EFFICIENCY OF CORN AND POPLAR BIOMASS SACCHARIFICATION AFTER PRETREATMENT WITH POTASSIUM HYDROXIDE

Abstract: Pretreatment is an essential step in the conversion of lignocellulosic biomass into valuable products. It aims to increase the biomass susceptibility to enzymatic saccharification to generate fermentable monosaccharides. In this study, the efficiency of 2% potassium hydroxide (KOH) solution used as a pretreating agent for various lignocellulosic feedstocks, such as corn straw, corncob, and poplar wood, was evaluated. The influence of the pretreatment time, which varied from 0.5 to 24 h at 50 °C, on the alteration of biomass composition was investigated, as well as the enzymatic digestibility. Finally, the overall sugar yields were determined. For corncob, the yield on average amounted to 453.9 ±18.9 mg·g⁻¹ raw (untreated) biomass, regardless of the pretreatment time. The overall sugar yield for both the corn straw and poplar wood biomass increased with increased pretreatment time and ranged from 333.0 to 438.4 mg·g⁻¹ raw biomass and from 123.2 to 215.7 mg·g⁻¹ raw biomass, respectively. Based on the results obtained, the most appropriate pretreatment times for all types of biomass were proposed. The results of this study may be useful for the development of lignocellulosic biomass processing technology.

Keywords: corn straw, corncob, pretreatment time, enzymatic digestibility, overall sugar yield

Introduction

The pretreatment of lignocellulosic biomass is the first step in the production of second generation biofuels and other valuable products in biorefineries [1–4]. It is performed to disrupt the complex lignocellulosic structure, which in turn contributes to enhancing the availability of cellulose and hemicellulose, i.e., polysaccharides, these being the main components of lignocellulose, to the enzymes that convert them into fermentable C5 and C6 monosaccharides (mainly glucose and xylose) [5]. Various pretreatment technologies developed and widely studied to date, including physical, chemical, and biological methods, have been described in literature and tested on a pilot scale [6–8]. Nevertheless, the pretreatment step is still considered a technological bottleneck in lignocellulose processing. More research is needed to develop methods for increasing the efficiency and cost effectiveness of the whole process. The choice of technology depends mainly on the type of biomass used as a feedstock and its structure and lignin content.

Among the various pretreatment methods, chemical ones have received the greatest attention and is used most often because it is effective, relatively cheap, and of low intensity in terms of time and energy [8, 9]. Alkaline pretreatment is used primarily due to its high efficiency of lignin removal from the biomass, thus improving the reactivity of

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1 Faculty of Chemical and Process Engineering, Warsaw University of Technology, ul. L. Waryńskiego 1, 00-645 Warszawa, Poland, phone +48 22 234 62 72, email: Katarzyna.Dabkowska@pw.edu.pl
polysaccharides by enhancing the exposure of cellulose and hemicellulose to enzyme attacks. Lignin acts as a physical barrier limiting the access of cellulolytic enzymes to substrate and has the capacity to non-productively adsorb the enzymes, resulting in reduced saccharification efficiency [10, 11]. Therefore, removing of lignin content is essential for the improvement of the enzymatic digestibility of lignocellulosic biomass.

Besides lignin removal, the advantage of using alkaline pretreatment is utilizing of non-polluting and non-corrosive reagents, as well as its effectiveness under relatively mild conditions. Furthermore, unlike acid pretreatment, this method does not generate by-products such as furfural and 5-hydroxymethylfurfural, which may act as inhibitors for enzymes and microorganisms [12]. The mechanism of alkaline pretreatment is suggested to be based on the breaking of intermolecular ester bonds between lignin and other polymers, particularly hemicellulose [13]. In addition, during alkaline process, the acetyl and uronic acid substitutions in hemicellulose are removed, which leads to enhanced accessibility of enzymes to its surface and improves biomass digestibility [14]. Alkaline delignification also tends to solubilize some hemicelluloses [15], which may be considered the main drawback of the process as it contributes to the loss of polysaccharides in biomass.

In most investigations carried out thus far, sodium hydroxide (NaOH) solutions have tended to be used as alkali pretreatment reagents and been extensively studied [16, 17]. In contrast, far less reports have concerned the application of potassium hydroxide (KOH), although it has many advantages over NaOH. For example, effluents generated during NaOH pretreatment are recognized as harmful chemicals for the environment because they may cause water pollution and soil salination; this limits its application on an industrial scale. Effluents of KOH pretreatments may be used in potassium fertiliser and thus are considered more environmentally friendly than NaOH [18]. Until now, KOH pretreatment has only been applied to a limited range of lignocellulosic materials, such as switchgrass [19], sugarcane bagasse [20], wheat straw [21], kallar grass and cotton stalk [22].

In this study, the effect of KOH pretreatment on different lignocellulosic biomass abundantly produced in Poland such as corn straw, corncob, and poplar wood, was investigated. The study focuses on the influence of the duration of pretreatment on the recovery of polysaccharides (glucan and xylan) and lignin reduction in biomass, as well as on the susceptibility of biomass to subsequent enzymatic hydrolysis. The main objective of the study is to determine the most appropriate pretreatment time for each tested biomass type at mild (50 °C, 2 % KOH) process conditions.

