Chemical Changes in Lignocellulosic Biomass (Corncob) Influenced by Pretreatment and Anaerobic Digestion (AD)

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Changes in chemical composition and structure of corncob lignocellulosic biomass were investigated relative to pretreatment and anaerobic digestion. The pretreatment involved 1% and 3% sodium hydroxide, 3% and 7% sulphuric acid, as well as medium and high temperature extrusion (in 110 °C and in the range from 140 °C to 160 °C). The chemical components content was studied using a gravimetric method, whereas structure and relations between the carbohydrate and lignin participation were investigated using Fourier transform infrared spectroscopy. It was determined that the chemical treatment, both acidic and alkaline, changed the chemical composition of corncob more significantly than the extrusion. Alkaline pretreatment contributed to significant delignification, while acidic pretreatment reduced the share of hemicelluloses and increased the proportion of lignin, the so-called "pseudolignin". The composition of corncob (control and after pre-treatment) was changed after anaerobic digestion, *i.e.*, a decreased carbohydrate substance content and a significantly increased lignin content. FTIR analysis showed changes in their structure. Although the control corncob differed from that processed by various pretreatment methods, the chemical composition of the digested pulp obtained from them was similar. The NaOH pretreatment was judged to be the preferred method for delignification of the raw material.

Keywords: Biomass; Anaerobic Digestion (AD); Pretreatment; Chemical composition; FTIR

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INTRODUCTION

Lignocellulosic biomass is a crucial sustainable energy source. Depending on the chemical composition, it may be subject to various conversion processes such as direct incineration or biofuel and biogas production. Anaerobic digestion (AD), during which biogas is produced, occurs in the presence of anaerobic microorganisms that decompose organic substances, producing methane and carbon dioxide. The process is divided into four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis; special environmental conditions and proper microorganism group participation are required for each stage. During hydrolysis, bacterial enzymes decompose organic polymers, such as carbohydrates, fats, and proteins, into soluble monomers, dimers, monosaccharides, fatty acids, and amino acids (Jędrczak 2008). Biomass conversion into methane is inhibited due to its poor biodegradability (Balat 2011). In particular, limitations apply to first phase of anaerobic digestion, *i.e.* hydrolysis (Shrestha *et al.* 2017).

Biomass hydrolysis is constrained by some factors, such as cellulose crystallinity, its high polymerisation degree, low surface availability, as well as lignin presence (Hendriks and Zeeman 2009). Lignin is considered the most resistant component to biological decomposition (Castro *et al.* 1994; Pu *et al.* 2013; Ragauskas *et al.* 2014). According to Hendriks and Zeeman (2009), it is favourable to pre-treat a material prior to its conversion, which changes a lignocellulosic biomass' physical and chemical structure (Kumar *et al.* 2009). As far as anaerobic digestion is concerned, glucose is the most desirable product of lignocellulose structure decomposition (Michalska and Ledakowicz 2012). The efficiency of the AD process can be improved by pretreatment of the raw material (Paudel *et al.* 2017). Pre-treatment of biomass may cause some changes: delignification, decrystallization, and depolymerization of cellulose, which in turn will promote the enzymes and bacteria involved in the process and can positively affect for biogas efficiency (Kumar *et al.* 2009; Witaszek *et al.* 2015; Robak and Balcerek 2017).

Mechanical treatment results in diminishing the material particles' size and decreasing the biomass crystallinity. Lignocellulose material fragmentation can reduce the anaerobic digestion period by 23% to 59% (Bernat *et al.* 2014). Physicochemical processes are also employed as methods of lignocellulosic material pretreatment. They include steam explosion, CO_2 or SO_2, ammonia action, hot water extraction, or wet oxidation (Chandel *et al.* 2015). Steam explosion leads to hydrolysis of the hemicellulose and structural changes of the lignin (Agbor *et al.* 2011; Robak and Balcerek 2017). Ammonia fibre/freeze explosion (AFEX) and ammonia recycle percolation (ARP*) processes, in which a lignocellulosic material is subjected to liquid ammonia, lead to swelling of the material. Swelling may influence a change in cellulose crystallinity degree and partial lignin degradation (Kim and Lee 2002; Agbor *et al.* 2011). Biological treatment occurs in the presence of microorganisms that decompose lignin and hemicellulose without cellulose degradation (Lang *et al.* 2001; Börjesson 2009). Lignin depolymerisation by fungi is not fast enough for industrial purposes (Hatakka *et al.* 1993; Agbor *et al.* 2011).

Pretreatment of a material is also conducted with chemical compounds such as alkali, acids, organic solvents, and ionic liquids. In contrast, alkaline pretreatment causes biomass swelling, which results in a reduced cellulose polymerisation and crystallinity degree. It breaks a lignin structure and the bonds between lignin and carbohydrates in biomass so that they become more accessible (Fan *et al.* 1987; Bjerre *et al.* 1996; Chandra *et al.* 2007; Agbor *et al.* 2011; Galbe and Zacchi 2012). It was noted that it was more useful to the hydrolysis of agriculture biomass with a lower lignin content than to wood biomass (Bjerre *et al.* 1996; Kumar *et al.* 2009; Zheng *et al.* 2009a; Agbor *et al.* 2011). Concentrated acids provide fast polysaccharide conversion with slight sugar degradation (Iranmahboob *et al.* 2002; Balat 2011). However, they are not as beneficial as they are corrosive, and they require retrieving so that fermentation could be profitable (Sivers and Zacchi 1995). Diluted acids, *e.g.*, sulphuric acid, hydrochloric acid, and phosphoric acid, of concentrations less than 4% under increased temperature conditions lead to the hemicellulose hydrolysis of monomers (Nguyen *et al.* 2000; Sun and Cheng 2005; Marzialetti *et al.* 2008; Zheng *et al.* 2009b; Agbor *et al.* 2011). The monomers may undergo subsequent reactions and form 'pseudo-lignin' (Hu *et al.* 2012). A disadvantage of diluted acid action under increased temperature conditions is the development of fermentation inhibitors (Galbe and Zacchi 2012). Among all acids, sulphuric acid has been investigated the most thoroughly due to its low price and high efficiency (Ballesteros *et al.* 2008; Zheng *et al.* 2009b). Organic solvents, including alcohols, esters, ketones, organic acids, phenols, or ethers, remove or degrade lignin as well as part of the hemicellulose (Curreli *et al*. 1997).

The alterations of chemical composition caused by pretreatment have been widely described in the literature. However, little information can be found on digested chemical composition, including information on the pretreatment effect on the chemical composition of the material. Sannigrahi and Ragauskas (2011) described the ash and lignin content in digested pulp from miscanthus, red maple, switchgrass, and hybrid poplar. The Klason lignin content in untreated biomass ranged from 17.0% to 27.2%. Following anaerobic digestion, its proportional content increased from 50.4% to 78.2%. Mulat *et al.* (2018) presented the potential to produce biogas from birch hydrolysis lignin. They confirmed earlier reports regarding an increase in lignin percentage participation in digested residue (from 29.2% to 79.8%). Due to anaerobic digestion, glucan content decreased from 36.5% to 9.7%, similarly to hemicellulose that decreased from 20.7% to 1.7%. Waliszewska *et al.* (2018) presented changes in cellulose structure that occurred as a result of anaerobic digestion. They showed that the cellulose content in various miscanthus and sorghum varieties dropped 1.8% to 32.7% due to anaerobic digestion. They proved that cellulose from miscanthus and sorghum varieties underwent various modifications at a structural level and the process changed sorghum more extensively. The changes in cellulose structure concerned its crystallinity, energy, and hydrogen bond distances, as well as its lateral order index (LOI) and hydrogen bond intensity (HBI). The changes that take place in lignin have been described by Stachowiak-Wencek *et al.* (2018) and Waliszewska *et al.* (2019). The authors, apart from the fact that they noticed an increase in the lignin content in the digested pulp, also found changes to the structure of this component; *e.g.* it contained fewer functional groups than the native lignin.

