After 72 h the reaction was complete, so permeate and retentate samples were taken for glucose and xylose content analysis. Each hydrolysis process was repeated three times, and the standard deviation was calculated.

7. Analytical methods

7.1. Determination of enzymatic protein concentrations and activity

Enzymatic protein concentrations in the samples taken from feed and permeate after ultrafiltration of Cellic® CTec2 were analyzed with a UV-VIS spectrophotometer (Helios Gamma 9423 UVG 1702E, Thermo Electron Corporation) measuring the UV absorbance at 280 nm using a previously described method [Dąbkowska-Susfał 2023]. Five concentrations of Cellic® CTec2 in the citric buffer were applied as standard solutions to prepare the calibration curve. Citric buffer was used as a blank.

The enzymatic activities in the samples were determined by the CellG5 method (Megazyme, Ireland) [Mangan et al. 2016] using the Cellulase Assay Kit.

7.2. Determination of glucose and xylose contents

Monosaccharide (glucose and xylose) contents in the samples after hydrolysis were determined by high-performance liquid chromatography (HPLC). The analysis used the method previously developed by Akus-Szylberg et al. [2020].

8. Calculations

The membrane permeability (L_v) was determined by measuring the distilled water permeate flux (J_w [$dm^3 \cdot m^{-2} \cdot h^{-1}$]) at different transmembrane pressures (TMP [bar]), applying the following equation [Conidi et al. 2014]:

$$L_v = \frac{J_w}{TMP} \quad (1)$$

The membrane retention coefficient towards enzymatic proteins (R[-]) was calculated according to equation 2:

$$R = 1 - \frac{C_{P,p}}{C_{P,f}} \quad (2)$$

where $C_{p,p}$ and $C_{P,f}$ are the enzymatic protein concentrations in the permeate and the feed [%], respectively.

The relative enzymatic activity of the feed ($a_{rel,f}$) was determined according to equation 3:

$$a_{rel,f} = \frac{a_{f,0}}{a_{f,60min}} \quad (3)$$

where $a_{f,0}$ and $a_{f,60min}$ are cellulolytic enzyme activities in the feed before and after 60 min of ultrafiltration, respectively, determined by the CellG5 method.

The glucose or xylose contents (G/K) were calculated based on the amounts of glucose and xylose released during the process ($m_{G/K}$ [g]) and the initial content (dry mass) of pretreated biomass (m_B [g]) in the reaction mixture, as follows:

$$G/K = \frac{(m_{G/K})}{m_B} \cdot 100\% \quad (4)$$

Amounts of glucose or xylose ($m_{G/K}$ [g]) released during enzymatic hydrolysis carried out in the membrane bioreactor were calculated according to the mass balance expressed by equation 5:

$$m_{G/K} = C_{G/K,r} \cdot V_r + C_{G/K,p} \cdot V_p \quad (5)$$

where $C_{G/K,r}$ and $C_{G/K,p}$ [g·dm^{-3}] are glucose or xylose concentrations in the retentate and collected permeate, respectively, and V_r and V_p [dm^3] are volumes of retentate and the whole of the collected permeate, respectively, at the end of hydrolysis after 72 h.

Results and discussion

1. Selection of membrane for hydrolysis experiments

To select a suitable membrane for enzymatic hydrolysis of lignocellulosic biomass, membrane retentions of catalytic proteins were considered. Since the applied membranes had cut-off values in the range 5–30 kDa, they all retained biomass particles in the reaction mixture due to their being larger than the membrane pores. Monosaccharides released during the reaction passed to the permeate site as low molecular weight sugars and thus were removed from the hydrolysis solution. It was desired that the membranes remove monosaccharides (products) in a permeate stream while retaining maximum enzymatic activity in the reactor.The obtained membrane water permeabilities and retention coefficient values for Cellic® CTec2 solution are presented in Table 1. As observed, the obtained L_v values were dependent on membrane cut-off according to manufacturer data (given in parentheses). For all membranes made of unmodified PES, permeability increased for higher MWCO. An exception was observed for the PESH 30 kDa membrane, made of hydrophilized polyethersulfone.

Considering the calculated retention coefficients, the results show that each membrane used retained most of the proteins included in the Cellic® CTec2 preparation. The highest R value (0.77 ± 0.06) was observed for the PES 5 kDa membrane. The R values were significantly lower for the other membranes and slightly decreased with membrane cut-off in the 10–30 kDa range. Besides, after ultrafiltration of Cellic® CTec2 solution using a 5 kDa membrane, the relative enzymatic activity in the feed was close to 1.0, meaning that the proteins removed in the permeate were not enzymatic. Slightly lower $a_{rel,f}$ values were obtained when the other membranes were used. However, it should be noted that the enzymatic preparation is a mixture containing different components besides enzymes. Thus, removing just some of them may cause a decrease in enzyme activity.