**Materials and methods**

**Feedstock**

Three different lignocellulosic raw (untreated) materials were used as feedstock: corn straw, corncob, and poplar wood (*Populus deltoides × maximowiczii*). The corn biomass was provided by Bioagra S.A. (Warsaw, Poland), while the poplar wood originated from the experimental cultivation conducted by the Warsaw University of Life Sciences (Warsaw, Poland). The biomass was dried, shredded with a knife mill (Type T2-SW; ZWM Trymet Sp. z o.o., Pilchowo, Poland), and screened to a particle size of 0.5 to 1.0 mm before use. The main biomass composition determined by the National Renewable Energy Laboratory (NREL) procedure [23] is summarized in Table 1.
Main composition of the studied feedstock

| Feedstock     | Glucan [% d.m.] | Xylan [% d.m.] | Total lignin [% d.m.] |
|---------------|-----------------|----------------|----------------------|
| Corn straw    | 34.6 ±3.0       | 26.6 ±2.8      | 18.8 ±1.5            |
| Corn cob      | 35.1 ±2.1       | 29.3 ±2.3      | 18.6 ±2.0            |
| Poplar wood   | 40.3 ±2.1       | 16.4 ±1.0      | 28.3 ±1.2            |

* Klason lignin + acid soluble lignin; d.m. - dry matter; data are presented as mean ± standard deviation

**Biomass pretreatment**

The pretreatment of lignocellulosic raw materials was carried out through an alkaline-based process using 2 % solutions of potassium hydroxide (Chempur, Piekary Slaskie, Poland). Each process involved soaking 5.0 g of dry matter (d.m.) raw material in 0.1 dm³ of 2 % KOH solution and mixing at 50 °C and 150 rpm for 0.5, 1, 2, 4, or 24 h. The temperature and concentration of KOH were selected based on our previous studies (data not shown). All assays were performed in triplicate. After pretreatment, the remaining biomass was separated under a vacuum from the liquid phase, washed with distilled water until the filtrate reached a neutral pH, and then dried at an ambient temperature for 24-48 h. The pretreated biomass (solid fraction) with at least 90 % of d.m. content was used as a substrate for enzymatic hydrolysis. The liquid phase was subjected to an analysis of monosaccharide content.

**Enzymatic hydrolysis**

The hydrolysis reaction mixtures consisted of 5.0 g of d.m. of pretreated biomass, 0.1 dm³ of 0.05 M citrate buffer (pH 5.4), and 0.5 g of a Cellic® CTec2 (64.6 FPU per g, filter paper unit (FPU)) enzyme cocktail (Novozymes, Bagsvaerd, Denmark). Cellic® CTec2 is a commercially available cellulolytic enzyme preparation that shows good efficiency in laboratory tests [24, 25]. It is composed of a mixture of hydrolytic enzymes necessary for saccharification of lignocellulosic polysaccharides, including cellulases, hemicellulases as well as β-glucosidases. The reaction was performed in a batch mode at 50 °C and 150 rpm for 72 h. During the process, 10 samples (0.5 cm³ each) were taken from the reaction mixtures and used to determine concentrations of glucose, xylose and arabinose. To stop the reaction, harvested samples were immediately cooled in ice water and then centrifuged for 10 min at 14,243 g. Supernatants were diluted 5 times with distilled water prior to analysis. All hydrolysis assays were performed in triplicate.

The concentrations of monosaccharides in hydrolysates were determined via high performance liquid chromatography (HPLC) using the Varian 635 CL System (Varian Inc., Palo Alto, CA, USA) equipped with an autosampler (6 °C), a refractive index (RI) detector (Smartline 2300; Knauer, Berlin, Germany) and the Aminex HPX-87H column (BioRad Laboratories Inc., Hercules, CA, USA) thermostated at 50 °C. 0.001 N H₂SO₄ at a flow rate of 0.4 cm³·min⁻¹ was used as a mobile phase.

The percentage content of glucan and xylan in the raw and pretreated biomass was analysed using a two-step sulfuric acid hydrolysis method according to the procedure developed by the NREL [23]. Based on this method, cellulose and hemicellulose content was determined based on the amount of respective monosaccharides released from the biomass during complete acid catalysed hydrolysis.
The dry matter content of biomass was determined using a moisture analyser (MA 50/1.R; Radwag, Radom, Poland) operating at 105 °C [26].

Calculations

The removal of biomass during pretreatment was expressed as the percentage biomass weight loss, \( W_{\text{loss}} \) calculated based on the fraction of solid dry weight recovered in the pretreated material, according to:

\[
W_{\text{loss}} = \left(1 - \frac{m_{\text{pret}}}{m_{\text{raw}}}\right) \cdot 100 \% 
\]  

where \( m_{\text{pret}} \) is the dry mass of biomass recovered after pretreatment [g d.m.] and \( m_{\text{raw}} \) is the dry mass of used raw (untreated) biomass [g d.m.].

The degree of delignification (\( D_{\text{lig}} \)), defined as the weight fraction of lignin removed during pretreatment, was calculated following equation:

\[
D_{\text{lig}} = \left(1 - \frac{C_{\text{lig}}^{\text{pret}} \cdot m_{\text{pret}}}{C_{\text{lig}}^{\text{raw}} \cdot m_{\text{raw}}}\right) \cdot 100 \% 
\]  

where \( C_{\text{lig}}^{\text{pret}} \) is the concentration of lignin in the pretreated biomass [% d.m.], \( C_{\text{lig}}^{\text{raw}} \) is the concentration of lignin in raw biomass [% d.m.], \( m_{\text{pret}} \) is the dry mass of biomass recovered after pretreatment [g d.m.], and \( m_{\text{raw}} \) is the dry mass of used raw (untreated) biomass [g d.m.].