Changes in biomass chemical composition caused by anaerobic digestion concern not only the main components, *i.e.*, cellulose, lignin, and hemicellulose, but extractives as well. Partial information on the main biomass component changes resulting from anaerobic digestion can be found in the literature, and they consider a few species. However, there is no information concerning, *e.g.*, extractives. One of the materials that has not been described so far is corncob, which can be applied to biogas production. In recent years, the area of corn growing for processing purposes has increased. This has resulted in the production of a significant amount of waste, *e.g.* straw and corncob. In the light of the growing demand for energy, the use of this waste seems to be right. Information on the chemical composition of corn and straw is available in the literature (Chaudhry 2000; Zheng *et al.* 2009b; Paul and Dutta 2018). However, there are no reports on the composition of corncob in the literature, which is also an important and attractive in terms of energy production waste. In addition, there is a lack of information on the chemical composition of digested pulp from this kind of lignocellulosic biomass, and this knowledge may be useful to recognize its further most beneficial use.

The main aim of this paper is to determine the quantitative changes in the main components and extractives of corncobs that result from pretreatment (*i.e.*, 1% and 3% sodium hydroxide, 3% and 7% sulphuric acid, and extrusion at 110 °C and 140 °C to 160 °C) and anaerobic digestion. The chemical component was researched using a gravimetric method, whereas changes of the carbohydrate and lignin structure were investigated using FTIR.

EXPERIMENTAL

Materials

The research material was corncob from Bioagra SA company (Nysa City, Poland), which was a post-production waste of different species of corn from Polish cultivation.

Methods

Pretreatment

To disintegrate lignin, the corncobs were pretreated. The following material pretreatment methods were applied: chemical and thermo-mechanical. The chemical pretreatment was performed with sodium hydroxide (NaOH) and sulphuric acid (H_2SO_4) as reagents. Alkaline hydrolysis was performed with 1% and 3% NaOH, and an acidic hydrolysis using 3% and 7% H_2SO_4. The pre-fragmented material was boiled in aqueous acid and alkali solutions of a 500 mL volume at 100 °C for 3 h. Following the completed hydrolysis, the material was filtered on a Büchner funnel. Raw material was filtered and then dried in a laboratory dryer at 40 °C for 24 h. The content of solids after filtration was approximately 40%. For chemical analysis and for anaerobic digestion it was cleansed with water until a neutral pH was achieved. Thermo-mechanical treatment was performed in a single-screw extruder (model S45; MetalChem, Gliwice, Poland) in two temperature variants: 110 °C as well as in the range from 140 °C to 160 °C.

Anaerobic digestion

Anaerobic digestion was conducted under the procedure described in the standard DIN 38 414-8 (1985). It was performed in a 21-chamber biofermentor (Institute of Biosystems Engineering, Poznan University of Life Science, Poznan, Poland) whose schematic is presented in Lewicki *et al.* (2013). Glass chambers (capacity 2 dm^3) with material were placed in water at 39 °C ± 1 in mesophilic conditions. Samples were tested in triplicate.

The material was treated with an inoculum in the amounts shown in Table 1. The inoculum used in the studies was taken from an agricultural biogas plant. Chemical composition of inoculum (analysis according to TAPPI methods for samples of raw material), was determined by measuring the pH (pH-PN-90 C-04540/01), concentration of ammonium nitrogen (PN-73 C-04576/02), dry matter content (PN-75 C-04616/01), and mineral substances (T 211om - 07). The inoculum contained: 29.7% of holocellulose 32.4% of cellulose, 6.6% of pentosans, 48.9% of lignin, 5.8% of extractives, 64.9% of substances soluble in 1% NaOH, 8.8% substances soluble in cold water, and 13.9% substances soluble in hot water. The concentration of ammonium nitrogen was lower than 2.5 g / dm^3. Dry matter content in aqueous inoculum solution amounted to approximately 2.7% to 2.9%, of which approximately 28% to 30% was minerals. The pH was approximately 7.

The digested residue was divided using a Büchner funnel into two fractions: solid and liquid. The solid fraction of digested pulp was initially dried in a drying apparatus (type FD 23; BINDER GmbH, Tuttlingen City, Germany) at a temperature of 50 °C and conditioning was later continued in 10 days under laboratory conditions (in temperatures of 22 °C ± 1 °C). Digested pulp prepared in this way was then chemically analysed.

Table 1. Quantity of Raw Material and Inoculum Used in the Anaerobic Digestion Process

Material	Amount of Raw Material (g)	Amount of Inoculum (g)
Control	13.5	1186.5
Hydrolysis 1% NaOH	51.0	1149.0
Hydrolysis 3% NaOH	60.0	1140.0
Hydrolysis 3% H_2SO_4	59.0	1141.0
Hydrolysis 7% H_2SO_4	62.0	1138.0
Extrusion 110 °C	10.0	1190.0
Extrusion 140 °C to 160 °C	10.0	1190.0

Gravimetric method

The corncob before and after pretreatment and digested pulp were ground in a laboratory mill (Pulverisette 15; Fritsch GmbH, Idar-Oberstein City, Germany). For chemical analyses the 0.5 to 1.0 mm fraction was used. Holocellulose, cellulose, pentosans, and lignin content were determined according to TAPPI T9 wd-75 (2015), the Seifert method (Seifert 1956), TAPPI T223 cm-01 (2001), and TAPPI T222 om-06 (2006), respectively. Extractive substances, soluble in 1% NaOH, in 95% ethanol, and in cold and hot water were analyzed according to TAPPI T204 cm-07 (2007), TAPPI T212 om-02 (2007), and TAPPI T207 cm-99 (2008), respectively. All analyses were repeated three times for each examined sample set.

Fourier transform infrared spectra (FTIR)

The structure spectroscopy analysis was performed for corncob before and after pretreatment and digested pulp *via* FTIR. The FTIR spectra of the researched materials were recorded with an Alpha FTIR spectrometer from Bruker Optics GmbH (Ettlingen city, Germany). Measurements were performed on the pellets obtained during homogenisation of approximately 200 mg of anhydrous potassium bromide (KBr) with 2 mg of the investigated material that was subsequently pressed under 10 MPa pressure. During the measurements, 36 pellets were scanned in the range of 4000 cm^{-1} to 400 cm^{-1} with the resolution of 4 cm^{-1}. All IR spectra were performed three times and particular indicators were determined based on their average.

Statistical analysis

Every determination was performed three times, and the mean values were reported. The statistical analysis was performed using STATISTICA 10 (StatSoft, Polska Sp. z o.o., Cracow, Poland), including analysis of variance (ANOVA) followed by a *post hoc* Tukey's honestly significant difference (HSD) test. Identical letters in columns represent no differences at the significance level of $p = 0.05$.

RESULTS AND DISCUSSION

Chemical Composition of Biomass After Pretreatment

The percentage of the main components and extractives included in the corncob waste are presented in Table 2.