In summary, the PES 5 kDa membrane exhibited the best retention ability towards the enzymatic proteins used, and thus was selected as the most suitable for further hydrolysis experiments.

The results obtained for the PES 5 kDa membrane were consistent with a previous study [Dąbkowska-Susfał 2023]. On the other hand, the determined R values were lower than those found for a 10 kDa PES membrane by [Zhang et al. 2011], which were in the range 0.97–0.98. However, this may arise from the different compositions of the enzymatic preparations used in the experiments.

2. Efficiency of enzymatic hydrolysis

Enzymatic hydrolysis experiments were performed in a membrane bioreactor to study the impact of product separation from the reaction mixture on the glucose and xylose content. Additionally, the results obtained were compared with the previous results for enzymatic hydrolysis carried out in batch mode. In these cases, the material used, the initial composition of the reaction mixture, as well as process conditions, including

Table 1. The determined values of membrane distilled water permeabilities (L_v), retention coefficients toward catalytic proteins in Cellic® CTec2 (R) and relative enzymatic activities in the feeds ($a_{rel,f}$) at 48°C

Membrane	L_v (dm^3·m^{-2}·h^{-1}·bar^{-1})*	R (-)	$a_{rel,f}$ (-)
PES 5 kDa	17.6 ± 0.9 (≥10)	0.77 ± 0.06	0.95 ± 0.03
PES 10 kDa	103.0 ± 1.5 (≥50)	0.68 ± 0.05	0.88 ± 0.06
PES 20 kDa	108.8 ± 1.7 (≥70)	0.67 ± 0.03	0.87 ± 0.05
PESH 30 kDa	83.4 ± 2.1 (≥35)	0.60 ± 0.02	0.75 ± 0.06

* Water permeability values at 22°C given by the membrane manufacturer are shown in parentheses

Table 2. The glucose and xylose contents after enzymatic hydrolysis of corn stover and poplar wood performed in batch mode and in membrane bioreactor both untreated and after LHW pretreatment process

Material	Untreated			LHW pretreatment					
	Batch mode hydrolysis			Batch mode hydrolysis			Membrane bioreactor mode hydrolysis		
	glucose (%)	xylose (%)	Σ (%)	glucose (%)	xylose (%)	Σ (%)	glucose (%)	xylose (%)	Σ (%)
Poplar wood	4.4 ± 0.4[a]	1.6 ± 0.1[a]	6.0	47.0 ± 0.4[a]	3.3 ± 0.0[a]	50.3	73.7 ± 0.4	13.1 ± 0.1	86.8
Corn stover	8.0 ± 0.1[b]	2.5 ± 0.0[b]	10.5	48.3 ± 0.5[b]	6.7 ± 0.1[b]	55.0	62.5 ± 0.3	15.3 ± 0.2	77.8

[a] Akus-Szylberg et al. [2020]
[b] Akus-Szylberg et al. [2018]

Table 3. The glucose and xylose contents after enzymatic hydrolysis of corn stover and poplar wood performed in batch mode and in membrane bioreactor both untreated and after SAA pretreatment process

Material	Untreated			SAA pretreatment					
	Batch mode hydrolysis			Batch mode hydrolysis			Membrane bioreactor mode hydrolysis		
	glucose (%)	xylose (%)	Σ (%)	glucose (%)	xylose (%)	Σ (%)	glucose (%)	xylose (%)	Σ (%)
Poplar wood	4.4 ± 0.4[a]	1.6 ± 0.1[a]	6.0	29.7 ± 0.5[d]	12.2 ± 0.4[d]	41.9	48.4 ± 0.5	22.9 ± 0.4	71.3
Corn stover	8.0 ± 0.1[b]	2.5 ± 0.0[b]	10.5	49.3 ± 0.8[c]	22.3 ± 0.4[c]	71.6	51.2 ± 0.5	23.5 ± 0.3	74.7

[a] Akus-Szylberg et al. [2020]
[b] Akus-Szylberg et al. [2018]
[c] Akus-Szylberg et al. [2021a]
[d] Akus-Szylberg et al. [2021b]

temperature, mixing, and pH, were the same. The results obtained at the end of the 72 h reaction performed for corn stover and poplar wood after SAA and LHW pretreatments are presented in Tables 2 and 3.

The results for the sum of glucose and xylose content presented in Tables 2 and 3 were much higher in the hydrolyzates of the treated biomass (between 41.9% and 86.8%) than in the untreated biomass (respectively 10.5% for corn stover and 6.0% for polar wood), regardless of whether the hydrolysis was carried out in batch mode or in a membrane bioreactor.