The percentage glucan (\( R_{\text{glu}} \)) and xylan (\( R_{\text{xyl}} \)) recovery were calculated based on their content in raw biomass and in the solid fraction retained after pretreatment, according to:

\[
R_{\text{glu}} = \frac{C_{\text{glu}}^{\text{pret}} \cdot m_{\text{pret}}}{C_{\text{glu}}^{\text{raw}} \cdot m_{\text{raw}}} \cdot 100 \% 
\]  

\[
R_{\text{xyl}} = \frac{C_{\text{xyl}}^{\text{pret}} \cdot m_{\text{pret}}}{C_{\text{xyl}}^{\text{raw}} \cdot m_{\text{raw}}} \cdot 100 \% 
\]

where \( C_{\text{glu}}^{\text{pret}} \) and \( C_{\text{xyl}}^{\text{pret}} \) are the concentrations of glucan and xylan in the pretreated biomass, respectively [% d.m.], \( C_{\text{glu}}^{\text{raw}} \) and \( C_{\text{xyl}}^{\text{raw}} \) are the concentrations of glucan and xylan in raw biomass, respectively [% d.m.], \( m_{\text{pret}} \) is the dry mass of biomass recovered after pretreatment [g d.m.] and \( m_{\text{raw}} \) is the dry mass of used raw (untreated) biomass [g d.m.]

The effectiveness of the enzymatic hydrolysis of pretreated biomass was expressed by the glucan (\( Y_{\text{glu}} \)) and xylan (\( Y_{\text{xyl}} \)) enzymatic digestibility, calculated using following equations:

\[
Y_{\text{glu}} = \frac{C_{\text{glu},72h} \cdot V_{h}}{X_{\text{glu}}^{\text{pret}} \cdot m_{h} \cdot 1.11} \cdot 100 \% 
\]  

\[
Y_{\text{xyl}} = \frac{C_{\text{xyl},72h} + C_{a,72h}}{X_{\text{xyl}}^{\text{pret}} \cdot m_{h} \cdot 1.14} \cdot 100 \% 
\]

where \( C_{a,72h} \), \( C_{g,72h} \), and \( C_{x,72h} \) are the concentrations of arabinose (a), glucose (g), and xylose (x), respectively, in the reaction mixture after 72 h of hydrolysis [g · dm\(^{-3}\)]. \( V_{h} \) is the
volume of the reaction mixture \([\text{dm}^3]\), \(m_h\) is the dry mass of pretreated biomass used as a substrate for hydrolysis reaction \([\text{g d.m.}]\), \(X_{\text{glu}}^{\text{pret}}\) and \(X_{\text{xyl}}^{\text{pret}}\) are the mass fractions of glucan (glu) and xylan (xyl) in the pretreated biomass, respectively; 1.11 is the stoichiometric conversion factor of glucan to glucose, and 1.14 is the stoichiometric conversion factor of xylan to xylose and arabinose.

To calculate the overall yield of biomass conversion into monosaccharides (overall sugar yield) \((Y_{\text{ov}})\) the following relationship was proposed:

\[
Y_{\text{ov}} = \frac{\left( m_{a,72h} + m_{g,72h} + m_{x,72h} \right) \cdot \alpha}{m_{\text{raw}}} \quad \text{[g \cdot g}^{-1}]\]  \(\text{(7)}\)

where \(m_{a,72h}\), \(m_{g,72h}\), and \(m_{x,72h}\) are the masses of arabinose (a), glucose (g), and xylose (x) released from the pretreated biomass during enzymatic hydrolysis [g], \(m_{\text{raw}}\) is the dry mass of used raw (untreated) biomass [g d.m.], and \(\alpha\) is the coefficient defined as the ratio of dry mass of the biomass recovered after pretreatment to the dry mass of the pretreated biomass used as a substrate for enzymatic hydrolysis, according to:

\[
\alpha = \frac{m_{\text{pret}}}{m_h} \quad \text{\(\text{(8)}\)}
\]

The introduction of the \(\alpha\) coefficient in Eq. (7) resulted from the fact that in the experiments only a part of the biomass obtained after the pretreatment process was subjected to enzymatic hydrolysis. The \(\alpha\) coefficient can take values in the range from 1 to \(\infty\).

### Results and discussion

#### Pretreatment of biomass - quantitative analysis

Alkaline pretreatment focuses on the dissolution of lignin due to the hydrolytic cleavage of ester bonds between its monomers. It is also accompanied by the removal of some polysaccharides through their endwise depolymerization reaction and the breakdown of glycosidic bonds [27]. This contributes to the reduction of the mass of the raw material during the process and to the alteration of its composition.