Determining the holocellulose percentage in corncobs showed that the component in the untreated material was 59.6%. After the alkaline and acid treatment, the holocellulose percentage increased and higher figures were obtained for the alkali-treated material, whereas the extrusion resulted in decreased carbohydrate substance content in comparison to the raw material; lower figures were obtained for a high temperature variant. Cellulose content alterations were similar in the investigated materials after the treatment. Both the NaOH and H_2SO_4 treatments led to an increased percentage of cellulose. The acid treatment caused larger changes than the alkaline treatment. The acid treatment resulted in decreased pentosan content. By contrast, pentosans increased with the alkali treatment, which was not consistent with literature reporting that alkaline treatment leads to partial hemicellulose degradation (Zheng *et al.* 2009a). Vital differences in the effect of the applied NaOH solutions were observed: 1% NaOH increased pentosan content 5.6% while 3% —NaOH increased pentosan content 36.0%. According to the researchers who have analysed an acid pretreatment effect (Zheng *et al.* 2009b; Jung *et al.* 2010), the action results in hemicellulose hydrolysation that interfered with the lignocellulose structure of a material. Through analysing the concentration effect on the changes in pentosan percentage, a pentosan participation alteration was similar regardless of the applied acid solution concentration.

Hemicellulose reduction due to H_2SO_4 was not a positive effect of pretreatment because hemicellulose is a compound that is decomposed more easily by microorganisms than cellulose and its decrease may lead to lower biogas efficiency, unlike the NaOH action. Lignin reduction may lead to an increase in access to carbohydrate substances by microorganisms, which may in turn lead to a biogas efficiency increase in AD.

Table 2. Chemical Composition of Corncob Before Anaerobic Digestion (in percent)

Component		Control	NaOH		H_2SO_4		Extrusion	
			1%	3%	3%	7%	110 °C	140 °C to 160 °C
Lignin**		11.98 ± 0.03^{BC}	1.74 ± 0.66^{A}	1.03 ± 0.16^{A}	20.55 ± 0.78^{D}	20.86 ± 0.49^{D}	12.41 ± 0.16^{C}	11.17 ± 0.28^{B}
Holocellulose		59.59 ± 1.09^{D}	89.14 ± 1.51^{E}	96.75 ± 0.14^{F}	79.59 ± 0.24^{A}	79.46 ± 0.17^{A}	50.70 ± 0.49^{C}	44.81 ± 0.16^{B}
Cellulose		31.21 ± 0.61^{A}	47.16 ± 0.41^{C}	56.51 ± 0.46^{D}	64.25 ± 0.67^{E}	68.67 ± 0.47^{F}	29.97 ± 0.31^{A}	26.35 ± 0.28^{B}
Pentosans		31.23 ± 0.62^{AB}	32.96 ± 0.59^{B}	42.45 ± 1.54^{F}	13.14 ± 0.62^{D}	13.69 ± 0.23^{C}	29.23 ± 0.59^{A}	27.36 ± 0.47^{E}
Extractives (EtOH)		5.76 ± 1.07^{A}	0.63 ± 0.04^{B}	1.02 ± 0.05^{B}	10.80 ± 0.22^{C}	15.37 ± 0.06^{C}	5.62 ± 0.30^{A}	5.13 ± 0.87^{A}
Substances soluble in:	NaOH	44.54 ± 0.99^{A}	11.34 ± 0.10^{D}	9.62 ± 0.03^{C}	42.42 ± 0.30^{A}	61.13 ± 0.12^{E}	52.01 ± 0.16^{B}	57.47 ± 0.07^{B}
	Hot water	12.77 ± 0.24^{B}	4.09 ± 0.03^{A}	1.30 ± 0.08^{A}	2.09 ± 0.06^{A}	1.68 ± 0.06^{A}	19.49 ± 0.78^{C}	30.44 ± 1.13^{D}
	Cold water	7.70 ± 0.16^{C}	0.66 ± 0.12^{AB}	0.93 ± 0.04^{AB}	1.52 ± 0.11^{B}	0.51 ± 0.03^{A}	10.36 ± 0.31^{D}	16.56 ± 0.26^{E}

*Homogeneity groups: same letters in each column indicate that there was no statistical difference between the samples according to the Tukey's HSD multiply range test at $p < 0.05$
**Stachowiak-Wencek *et al.* (2018)

The extrusion led to the smallest cellulose and pentosan quantitative changes of the applied pretreatment methods. Their content slightly dropped, but the extent of the changes depended on the process temperature. A rise in extrusion temperature resulted in intensified of changes, where the cellulose content in 110 °C only decreased from 31.2% to 30.0%, while in the range of 140 °C to 160 °C it decreased to 26.4%. A similar tendency was reported for pentosans. An applied temperature of 110 °C led to a decrease in pentosan content from 31.2% to 29.2% and the higher temperature range of 140 °C through 160 °C resulted in a decrease to 27.4%. According to Camire (1998) and Karunanithy and Muthukumarappan (2009), extrusion leads to the depolymerisation of cellulose, hemicellulose, lignin, and protein. Moreover, depending on the intensity of stress related to it, it can cause thermal degradation of carbohydrates and protein. Hjorth *et al.* (2011) conducted extrusion to enhance the anaerobic digestion efficiency of five different types of agriculture biomass and they did not observe a cellulose, hemicellulose, or lignin content decrease after extrusion. Moreover, they demonstrated that extrusion considerably reduced the molecular size and extended the biomass specific surface area, which intensified hydrolysis and led to an enhanced biomass efficiency.

Carbohydrate substance alterations were also accompanied by alterations in lignin content. Amounts of 1% as well as 3% NaOH resulted in significant delignification. The lignin content dropped from 12.0% prior to the treatment to 1.03% and 1.74% following the 1% and 3% NaOH treatments, respectively. The observed changes were consistent with literature illustrating that lignin is soluble in alkali (Millet *et al.* 1976; Iyer *et al.* 1996; Kim and Holzapple 2005; Zheng *et al.* 2009a). In contrast, a lignin percentage increase from 12.0% to 20.6% and 20.9% was reported in a material due to the acid. According to literature, pretreatment with diluted acids does not result in material delignification as it does with alkali. In contrast, research by numerous authors (Sannigrahi *et al.* 2008; Sievers *et al.* 2009; Jung *et al.* 2010; Mao *et al.* 2010) has shown that lignin's proportional content after diluted acid treatment is higher than in the raw material. An acidic medium catalyses hemicellulose hydrolysis and the further degradation to compounds of a lower molecular mass (Mulat *et al.* 2018). Similar changes were observed in the analysed corncob in which a lignin increase was accompanied by a decrease in hemicellulose content. The compounds that formed after the hemicellulose breakdown may undergo repolymerisation reactions and/or polymerisation reactions with lignin, forming pseudo-lignin (Li *et al.* 2007; Hu *et al.* 2012). Pseudo-lignin is an aromatic compound of a structure similar to lignin (Sannigrahi *et al.* 2011; Hu *et al.* 2012) consisting of carbonyl, carboxyl, aromatic, and aliphatic structures (Hu *et al.* 2012). Sannigrahi *et al.* (2011) showed that pseudo-lignin may be formed from carbohydrates without a lignin contribution during diluted acids material treatment.

The effect of the extrusion was relatively small. At 110 °C, a slight increase (3.52%) in lignin content was observed, whereas temperatures of 140 °C to 160 °C resulted in a 6.79% decrease.

Applying a pretreatment method to the research led also to considerable alternations in the percentage content of extractive substances that are soluble in EtOH, NaOH, as well as cold and hot water.

The content of substances extracted in EtOH (5.76% in a raw material) significantly decreased after the pretreatment with NaOH. However, it increased after the sulphuric acid treatment. The extrusion did not cause any statistically significant changes in the extractive substance content in the investigated corn material. Raw material solubility in cold water was 7.7%, whereas in hot water it reached up to 12.8%. The corncob chemical treatment resulted in a considerate drop in the amount of a substance soluble in water. However, the content of a substance soluble in water increased as a result of the extrusion.