The monosaccharides content in hydrolyzates of poplar wood pretreated with the LHW amounted to 50.3% after batch mode hydrolysis and a remarkable 86.8% after hydrolysis performed in the membrane bioreactor (Table 2). Not only was the sum of monosaccharides higher due to product separation from the reaction mixture, but also both glucose and xylose contents separately were higher than in the case of batch mode hydrolysis. Analogous results were also achieved for poplar wood treated with the SAA method (41.9% after batch mode and 71.3% after hydrolysis in the membrane bioreactor), which suggests hydrolysis performed in the membrane bioreactor was very effective for the tested wood (Table 3). Also, according to the results, hydrolysis performed in a membrane bioreactor was an effective method for corn stover. After the LHW pretreatment, monosaccharides content in corn stover hydrolyzates amounted to 55.0% in batch mode and 77.8% in the membrane bioreactor mode (Table 2). From the corn biomass treated in alkaline conditions, higher monosaccharides content was also obtained after hydrolysis in the membrane bioreactor (74.7%) than after batch mode hydrolysis (71.6%) (Table 3). Assessing the pretreatment methods, regardless of the enzymatic hydrolysis mode and the type of lignocellulosic raw material, the LHW method led to higher glucose contents (47.0–73.7%) than the SAA method (29.7–51.2%). In turn, the SAA method led to higher xylose contents after enzymatic hydrolysis (12.2–23.5%) than with

the applied hydrothermal pretreatment (3.3–15.3%). Analogous relationships have been observed in other studies in which similar pretreatment methods were tested. Wyman et al. [2011] treated switchgrass by the LHW and SAA methods and also found that as a result of enzymatic hydrolysis in batch mode, more glucose was produced from LHW-pretreated biomass (47.3%) than from SAA-pretreated biomass (39.8%). They obtained an opposite relationship in the case of xylose, for which the SAA method turned out to be more effective (17.8%) than the LHW treatment (5.3%). In turn, analogous studies of hydrolysis in batch mode on corn stover and poplar wood conducted by Antczak et al. [2023] also showed that high glucose content (50.5–60.1%) was obtained from biomass pretreated by the LHW method. However, biomass processing in an alkaline environment (2% NaOH) led to high xylose contents (15.2–19.2%) after hydrolysis. The presented efficient methods of releasing glucose and xylose from lignocellulosic raw materials may be of interest in the future in the context of their conversion to valuable chemical substances such as ethanol, sorbitol, xylitol, furfural or furfuryl alcohol, etc.

The observed increase in monosaccharides release in the membrane bioreactor may be explained by the removal of inhibitory products and acceleration of the reaction rate due to the larger substrate concentration at the membrane surface than inside the reactor [Dąbkowska-Susfał et al. 2024]. Besides, based on our results, it can be stated that the improvement of enzymatic hydrolysis efficiency by end-product separation using a membrane depends on the biomass type and pretreatment methods. In general, the process efficiency increase was up to 72.6% compared with batch mode. Only in the case of corn stover after SAA pretreatment were the monosaccharides contents obtained in the membrane reactor similar to those obtained in batch mode (the improvement was only 4.3%). However, it can be observed that this biomass's hydrolysis yield was appropriately high (a monosaccharides content of 71.6%) even when the reaction was conducted in batch mode.

The positive influence of membrane separation on hydrolysis efficiency of lignocellulosic biomass is consistent with many previous studies. For example, in the case of enzymatic hydrolysis of SAA-pretreated corn stover conducted in a membrane bioreactor, cellulose conversion to glucose increased by 5% in comparison to a batch reactor [Zhang et al. 2011]. A much higher improvement of up to 206% was reported by Yang et al. [2009] for the hydrolysis of corn stalks. Moreover, our previous results [Dąbkowska-Susfał et al. 2024] obtained for corn stover hydrolysis performed in a reactor with tubular ceramic microfiltration membranes placed vertically indicated that the final reaction yield was ca. 6–21% higher than in batch mode, depending on the transmembrane pressure used. However, the pretreatment method and enzyme preparations in all of the above referenced cases differed from the present study. To our knowledge, no data on poplar wood hydrolysis in membrane reactors are available in the literature.

Conclusions

This is the first study in which various lignocellulosic feedstocks with different susceptibilities to enzymatic hydrolysis in a membrane bioreactor have been used for comparative purposes in the same experimental set-up and process conditions. The following main conclusions may be drawn from the results:

1. The PES 5 kDa membrane exhibited the best retention ability towards the enzymatic proteins used, and thus was selected as the most suitable for further hydrolysis experiments using Cellic® CTec2 enzyme solution.
2. Remarkable improvements (up to 72.6%) in monosaccharides contents in hydrolyzates obtained in the membrane bioreactor were observed for poplar wood biomass and corn stover after LHW pretreatment. Only in the case of corn stover after SAA pretreatment were the reaction efficiencies in the membrane bioreactor similar to those obtained in batch mode, with an improvement of 4.3%.
3. Regardless of the enzymatic hydrolysis mode and the type of lignocellulosic raw material, the LHW treatment led to relatively high glucose contents (47.0–73.7%). In turn, the SAA method led to higher xylose contents (12.2–23.5%) in hydrolyzates than were obtained after LHW.

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