Table 2 summarizes the percentage values of biomass weight loss, \(W_{\text{loss}}\) and the compositional changes of polysaccharides and lignin in the corn straw, corncob, and poplar wood caused by pretreatment performed in 2 % KOH solutions at 50 °C. In each case, the pretreatment reduced the biomass weight: \(W_{\text{loss}}\) values ranged from 12.0 to 48.9 % and were dependent on the type of raw material and duration of the process. The lowest losses of biomass weight were observed for the experiments performed with the shortest residence time, i.e., 0.5 h, and became higher as the pretreatment time increased. The highest \(W_{\text{loss}}\) values, reaching up to 48.9 % after 24 h, were observed for corn straw, whereas pretreatment of both corncob and poplar wood led to much lower percentages of biomass removal. In the case of corncob, the \(W_{\text{loss}}\) values ranged from 18.9 to 32.2 % depending on the duration of the process, whilst for poplar wood the \(W_{\text{loss}}\) was between 12.0 and 22.3 %. The influence of the duration of pretreatment on the biomass weight reduction observed confirmed findings from other research studies [19, 28, 29].

The degree of delignification caused by the action of 2 % KOH solution on the biomass at 50 °C (Table 2) was highly dependent on the type of raw material and the duration of pretreatment. The highest \(D_{\text{lig}}\) values (49.7 to 87.7 %) were found for corn
straw and increased with the duration of the process. Additionally, the influence of time on delignification was noticeable in the corncob and poplar wood pretreatments, for which the $D_{lig}$ values were in the ranges of 31.6 to 62.0 % and 3.7 to 37.7 %, respectively. The higher removal of lignin with a longer residence time in alkaline medium has previously been reported [19, 30]. In the case of switchgrass biomass pretreated in KOH solutions [19] under process conditions like those used in this work, the lignin reduction after 24 h reached 41.7 %, which was lower than the results obtained in this study for corn straw and corncob biomass. In contrast, the results obtained for the alkaline hydrogen peroxide pretreatment of corncob at 50 °C in a previous study [30] showed a higher percentage of lignin removal (76.5 %) than that achieved in this study, even after just 8 h of the process. This demonstrates that the degree of delignification varies with the type of biomass being pretreated and the pretreatment medium used.

The glucan recovery ($R_{glu}$) (Table 2) was high for all types of the biomass investigated and ranged between 80.4 and 99.2 %. This indicated that most of the glucan was insoluble in the 2 % KOH solution at 50 °C and remained in solid fraction. This agrees with previous studies concerning the alkaline pretreatment of lignocellulosic biomass [31, 32]. The impact of the process duration on glucan recovery was indiscernible. Regarding xylan recovery, the $R_{xyl}$ values were highly dependent upon the type of biomass and duration of the process and ranged between 44.6 and 99.6 %. The lowest $R_{xyl}$ values were determined for corn straw, which suggested that a relatively high amount of xylan was removed from this raw material during pretreatment and this may be considered as the main drawback of the method. Compared to the glucan recovery, the influence of the process duration was major in this case; thus, a longer pretreatment in all experiments provided a higher removal of xylan. These results confirmed previous observations, such as for corncob pretreated by alkaline hydrogen peroxide [30]. It should be noted that a quantitative analysis of the liquid

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Table 2

| Pretreatment time [h] | $W_{loss}$ [% d.m.] | $R_{glu}$ [% d.m.] | $R_{xyl}$ [% d.m.] | $D_{lig}$ [% d.m.] |
|-----------------------|---------------------|--------------------|---------------------|---------------------|
| Corn straw            |                     |                    |                     |                     |
| 0.5                   | 36.7 ±3.2           | 85.7 ±3.2          | 55.3 ±3.2           | 49.7 ±3.2           |
| 1.0                   | 41.6 ±1.9           | 87.8 ±2.1          | 53.7 ±1.3           | 73.8 ±2.5           |
| 2.0                   | 43.7 ±2.7           | 86.8 ±3.2          | 52.4 ±2.1           | 79.3 ±3.1           |
| 4.0                   | 46.4 ±2.1           | 82.7 ±1.9          | 49.7 ±2.0           | 80.0 ±2.9           |
| 24.0                  | 48.9 ±1.8           | 84.5 ±2.1          | 44.6 ±2.5           | 87.7 ±3.2           |
| Corncob               |                     |                    |                     |                     |
| 0.5                   | 18.9 ±1.2           | 81.1 ±3.1          | 92.7 ±4.3           | 31.6 ±2.1           |
| 1.0                   | 21.6 ±1.0           | 81.2 ±2.2          | 82.8 ±3.6           | 34.5 ±1.6           |
| 2.0                   | 26.2 ±2.0           | 81.0 ±1.0          | 80.5 ±3.4           | 31.6 ±2.0           |
| 4.0                   | 28.1 ±2.1           | 79.9 ±2.0          | 78.5 ±3.0           | 49.6 ±3.1           |
| 24.0                  | 32.2 ±1.9           | 80.4 ±1.6          | 76.3 ±2.9           | 62.0 ±3.0           |
| Poplar wood           |                     |                    |                     |                     |
| 0.5                   | 12.0 ±1.0           | 99.2 ±2.0          | 99.6 ±2.1           | 3.7 ±0.2            |
| 1.0                   | 13.1 ±0.7           | 97.3 ±2.2          | 99.7 ±3.0           | 4.6 ±0.2            |
| 2.0                   | 14.8 ±1.8           | 98.0 ±1.8          | 99.1 ±2.4           | 10.8 ±0.4           |
| 4.0                   | 15.0 ±0.9           | 96.9 ±2.4          | 97.6 ±1.8           | 9.2 ±0.4            |
| 24.0                  | 22.3 ±1.7           | 97.7 ±1.3          | 92.8 ±2.0           | 37.7 ±1.1           |
fractions obtained after pretreatment showed only trace amounts of dissolved glucose and xylose (average 0.3 g · dm⁻³) for all types of the biomass investigated. Thus, the observed loss of glucan and xylan during pretreatment can be explained either by the basic instability and degradation of reducing sugars in a highly alkaline medium [33] or by the partial depolymerization of cellulose and hemicellulose resulting in the formation of oligosaccharides dissolved in the alkaline environment [34, 35]. The latter hypothesis can also be supported by the results of the author’s preliminary studies, in which a noticeable release of monosaccharides (especially xylose) was observed after the addition of a cellulolytic enzyme preparation (Cellic CTec2) to the neutralized liquid fraction collected after biomass pretreatment in the 2% KOH solution.