Chemical Composition of Biomass After Anaerobic Digestion

The changes in the corncob chemical composition that resulted from the digestion are presented in Table 3. The holocellulose content in the material dropped significantly. For the non-pretreated raw material, the holocellulose content decreased by 53.4% (from 59.6% to 27.8%). For all materials after the pretreatment, the decrease ranged from 35.2% to 69.4%. The smallest carbohydrate decrease was reported for the material after the extrusion (by 41.9% and by 35.2%) and for the materials after the chemical treatment, it experienced a similar trend (the percent lost was from 62.7% to 69.4%).

Table 3. Chemical Composition of Corncob After Anaerobic Digestion (in %)

Component		Control	NaOH		H2SO4		Exstrusion	
			1%	3%	3%	7%	110 °C	140 °C to 160 °C
Lignin**		51.65 ± 0.51^{D}	48.44 ± 0.18^{A}	46.91 ± 0.27^{BC}	50.87 ± 0.17^{BD}	49.58 ± 0.37^{AB}	49.19 ± 0.88^{AC}	49.36 ± 0.12^{AB}
Holocellulose		27.79 ± 0.37^{A}	29.32 ± 0.13^{A}	29.56 ± 0.04^{A}	29.58 ± 0.25^{A}	29.66 ± 0.89^{A}	29.48 ± 0.47^{A}	29.02 ± 0.34^{A}
Cellulose		26.39 ± 2.27^{A}	29.21 ± 0.07^{AB}	26.84 ± 0.96^{AB}	31.08 ± 0.17^{AB}	36.62 ± 0.98^{B}	27.93 ± 0.15^{AB}	27.10 ± 1.21^{AB}
Pentozans		11.84 ± 0.10^{B}	7.14 ± 0.18^{A}	7.11 ± 0.48^{A}	7.79 ± 0.17^{A}	7.08 ± 1.23^{A}	7.57 ± 0.05^{A}	7.51 ± 0.30^{A}
Extractives (EtOH)		5.22 ± 0.48^{A}	7.00 ± 0.29^{BC}	7.56 ± 0.30^{C}	7.04 ± 0.47^{BC}	5.65 ± 0.14^{AB}	5.44 ± 0.15^{A}	6.27 ± 0.13^{AB}
Subs-tances soluble in:	NaOH	66.80 ± 0.36^{B}	64.99 ± 0.06^{AB}	63.05 ± 0.27^{A}	61.49 ± 0.81^{A}	59.61 ± 0.29^{C}	62.52 ± 0.16^{A}	64.66 ± 0.25^{AB}
	Hot water	17.87 ± 0.11^{A}	17.14 ± 0.10^{D}	21.06 ± 0.29^{F}	13.84 ± 0.05^{B}	15.88 ± 0.01^{C}	17.95 ± 0.24^{A}	20.53 ± 0.02^{E}
	Cold water	8.36 ± 0.01^{B}	11.31 ± 0.16^{D}	12.85 ± 0.12^{F}	9.32 ± 0.12^{C}	10.11 ± 0.010^{A}	10.46 ± 0.28^{A}	12.26 ± 0.28^{E}

*Homogeneity groups: same letters in each column indicate that there was no statistical difference between the samples according to the Tukey's HSD multiply range test at $p < 0.05$
** Stachowiak-Wencek *et al.* (2018)

The pentosan content was similar to each other (7.1% to 7.8%) and was lower than in the control material (11.8%). The most significant pentosan drop was recorded for the material after the NaOH treatment. The decrease was slightly lower for the corncob after the extrusion and the lowest after the treatment with H_2SO_4. This material prior to the anaerobic digestion was characterised by the lowest pentosan content.

The residue obtained from corncobs following the extrusion was characterised by the lowest cellulose loss. For the material after the extrusion in a temperature of 110 °C, it amounted to 6.8%, whereas in a temperature of 140 °C to 160 °C no cellulose loss was recorded. In contrast, a slight increase of approximately 2% of its content occurred. The cellulose loss reported for the control material was a little higher in comparison to the material after the extrusion (15.4%), but much higher when compared to the chemically treated material (38.1% to 52.5%). The cellulose loss can be explained both by constraints resulting from its crystalline structure and by the pseudo-lignin presence that inhibits its hydrolysis (Hu *et al.* 2012). Hu *et al.* (2012) showed that pseudo-lignin not only led to an increase in Klason lignin content, but also inhibited cellulose hydrolysis. The changes resulting from anaerobic digestion in cellulose were investigated by Waliszewska *et al.* (2018), who reported a cellulose percentage content decrease in both miscanthus and sorghum varieties.

According to literature, due to its complex structure, lignin is the most difficult lignocellulosic material component to decompose (Pu *et al.* 2013; Ragauskas *et al.* 2014; Theuretzbacher *et al.* 2015; Mulat *et al.* 2018). It constituted 46.9% to 51.6% in digested pulp from corncobs. Its largest amount was found in the control material, in which it increased from 12.0% to 51.6%. The smallest lignin amount was found in the residue that had been treated with alkali prior to the digestion. The raw material NaOH treatment led to nearly complete lignin degradation to the level of 1.03% to 1.74%. Lignin content in the digested residue, which was treated with 1% and 3% NaOH prior to the digestion, amounted to 48.4% and 46.9%, respectively. A slightly bigger lignin amount of 49.6% to 50.9% was found in the digested residue, which had been treated with sulphuric acid. Lignin content in the residue obtained following the extrusion did not vary much from the amounts determined for other digested pulp. Mulat *et al.* (2018) investigated birch residue (*Betula pubescens*) wood chips and reported an increase in lignin content compared with to the raw material.

The content of substances soluble in EtOH, 1% NaOH, and water, due to the digestion for a control material and after the extrusion, did not change significantly. The changes were recorded for a material after its chemical treatment. The residue treated with NaOH solutions was characterised by a bigger amount of extraction substances, whereas the material treated with H_2SO_4 acid was characterised by a lower amount than in a control material.

Based on the obtained results, it can be stated that each of the applied pretreatment methods resulted in a different character of changes in the chemical composition. Changes observed after the thermo-mechanical pretreatment were evidently smaller when compared to those caused by the chemical pretreatment. However, a significant solution concentration and temperature of chemical pretreatment effect was not shown.

FTIR Interpretation

The FTIR spectra of materials before and after the pretreatment and after the anaerobic digestion are presented in Figs. 2 and 3. The recorded bands were attributed to the material main components, *i.e.*, cellulose, lignin, and hemicellulose.

There were two regions of the FTIR spectra of the researched samples that were rich in information and fit in the band range from 3700 cm^{-1} to 2780 cm^{-1} and from 1780 cm^{-1} to 840 cm^{-1}. On the spectra of all of the materials, a wide band was present at 3500 cm^{-1} to 3400 cm^{-1}, which was typical for –OH group stretching vibrations that stemmed from water present in the samples and biomass components. The band at 2924 cm^{-1} was attributed to C-H symmetrical stretching vibrations in aromatic methoxy as well as methyl and methylene groups. An absorption band at 1736 cm^{-1} (C=O) was present in hemicellulose but not extensively in lignin. It was also found in most of the materials both prior to and following the pretreatment. The only exception was in the samples treated with 3% NaOH solution, in which the peak was not reported. For the materials after the sulphuric acid treatment, the presence of the band at 1705 cm^{-1} was noted to correspond to C=C stretching vibrations of an aromatic nucleus in syringyl units (S) (Todorciuc *et al.* 2009).

At 1654 cm^{-1}, the band corresponded to C=O unconjugated stretching vibrations, and at approximately 1600 cm^{-1} and 1510 cm^{-1} the bands of vibrations of C=C bonds in lignin aromatic nuclei were observed. The band at 1270 cm^{-1} also stemmed from guaiacyl unit vibrations of a lignin aromatic nucleus (Sills and Gossett 2012).

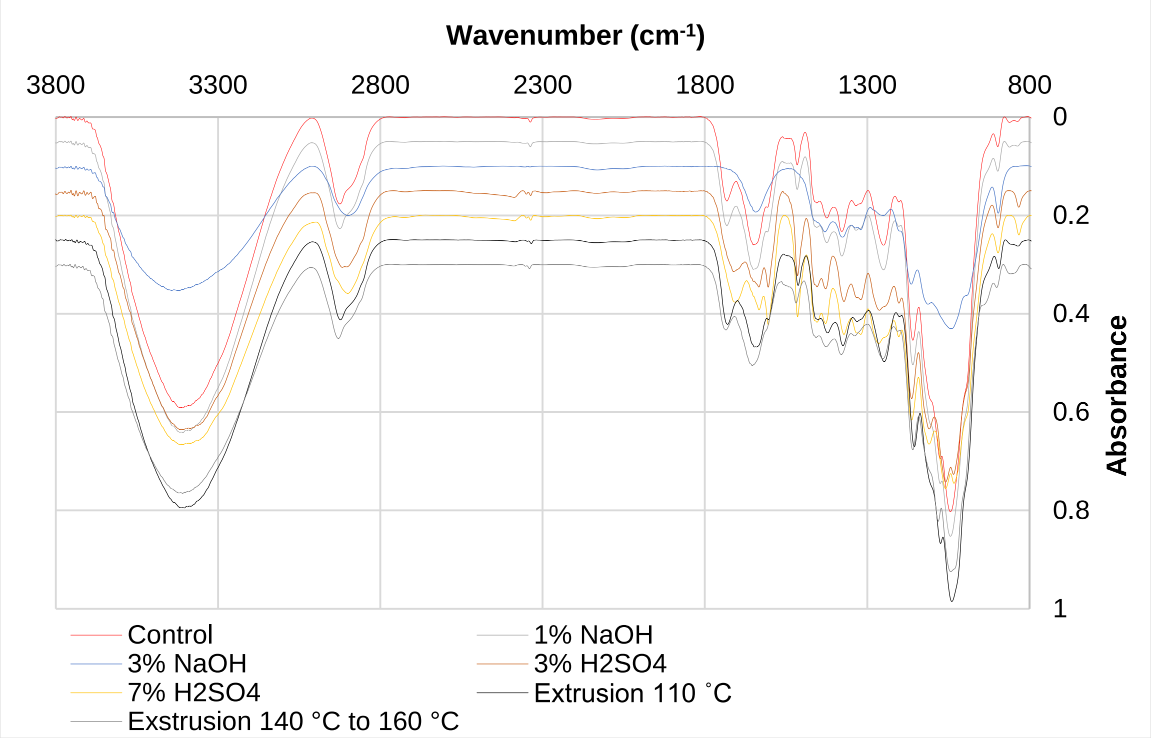

Fig. 2. FTIR spectra of corncob after pretreatment

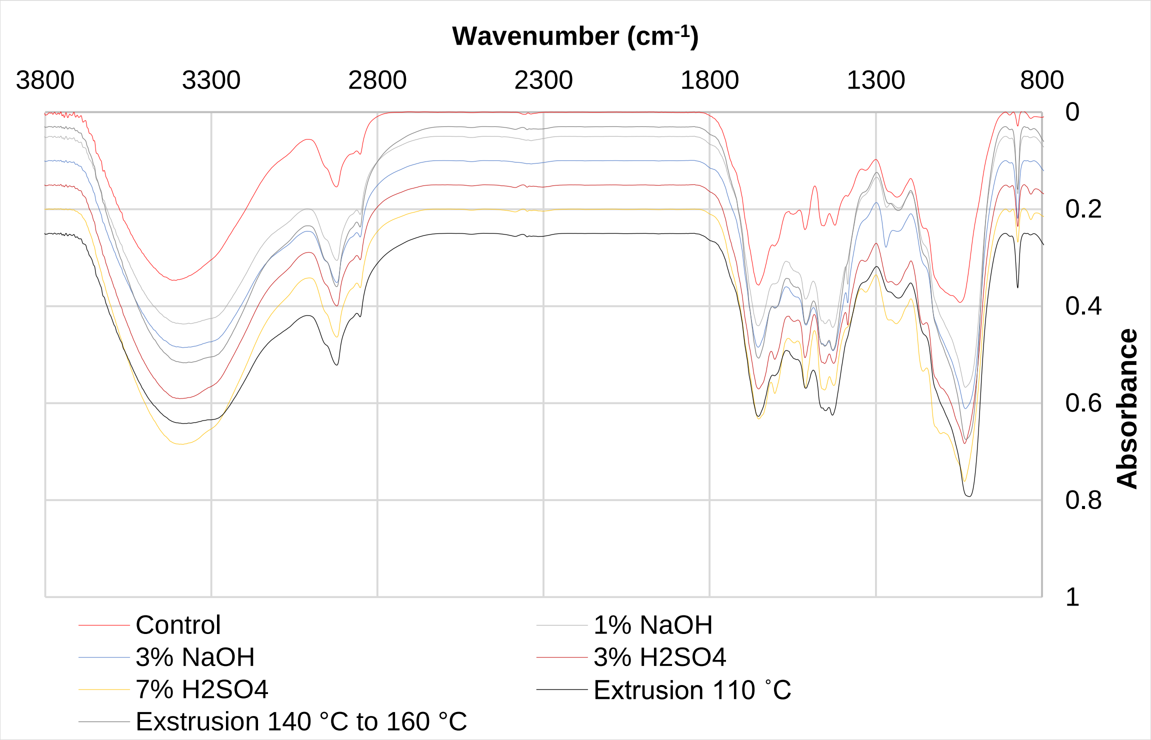

Fig. 3. FTIR spectra of corncob after anaerobic digestion

The bands at 1454 cm^{-1}, 1430 cm^{-1}, 1330 cm^{-1}, and 1110 cm^{-1} were characterised for a C-H and C-O deformation that bent and stretched many groups in the lignin and carbohydrates (Yokoi *et al.* 2003; Mészáros *et al.* 2007). Whereas bands at 1375 cm^{-1}, 1240

cm^{-1}, 1165 cm^{-1}, 1060 cm^{-1}, and 1030 cm^{-1} were attributed to C=O, C-H, C-O-C, and C-O deformations or stretching vibrations of various groups in carbohydrates (Yokoi *et al.* 2003; Popescu *et al.* 2009; Poletto *et al.* 2012). The band at 1420 cm^{-1} to 1430 cm^{-1} was connected to the amount of cellulose crystalline structures, while the band at 898 cm^{-1} was connected to an amorphous area in cellulose (Proniewicz *et al.* 2001; Akerholm *et al.* 2004).