The influence of the duration of pretreatment on the main composition of corn straw, corncob, and poplar wood is presented in Figure 1. Regardless of the type of biomass used, the concentration of glucan in the recovered solid fractions was higher compared to its content in untreated material and generally increased gradually with longer duration of the process. At the same time, in all cases, the xylan content in the pretreated biomass was almost the same as in the raw material. This can be explained by the simultaneous removal of part of the xylan and lignin fractions from the biomass during pretreatment (see Table 2).

![Fig. 1. Influence of pretreatment time on the concentration of main biomass components](image)

**Enzymatic hydrolysis - glucan and xylan digestibilities**

Glucose and xylose were the main final products found in the hydrolysates of corn straw and corncob. These products comprised more than 98% of the total reducing sugars determined in the reaction mixtures after 72 h of hydrolysis for all studied durations of the prior pretreatment, whereas the remaining approximate 2% was arabinose. In the case of poplar wood hydrolysis, no other monosaccharides besides glucose and xylose were detected in hydrolysates. Final concentrations of monosaccharides in obtained hydrolysates equalled from 26.3 to 40.6 g·dm⁻³ for corn biomass and from 7.0 to 13.9 g·dm⁻³ for poplar wood, depending on the duration of the pretreatment. It should be noted that for all studied reactions, the cellobiose concentration in the reaction mixtures was close to zero. This is advantageous because cellobiose exhibits an inhibitory effect on cellulolytic enzymes [36]. This was related to the high activity of β-glucosidase present in the CelliC® CTec2 enzymatic cocktail applied as a catalyst.
Based on the concentrations of monosaccharides determined in the reaction mixtures after 72 h of hydrolysis of raw (untreated) and pretreated corn straw, corncob, and poplar wood, the glucan and xylan enzymatic digestibilities were calculated according to Eqs. (5) and (6). The results are presented in Figure 2. On average, the glucan digestibilities of raw corn straw, corncob, and poplar wood biomass amounted to 24.5, 27.2, and 8.1 %, respectively, whilst the xylan digestibilities were 19.8, 16.8, and 10.7 %, respectively. This indicated that the untreated biomass was hardly hydrolysable. Pretreatment in 2 % KOH solution notably increased the enzymatic hydrolysis of all lignocellulosic substrates used in this study, as demonstrated by the much higher digestibilities obtained for the pretreated corn straw, corncob, and poplar wood in comparison to the raw biomass. This proved the effectiveness of the alkaline pretreatment applied for all types of biomass before their enzymatic hydrolysis. Moreover, the enzymatic digestibility in most cases, except for the corncob glucan, increased remarkably with an increase in the pretreatment time, which suggested that a longer process duration facilitated the disruption of the rigid structure of the biomass. These results agreed with previous reports for different types of biomass and pretreating agents [37-39]. However, there are also some studies showing an indiscernible influence of the duration of pretreatment on the final yield of saccharification, which was observed after exceeding an optimal processing time, for example in the case of the hydrolysis of corn stover pretreated in aqueous ammonia [37] or alkaline organosolv solutions [39].

![Fig. 2. Glucan and xylan saccharification yields after 72 h of hydrolysis for all pretreated biomass used as a substrate](image)

In this study, in the case of pretreated corn straw hydrolysis, the average glucan enzymatic digestibility ranged from 69.6 to 92.8 % depending on the pretreatment time, while the values of xylan digestibilities were slightly lower and were in the range of 65.6 to 85.9 %. The results, especially for xylan hydrolysis, were better than many previously reported values for this type of biomass. For example, in the case of corn stover pretreated by alkali-assisted extrusion, the highest glucan and xylan enzymatic digestibilities amounted to 86.8 and 50.5 %, respectively [40]. In another study concerning the pretreatment of corn stover in phosphoric acid, the glucan and xylan saccharification yields do not exceed 70 and 45 %, respectively [41]. For corncob hydrolysis, the average glucan enzymatic digestibilities achieved in this study ranged from 84.7 to 93.7 %, while the xylan
digestibilities ranged from 57.9 to 78.5 %, depending on the pretreatment time. These results are superior compared to those of other studies, e.g., concerning hydrolysis of corncob pretreated by acid-catalysed steam explosion [42].