Following digestion, the analysed relation values rose considerably and did not show the same tendencies in certain areas as prior to the process. On the spectra obtained for the material after digestion, the loss of a few bands and the occurrence of a few new ones were observed. The occurrence of the band at 1735 cm^{-1} (C=O) present in hemicellulose and lignin was not observed. As shown by the quantitative determination of the investigated biomass component percentage, hemicellulose content dropped as a result of digestion. An absorbance decrease of the band may have been related to the loss of its primary source, *i.e.*, hemicellulose itself. In lignin, there is merely slight participation of C=O groups. It was concluded that despite a lignin increase resulting from digestion, the content of those groups did not rise; therefore, changes of absorbance at 1735 cm^{-1} should not to be related to lignin. The loss of the band at 1705 cm^{-1} was also reported, which corresponded to lignin aromatic nucleus vibrations. The band at 1060 cm^{-1} was present only on the spectra obtained for the material after the alkali treatment. There was a lack of an intense absorption band at 1060 cm^{-1}, *i.e.*, the band represented C-O and C-C stretching, or C-OH bending in non-cellulosic polysaccharides (Kacurakova *et al.* 2002), which was characteristic for samples of low cellulose content (Siengchum *et al.* 2012). The bands attributed to cellulose at 1315 cm^{-1} and 896 cm^{-1} were also not found. The band at 1315 cm^{-1} corresponded to CH_2 that is present in cellulose and hemicellulose, and the band at 896 cm^{-1} was characteristic for cellulose amorphous structures.

Additional bands were observed at 840 cm^{-1}, 1445 cm^{-1}, 1124 cm^{-1}, and 875 cm^{-1}. The band at 2840 cm^{-1} corresponded to C-H asymmetric stretching vibrations in lignin, and the band at 1124 cm^{-1} corresponded to C-H aromatic unit deformation syringyl vibrations and C=O stretching vibrations (Popescu *et al.* 2007). The band at 1445 cm^{-1} was attributed to O-H stretching vibrations (Sills and Gossett 2012), and the band at 875 cm^{-1} was attributed to glycosidic bonds in hemicellulose (Sills and Gossett 2012).

The intensity of a few characteristic cellulose IR absorption bands at 1375 cm^{-1} (bending vibrations in cellulose I and cellulose II as well as in hemicellulose) (Ishida *et al.* 2007), 1160 cm^{-1} (C-O-C asymmetric stretching vibrations in cellulose and hemicellulose) (Ishida *et al.* 2007), and 895 cm^{-1} (cellulose amorphous structures) (Proniewicz *et al.* 2001) were compared to the 1510 cm^{-1} band intensity that is often applied as an internal standard, which corresponded to a stretching lignin benzene nucleus (Fengel and Wegener 1984). The results are presented in Table 4. Table 4 also shows the ratio between the lignin and cellulose percentages (L/C) calculated based on the data from Tables 2 and 3. Following pretreatment, the ratio between the characteristic lignin band (1510 cm^{-1}) and the bands attributable to cellulose (1375 cm^{-1}, 1158 cm^{-1}, and 895 cm^{-1}) was the lowest for the material after alkali treatment and the highest after acid treatment. For the control material and after the extrusion the ratios were similar. The FTIR results overlap partially with the L/C results obtained from gravimetric methods. The material treated with alkali was characterised with the lowest L/C ratio, as alkali caused considerable lignin degradation. The highest L/C was reported for the material after the extrusion. For the control material and after the H_2SO_4 treatment, the ratios were similar. Following digestion, the L/C value rose significantly from 0.018 to 0.458 to 1.354 to 1.957. The highest L/C value after digestion was reported for the control material.

Table 4. Lignin/Carbohydrate Ratio Calculated from FTIR Spectra for the Corncob Before and After Anaerobic Digestion

Bands (cm^{-1})	1510/1375	1510/1158	1510/895	L/C*
Before AD				
Control	0.418	0.214	1.615	0.384
Hydrolysis 1% NaOH	0.111	0.061	0.162	0.037
Hydrolysis 3% NaOH	0.148	0.090	0.223	0.018
Hydrolysis 3% H_2SO_4	0.758	0.404	2.274	0.320
Hydrolysis 7% H_2SO_4	0.843	0.489	2.673	0.304
Extrusion 110 °C	0,430	0.220	1.596	0.414
Extrusion 140 °C to 160 °C	0.428	0.208	1.682	0.458
After AD				
Control	1.418	1.011	40.421	1.957
Hydrolysis 1% NaOH	1.216	1.502	71.970	1.658
Hydrolysis 3% NaOH	1.258	1.567	69.571	1.748
Hydrolysis 3% H_2SO_4	1.317	1.239	77.788	1.636
Hydrolysis 7% H_2SO_4	1.537	1.102	66.678	1.354
Extrusion 110 °C	1.571	1.525	53.098	1.761
Extrusion 140 °C to 160 °C	1.527	1.530	66.120	1.821

*L/C was calculated based on data from Tables 1 and 2

CONCLUSIONS

1. The investigated corncob material was chemically changed due to both the pretreatment and anaerobic digestion. Among the applied pretreatment methods, the smallest changes in the chemical composition were as a result of the extrusion. The effects of the NaOH and H_2SO_4 action were higher. The NaOH led to considerable lignin degradation, whereas H_2SO_4 resulted in hemicellulose hydrolysis. Moreover, due to NaOH action, extractive substance content, as well as content of the substances soluble in NaOH, cold, and hot water also decreased. The H_2SO_4 led mainly to changes in the amount of substances soluble in cold and hot water.

2. In the digested pulp, carbohydrate substance content dropped considerably, especially pentosans, while smaller changes were reported for cellulose. Moreover, increased lignin content was recorded. It is worth noting that the chemical composition in all digested pulp, regardless of the pre-treatment method, was similar.

~~3.~~ FTIR analysis showed significant changes in the structure of the raw material after anaerobic digestion. A loss of C=O groups and amorphous cellulose structures was observed. On the other hand, the share of aromatic structures increased.

4. It has been found that in the case of waste of corn production such as corncob, NaOH treatment is the more preferred pretreatment method. The significant delignification of this material caused by the action of NaOH can significantly contribute to the improvement of biogas efficiency.

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REFERENCES CITED

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., and Levin, D.-B. (2011). “Biomass pretreatment: Fundamentals toward application,” *Biotechnology Advances* 29(6), 675-685. DOI: 10.1016/j.biotechadv.2011.05.005

Akerholm, M., Hinterstoisser, B., and Salmén, L. (2004). “Characterization of the crystalline structure of cellulose using static and dynamic FT-IR spectroscopy,” *Carbohydrate Research* 339(3), 569-578. DOI: 10.1016/j.carres.2003.11.012

Balat, M. (2011). “Production of bioethanol from lignocellulosic materials *via* biochemical pathway: A review,” *Energy Conversion and Management* 52(2), 858-875. DOI: 10.1016/j.enconman.2010.08.013

Ballesteros, I., Ballesteros, M., Manzanares, P., Negro, M. J., Oliva, J. M., and Sáez, F. (2008). “Dilute sulfuric acid pretreatment of cardoon for ethanol production,” *Biochemical Engineering Journal* 42(1), 84-91. DOI: 10.1016/j.bej.2008.06.001

Bernat, K., Wojnowska-Baryła, I., Kasiński, S., and Szatkowski, M. (2014). “Metody wstępnego przygotowania biomasy lignocelulozowej do fermentacji metanowej [Methods of pre-treatment of lignocellulosic biomass for AD],” *Gaz, Woda i Technika Sanitarna* [*Gas, Water and Sanitary Technology*] 7, 269-273.

Bjerre, A. B., Olesen, A. B., Ploger, A., Schmidt, A. S., and Fernqvist, T. (1996). “Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose,” *Biotechnology and Bioengineering* 49(5), 568-577. DOI: 10.1002/(SICI)1097-0290(19960305)49:5<568::AID-BIT10>3.0.CO;2-6

Börjesson, P. (2009). “Good or bad bioethanol from a greenhouse gas perspective – What determines this?,” *Applied Energy* 86(5), 589-594. DOI: 10.1016/j.apenergy.2008.11.025

Camire, M.-E. (1998). “Chemical changes during extrusion cooking,” in: *Process-Induced Chemical Changes in Food*, F. Shahidi, C.-T. Ho, and H. van Chuyen (eds.), Plenum Press, New York, NY, USA, pp. 109-121.

Castro, F. B., Hotten, P. M., Ørskov, E. R., and Rebeller, M. (1994). “Inhibition of Rumen microbes by compounds formed in the steam treatment of wheat straw,” *Bioresource Technology* 50(1), 25-30. DOI: 10.1016/0960-8524(94)90216-X

Chandel, A. K., Gonçalves, B. C. M., Strap, J. L., and Da Silva, S. S. (2015). “Biodelignification of lignocellulose substrates: An intrinsic and sustainable pretreatment strategy for clean energy production,” *Critical Reviews in Biotechnology* 35(3), 281-293. DOI: 10.3109/07388551.2013.841638

Chandra, R. P., Bura, R., Mabee, W. E., Berlin, A., Pan, X., and Saddler, J. N. (2007). “Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics?,” in: *Biofuels*, L. Olsson (ed.), Springer-Verlag, Berlin, Germany, pp. 67-93. DOI: 10.1007/10_2007_064

Chaudhry, A. S. (2000). “Rumen degradation in sacco in sheep of wheat straw treated with calcium oxide, sodium hydroxide and sodium hydroxide plus hydrogen peroxide,” *Animal Feed Science and Technology* 83(3-4), 313-323. DOI: 10.1016/S0377-8401(99)00134-0

Curreli, N., Fadda, M. B., Rescigno, A., Rinaldi, A. C., Soddu, G., Sollai, F., Vaccargiu, S., Sanjust, E., and Rinaldi, A. (1997). “Mild alkaline/oxidative pretreatment of wheat straw,” *Process Biochemistry* 32(8), 665-670. DOI: 10.1016/S0032-9592(97)00020-4

DIN 38414-8 (1985). “Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung; Schlamm und Sedimente (gruppe S); Bestimmung des Faulverhaltens (S 8) [German standard methods for the examination of water, waste water and sludge; sludge and sediments (group S); determination of the amenability to anaerobic digestion (S 8)],” German Institute for Standardization, Berlin, Germany.

Fan, L.-T., Gharpuray, M. M., and Lee, Y.-H. (1987). *Cellulose Hydrolysis*, Springer-Verlag, Heidelberg, Germany. DOI: 10.1007/978-3-642-72575-3

Fengel, D., and Wegener, G. (1984). *Wood - Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, Germany.

Galbe, M., and Zacchi, G. (2012). “Pretreatment: The key to efficient utilization of lignocellulosic materials,” *Biomass and Bioenergy* 46, 70-78. DOI: 10.1016/j.biombioe.2012.03.026

Hatakka, A. I., Varesa, T., and Lundell, T. K. (1993). “Production of multiple lignin peroxidases by the white-rot fungus *Phlebia ochraceofulva*,” *Enzyme and Microbial Technology* 15(8), 664-669. DOI: 10.1016/0141-0229(93)90066-B

Hendriks, A. T., and Zeeman, G. (2009). “Pretreatments to enhance the digestibility of lignocellulosic biomass,” *Bioresource Technology* 100(1), 10-18. DOI: 10.1016/j.biortech.2008.05.027

Hjorth, M., Gränitz, K., Adamsen, A. P. S., and Møller, H. B. (2011). “Extrusion as a pretreatment to increase biogas production,” *Bioresource Technology* 102(8), 4989-4994. DOI: 10.1016/j.biortech.2010.11.128

Hu, F., Jung, S., and Ragauskas, A. J. (2012). “Pseudo-lignin formation and its impact on enzymatic hydrolysis,” *Bioresource Technology* 117, 7-12. DOI: 10.1016/j.biortech.2012.04.037

Iranmahboob, J., Nadim, F., and Monemi, S. (2002). “Optimizing acid-hydrolysis: A critical step for production of ethanol from mixed wood chips,” *Biomass and Bioenergy* 22(5), 401-404. DOI: 10.1016/S0961-9534(02)00016-8

Ishida, Y., Goto, K., Yokoi, H., Tsuge, S., Ohtani, H., Sonoda, T., and Ona, T. (2007). “Direct analysis of phenolic extractives in wood by thermochemolysis-gas chromatography in the presence of tetrabutylammonium hydroxide,” *Journal of Analytical and Applied Pyrolysis* 78(1)**,** 200-206. DOI: 10.1016/j.jaap.2006.06.009

Iyer, P. V., Wu, Z.-W., Kim, S. B., and Lee, Y. Y. (1996). “Ammonia recycled percolation process for pretreatment of herbaceous biomass,” *Applied Biochemistry and Biotechnology* 57-58(1), 121-132. DOI: 10.1007/BF02941693

Jędrczak, A. (2008). *Biologiczne Przetwarzanie Odpadów [Biological Waste Treatment]*, PWN Scientific Publisher, Warszawa, Poland.

Jung, S., Foston, M., Sullards, M. C., and Ragauskas, A. J. (2010). “Surface characterization of dilute acid pretreated *Populus deltoides* by ToF-SIMS,” *Energy & Fuels* 24(2), 1347-1357. DOI: 10.1021/ef901062p

Kacurakova, M., Smith, A. C., Gidley, M. J., and Wilson, R. H. (2002). “Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D

FT-IR spectroscopy," *Carbohydrate Research* 337(12), 1145-1153. DOI: 10.1016/S0008-6215(02)00102-7

Karunanithy, C., and Muthukumarappan, K. (2009). "Influence of extruder temperature and screw speed on pretreatment of corn stover while varying enzymes and their ratios," *Applied Biochemistry and Biotechnology* 162(1), 264-279. DOI: 10.1007/s12010-009-8757-y

Kim, S., and Holtzapple, M. T. (2005). "Lime pretreatment and enzymatic hydrolysis of corn stover," *Bioresource Technology* 96(18), 1994-2006. DOI: 10.1016/j.biortech.2005.01.014

Kim, S. B., and Lee, Y. Y. (2002). "Diffusion of sulfuric acid within lignocellulosic biomass particles and its impact on dilute-acid pretreatment," *Bioresource Technology* 83(2), 165-171. DOI: 10.1016/S0960-8524(01)00197-3

Kumar, P., Barrett, D. M., Delwiche, M. J., and Stroeve, P. (2009). "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Industrial and Engineering Chemistry Research* 48(8), 3713-3729. DOI: 10.1021/ie801542g

Lang, X., Macdonald, D. G., and Hill, G. A. (2001). "Recycle bioreactor for bioethanol production from wheat starch II. Fermentation and economics," *Energy Sources* 23(5), 427-436. DOI: 10.1080/009083101300058426

Lewicki, A., Pilarski, K., Janczak, D., Czekała, W., Rodríguez Carmona, P. C., Cieślik, M., and Witaszek, K. (2013). "The biogas production from herbs and waste from herbal industry," *Journal of Research and Applications in Agricultural Engineering* 58(1), 114-117.