Among all of the types of biomass used in this study, poplar wood was the least susceptible to enzymatic hydrolysis. The average glucan digestibility obtained after 24 h of pretreatment barely amounted to 30.2 %, whereas the biomass subjected to a shorter process time did not exceed 10 %. At the same time, xylan digestibility ranged between 15.1 and 48.5 %, depending on the pretreatment time. The results obtained after 24 h of pretreatment were better than previously reported values for this type of biomass [43]. The reason for such low values compared to corn straw and corncob may have been associated with a limited access of enzymes to the polysaccharide chains in the pretreated poplar wood biomass, which indicated a low efficiency of the pretreatment process applied for this type of feedstock. This confirmed previous reports suggesting that more severe methods are required to enhance the enzymatic digestibility of poplar wood biomass [44].

The poor hydrolysis yields of poplar wood can be explained by the relatively high residual lignin content of this material, which was in the range of 30.9 to 22.7 % d.m., depending on the pretreatment time. As shown in Figure 3 an enzymatic digestibilities of each type of biomass used as a substrate tent to be inversely proportional to the residual lignin content, which indicated that the elevation in lignin removal significantly enhanced the enzymatic saccharification. The results suggested that further improvement of delignification of poplar wood were critical to increase its enzymatic digestibility.

The glucan and xylan digestibilities shown in Figure 2 are only a measure of the susceptibility of the pretreated biomass to enzymatic hydrolysis and do not describe the overall efficiency of the production of monosaccharides from the investigated feedstock. The monosaccharides production efficiency needs to be considered to estimate the benefits of using the proposed methodology for industrial application. The overall yields of corn straw, corncob, and poplar wood conversion into monosaccharides, calculated using Eq. (7), are presented in Figure 4.
The overall sugar yields were higher (up to 2.69, 3.04, and 3.85 times for pretreated corn straw, corncob, and poplar wood, respectively) than the yields determined for raw biomass. Among all of the investigated types of lignocellulosic feedstock, the highest process efficiency was observed for corncob. The overall monosaccharide yields obtained for the pretreated corncob were almost constant, regardless of the pretreatment time, and amounted to 453.9 ±18.9 mg·g⁻¹ raw biomass on average. This meant that the pretreatment time used in the study had no important influence on the overall process of monosaccharide production for this type of feedstock. Therefore, there was no justification for pretreating corncob for more than 0.5 h, at 50 °C, and using 2 % KOH as the pretreating agent. The process yield was higher than that found by Boonsombuti et al. [45] for corncob pretreated in 2 % NaOH at 100 °C for 30 min in a microwave oven. Although these authors reported monosaccharide yields of 683.97 mg·g⁻¹ for pretreated corncob, considering the loss of biomass weight (54.25 %) during the process, it can be calculated that the overall yield was 312.9 mg·g⁻¹ for raw biomass.

Figure 4 shows that the overall process yield for corn straw biomass increased with pretreatment time and ranged from 333.0 to 438.4 mg·g⁻¹ raw biomass; thus, in general, it was lower than that of corncob. In addition, it was observed that the maximum overall monosaccharide yield determined in this study for this type of biomass was approximately 20 % below the value reported by Yuan et al. [39] for corn stover pretreated with a potassium hydroxide-methanol solution at 80 °C. However, the differences may have arisen not only from the differing efficiency of the pretreatment processes applied, but also from the less precise dinitrosalicylic acid (DNS) method used previously to determine the sugar content in hydrolysate.

It should be noted, that the glucan and xylan content in both pretreated corn straw and corncob biomass was similar and equalled from 67.2 to 77.6 % (for corn straw) and from 63.7 to 74.6 % (for corncob), depending on pretreatment time. Therefore, observed noticeable differences in overall sugar yields for these raw materials indicate, that the efficiency of biomass saccharification depends not only on its percentage composition but is also influenced by the other structural features.

The influence of pretreatment time on the overall process efficiency was also observed for the poplar wood biomass. In this case, the overall sugar yields were the lowest among
all types of biomass studied and ranged between 123.2 and 215.7 mg · g\(^{-1}\) raw biomass (Fig. 4). The slight increase (14.4 and 15.4 %, respectively) in the yields of both corn straw and poplar wood biomass observed after 24 h pretreatment compared to that of 4 h indicated that using a process time longer than 4 h, especially for corn straw, should be considered. Nevertheless, the low process efficiency observed for poplar wood demonstrated the need for more serious pretreatment conditions in the case of this type of feedstock, as recognized by other previous studies. As it was mentioned before, the lower efficiency of poplar wood saccharification was associated with higher lignin content of this material compared to corn straw and corncob.

A noteworthy finding was that the total sugar yield could further be increased by recovering the glucose and xylose from the liquid phase that remained after pretreatment, e.g., by acidic or enzymatic hydrolysis of dissolved xylooligosaccharides. However, this would require the introduction of additional steps, thereby increasing the cost and time of the whole process. In addition, under acidic conditions, oligosaccharides and monosaccharides are degraded into by-products [46], resulting in sugar losses and the formation of inhibitors for enzymes and microorganisms [47]. Moreover, enzymatic hydrolysis is also negatively affected by the presence of lignin degradation products, which makes the process inefficient [48]. Therefore, other uses of xylooligosaccharides remaining in the liquid phase after pretreatment, such as in the food or pharmaceutical industries [49, 50], seem to be more reasonable.

### Conclusion

A study was successfully conducted on the influence of potassium hydroxide pretreatment time (0.5 h-24 h) on the efficiency of corn straw, corncob, and poplar wood biomass conversion into fermentable monosaccharides at mild process conditions (50 °C, 2 % KOH). In the case of corncob biomass, the influence of the pretreatment time on the overall efficiency in monosaccharide production was negligible, whereas the overall sugar yields for both the corn straw and poplar wood biomass increased with the duration of the pretreatment process. Based on the results obtained, the most appropriate pretreatment time for each biomass type investigated was suggested. The results also indicate that applied pretreatment method is efficient for corn straw and corncob, whilst in the case of poplar wood biomass more severe pretreatment conditions should be applied.

### Acknowledgements

The author is grateful for the financial support of the Polish National Centre for Research and Development Project BIOSTRATEG 2, No. 298241/10/NCBR/2016: "Intelligent systems for breeding and cultivation of wheat, maize, and poplar for optimized biomass production, biofuels, and modified wood".

Poplar material used in presented work was obtained in Welcome 2008/1 project of the Foundation for Polish Science given to Prof. Stanisław Karpiński.

### References

[1] Bilal M, Asgher M, Iqbal H, Zhang X. Int J Biol Macromol. 2017;98:447-58. DOI: 10.1016/j.ijbiomac.2017.01.133.

[2] Raud M, Kikas T, Sippula O, Shurpali N. Renew Sust Energy Rev. 2019;111:44-56. DOI: 10.1016/j.rser.2019.05.020.
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[3] Kołtuniewicz AB, Dąbkowska K. Chem Process Eng. 2016;37:109-19. DOI: 10.1515/cpe-2016-0011.

[4] Arevalo-Gallegos A, Ahmad Z, Asgher M, Parra-Saldivar R, Iqbal HMN. Int J Biol Macromol. 2017;99:308-18. DOI: 10.1016/j.ijbiomac.2017.02.097.

[5] Seidl PR, Goulart AK. Curr Opin Green Sustain Chem. 2016;2:48-53. DOI: 10.1016/j.cogsc.2016.09.003.

[6] Chen H, Liu J, Chang X, Chen D, Xue Y, Liu P, et al. Fuell Process Technol. 2017;160:196-206. DOI: 10.1016/j.fuproc.2016.12.007.

[7] Kuhn EM, O’Brien MH, Ciesielski PN, Schell DJ. ACS Sustain Chem Eng. 2016;4:944-56. DOI: 10.1021/acssuschemeng.6b01041.

[8] Kumar AK, Sharma S. Bioreour Bioprocess. 2017;4:7. DOI: 10.1186/s40643-017-0137-9.

[9] Bensah EC, Mensah M. Int J Chem Eng. 2013:1-21. DOI: 10.1155/2013/719607.

[10] Li X, Zheng Y. Biotechnol Adv. 2017;35;466-89. DOI: 10.1016/j.biotechadv.2017.03.010.

[11] Santos AC, Ximenes E, Kim Y, Ladisch MR. Trends Biotechnol. 2019;37:518-31. DOI: 10.1016/j.tibtech.2018.10.010.

[12] Yuan Z, Wen Y, Kapu NS. Bioreour Technol. 2018;247:242-9. DOI: 10.1016/j.biortech.2017.09.080.

[13] El-Naggar NE, Deraz S, Khalil A. Biotechnology. 2014;13(1):1-21. DOI: 10.3923/biotech.2014.1.21.

[14] Baruah J, Nath BK, Sharma R, Kumar S, Deka RC, Baruah DC, et al. Front Energy Res. 2018;6(141):1-19. DOI: 10.3389/fenrg.2018.00141.

[15] Brienzo M, Siqueira AF, Milagres AMF. Biochem Eng J. 2009;46:199-204. DOI: 10.1016/j.bej.2009.05.012.

[16] Michalska K, Bizukojc M, Ledakowicz S. Biomass Bioenergy. 2015;80:213-21. DOI: 10.1016/j.biombioe.2015.05.022.

[17] Kumari D, Singh R. Renew Sust Energy Rev. 2018;90:877-91. DOI: 10.1016/j.rser.2018.03.111.

[18] Siddhu MAH, Li J, Zhang R, Liu J, Ji J, He Y, et al. BioResources. 2016;11:4550-63. DOI: 10.15376/biores.11.2.4550-4563.

[19] Sharma R, Palled V, Sharma-Shivappa RR, Osborne J. Appl Biochem Biotechnol. 2013;169:761-72. DOI: 10.1007/s12010-012-0009-x.

[20] Paixão SM, Ladeira SA, Silva TP, Arez BF, Roseiro JC, Martins MLL, et al. RSC Advance. 2016;6:1042-52. DOI: 10.1039/C5RA14908H.

[21] Liu X, Zicari SM, Liu G, Li Y, Zhang R. Bioreour Technol. 2015;185:150-7. DOI: 10.1016/j.biortech.2015.02.047.

[22] Asghar U, Irfan M, Nadeem M, Nelofer R, Syed Q. Iran J Sci Technol Trans Sci. 2017;41:659-63. DOI: 10.1007/s40995-017-0284-z.

[23] Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. Determination of Structural Carbohydrates and Lignin in Biomass [Report No. NREL/TP-510-42618]. Golden, CO, USA: National Renewable Energy Laboratory; 2008. https://www.nrel.gov/docs/gen/fy13/42618.pdf.

[24] Dąbkowska K, Mech M, Kopeć K, Pilarek M. Ecol Chem Eng S. 2017;24:9-18. DOI: 10.1515/eces-2017-0001.

[25] Kuglarz M, Alvarado-Morales M, Dąbkowska K, Angelidaki I. Bioreour Technol. 2018;265:191-9. DOI: 10.1016/j.biortech.2018.05.099.

[26] Li E, Mira de Orduña R. Lett Appl Microbiol. 2010;50:283-8. DOI: 10.1111/j.1472-765X.2009.02789.x.

[27] Xu JK, Sun RC. Recent advances in alkaline pretreatment of lignocellulosic biomass. In: Mussatto SI, editor. Biomass Fractionation Technologies for a Lignocellulosic Feedstock Based Biorefinery. Amsterdam, NL: Elsevier; 2016. ISBN 978-0-12-802323-5.

[28] Qi G, Xion L, Tian L, Luo M, Chen X, Huang C, et al. Sustainable Energy Technol Assess. 2018;29:12-8. DOI: 10.1016/j.seta.2018.06.014.

[29] Yuan Z, Wen Y, Li G. Bioreour Technol. 2018;259:228-36. DOI: 10.1016/j.biortech.2018.03.044.

[30] Su Y, Du R, Guo H, Cao M, Wu Q, Su R, et al. Food Bioprod Process. 2015;94:322-30. DOI: 10.1016/j.fbp.2014.04.001.

[31] Chen Y, Stevens MA, Zhu Y, Holmes J, Xu H. Biotechnol Biofuels. 2013;6:1-10. DOI: 10.1186/1754-6834-6-8.

[32] Ma L, Cui Y, Cai R, Liu X, Zhang C, Xiao D. Bioreour Technol. 2015;180:1-6. DOI: 10.1016/j.biortech.2014.12.078.

[33] Isbell HS, Frush HL, Wade CWR, Hunter CE. Carbohydr Res. 1969;9:163-75. DOI: 10.1016/S0008-6215(00)82132-1.

[34] Ibrahim IH, Jahim JM, Harun S, Nor MTM, Hassan O. Int J Chem Eng Appl. 2013;4:101-5. DOI: 10.7763/IJCEA.2013.V4.272.

[35] Sporck D, Reinoso FAM, Rencoret J, Gutiérrez A, Del Rio JC, Ferraz A, et al. Biotechnol Biofuels. 2017;10:1-11. DOI: 10.1186/s13068-017-0981-z.

[36] Hsieh CC, Cannella D, Jørgensen H, Felby C, Thygesen LG. J Agr Food Chem. 2014;62:3800-5. DOI: 10.1021/jf5012962.
[37] Xu Q-Q, Zhao M-J, Yu Z-Z, Yin J-Z, Li G-M, Zhen M-J, et al. Ind Crop Prod. 2017;109:220-6. DOI: 10.1016/j.indcrop.2017.08.038.
[38] Chong G, Di J, Qian J, Wang C, He Y, Huo X, et al. Process Biochem. 2018;68:121-30. DOI: 10.1016/j.procbio.2018.02.022.
[39] Yuan W, Gong Z, Wang G, Zhou W, Liu Y, Wang X, et al. Bioresource Technol. 2018;265:464-70. DOI: 10.1016/j.biortech.2018.06.038.
[40] Zhang Q, Keshwani DR, Xu Y, Hanna MA. Ind Crop Prod. 2012;37:352-7. DOI: 10.1016/j.indcrop.2011.12.001.
[41] Wang Q, Wei W, Li X, Sun J, He J, He M. BioResources. 2016;11:482-91. DOI: 10.15376/biores.11.1.482-491.
[42] Zhang X, Yuan Q, Cheng G. Carbohydr Polym. 2017;156:351-6. DOI: 10.1016/j.carbpol.2016.09.044.
[43] Marchwicka MM, Radomski A, Antczak A, Szadkowski J, Lewandowska A, Szadkowska D, et al. Przem Chem. 2015;94:814-7. DOI: 10.15199/62.2015.5.34.
[44] Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY, et al. Biotech Progress. 2009;25:333-9. DOI: 10.1021/bp100142u.
[45] Boonsombuti A, Luengnaruemitchai A, Wongkasemjit S. Cellulose. 2013;20:1957-66. DOI: 10.1007/s10570-013-9958-7.
[46] Kumar R, Wyman CE. Carbohydr Res. 2008;343:290-300. DOI: 10.1016/j.carres.2007.10.022.
[47] Palmqvist E, Hahn-Hagerdal B. Bioresource Technol. 2000;74:25-33. DOI: 10.1016/S0960-8524(99)00161-3.
[48] Zhai R, Hu J, Saddler JN. Sustainable Energy Fuels. 2018;2:1048-56. DOI: 10.1039/C7SE00569E.
[49] Samantha AK, Jayapal N, Jayaram C, Roy S, Kolte AP, Senani S, et al. Bioact Carbohydrates Dietary Fibre. 2015;5:62-71. DOI: 10.1016/j.bcdf.2014.12.003.
[50] Álvarez C, González A, Negro MJ, Ballesteros I, Oliva JM, Sáez F. Ind Crop Prod. 2017;99:41-8. DOI: 10.1016/j.indcrop.2017.01.034.