Li, J. B., Henriksson, G., and Gellerstedt, G. (2007). "Lignin depolymerization/ repolymerization and its critical role for delignification of aspen wood by steam explosion," *Bioresource Technology* 98(16), 3061-3068. DOI: 10.1016/j.biortech.2006.10.018

Mao, J. D., Holtman, K. M., and Franqui-Villanueva, D. (2010). "Chemical structures of corn stover and its residue after dilute acid prehydrolysis and enzymatic hydrolysis: Insight into factors limiting enzymatic hydrolysis," *Journal of Agriculture and Food Chemistry* 58(22), 11680-11687. DOI: 10.1021/jf102514r

Marzialetti, T., Olarte, M. B. V., Sievers, C., Hoskins, T. J. C., Agrawal, P. K., and Jones, C. W. (2008). "Dilute acid hydrolysis of loblolly pine: A comprehensive approach," *Industrial & Engineering Chemistry Research* 47(19), 7131-7140. DOI: 10.1021/ie800455f

Mészáros, E., Jakab, E., and Várhegyi, G. (2007). "TG/MS, Py-GC/MS and THM-GC/MS study of the composition and thermal behavior of extractive components of *Robinia pseudoacacia*," *Journal of Analytical and Applied Pyrolysis* 79(1-2), 61-70. DOI: 10.1016/j.jaap.2006.12.007

Michalska, K., and Ledakowicz, S. (2012). "Degradacja struktur lignocelulozowych oraz produktów ich hydrolizy [Degradation of lignocellulosic structures and products of their hydrolysis]," *Inżynieria i Aparatura Chemiczna* [*Engineering and Chemical Apparatus*] 51(4), 157-159.

Millet, M. A., Baker, A. J., and Satter, L. D. (1976). "Physical and chemical pretreatments for enhancing cellulose saccharification," *Biotechnology and Bioengineering Symposium* 6, 125-153.

Mulat, D. G., Dibdiakova, J., and Horn, S. J. (2018). "Microbial biogas production from hydrolysis lignin: Insight into lignin structural changes," *Biotechnology for Biofuels* 11, 61-77. DOI: 10.1186/s13068-018-1054-7

Nguyen, Q. A., Tucker, M. P., Keller, F. A., and Eddy, F. P. (2000). "Two-stage dilute-acid pretreatment of softwoods," in: *Applied Biochemistry and Biotechnology*, M. Finkelstein and B. H. Davison (eds.), Humana Press, Totowa, NJ, USA, pp. 84-86. DOI: 10.1007/978-1-4612-1392-5_44

Paudel, S. R., Banjara, S. P., Choi, O. K., Young, P. K., Kim, Y. M., and Lee, J. W. (2017). "Pretreatment of agricultural biomass for anaerobic digestion: Current state and challenges," Bioresource Technology 245(Part A), 1194-1205. DOI: 10.1016/j.biortech.2017.08.182

Paul, S., and Dutta, A. (2018). "Challenges and opportunities of lignocellulosic biomass for anaerobic digestion," *Resources, Conservation and Recycling* 130, 164-174. DOI: 10.1016/j.resconrec.2017.12.005

PN-EN 12879:2004: Charakterystyka osadów ściekowych. Oznaczanie strat przy prażeniu suchej masy osadu [Characteristics of sewage sludge. Determination of losses during roasting of dry sludge]

PN-73C-04576/02: Woda i ścieki. Badania zawartości związków azotu. Oznaczanie azotu amonowego metodą miareczkową [Water and sewage. Research on the content of nitrogen compounds. Determination of ammonium nitrogen by the titration method]

PN-90/C-04540.01: Woda i ścieki. Badania pH, kwasowości i zasadowości. Oznaczanie pH wód i ścieków o przewodności elektrolitycznej właściwej 10 mikrosimensów/cm i powyżej metodą elektrometryczną [Water and sewage. Research on pH, acidity and alkalinity. Determination of pH of water and sewage with electrolytic conductivity of 10 microsiemens / cm and above by electrometric method]

Poletto, M., Zattera, A. J., and Santana, R. M. C. (2012). "Structural differences between wood species: Evidence from chemical composition, FTIR spectroscopy, and thermogravimetric analysis," *Journal of Applied Polymer Science* 126(S1), 336-343. DOI: 10.1002/app.36991

Popescu, C.-M., Singurel, G., Popescu, M.-C., Vasile, C., Argyropoulos, D. S., and Willför, S. (2009). "Vibrational spectroscopy and X-ray diffraction methods to establish the differences between hardwood and softwood," *Carbohydrate Polymers* 77(4), 851-857. DOI: 10.1016/j.carbpol.2009.03.011

Popescu, C.-M., Singurel, G., Vasile, C., Argyropoulos, D. S., and Willfor, S. (2007). "Spectral characterization of eucalyptus wood," *Applied Spectroscopy* 61(11), 1168-1177. DOI: 10.1366/000370207782597076

Proniewicz, L. M., Paluszkiewicz, C., Weselucha-Birczynska, A., Majcherczyk, H., Baranski, A., and Konieczna, A. (2001). "FT-IR and FT-Raman study of hydrothermally degradated cellulose," *Journal of Molecular Structure* 596(1-3), 163-169. DOI: 10.1016/S0022-2860(01)00706-2

Pu, Y., Hu, F., Huang, F., Davison, B. H., and Ragauskas, A. J. (2013). "Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments," *Biotechnology for Biofuels* 6(1), 1-13. DOI: 10.1186/1754-6834-6-15

Ragauskas, A. J., Beckham, G. T., Biddy, M. J., Chandra, R., Chen, F., Davis, M. F., Davison, B. H., Dixon, R. A., Gilna, P., Keller, M., *et al.* (2014). "Lignin valorization: Improving lignin processing in the biorefinery," *Science* 344(6185), Article ID 1246843. DOI: 10.1126/science.1246843

Robak, K., and Balcerek, M. (2017). "Rola obróbki wstępnej biomasy lignocelulozowej w produkcji bioetanolu II generacji [The role of pre-treatment of lignocellulosic biomass in the production of 2nd generation bioethanol]," *Acta Agrophysica* 24(2), 301-318.

Sannigrahi, P., Kim, D. H., Jung, S., and Ragauskas, A. (2011). "Pseudo-lignin and pretreatment chemistry," *Energy & Environmental Science* 2011(4), 1306-1310. DOI: 10.1039/C0EE00378F

Sannigrahi, P., and Ragauskas, A. J. (2011). "Characterization of fermentation residues from the production of bio-ethanol from lignocellulosic feedstocks," *Journal of Biobased Materials and Bioenergy* 5(4), 514-519. DOI: 10.1166/jbmb.2011.1170

Sannigrahi, P., Ragauskas, A. J., and Miller, S. J. (2008). "Effects of two-stage dilute acid pretreatment on the structure and composition of lignin and cellulose in loblolly pine," *BioEnergy Research* 1(3-4), 205-214. DOI: 10.1007/s12155-008-9021-y

Shrestha, S., Fonoll, X., Khanal, S.-K., and Raskin, L. (2017). "Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: Current status and future perspectives," *Bioresource Technology* 245, Part A, 1245-1257. DOI: 10.1016/j.biortech.2017.08.089

Siengchum, T., Isenberg, M., and Chuang, S. S. C. (2012). "Fast pyrolysis of coconut biomass – An FTIR study," *Fuel* 105, 559-565. DOI: 10.1016/j.fuel.2012.09.039

Sievers, C., Marzialetti, T., Hoskins, T. J. C., Olarte, M. B. V., Agrawal, P., and Jones, C. W. (2009). "Quantitative solid state NMR analysis of residues from acid hydrolysis of loblolly pine wood," *Bioresource Technology* 100(20), 4758-4765. DOI: 10.1016/j.biortech.2008.11.060

Sills, D. L., and Gossett, J. M. (2012). "Using FTIR spectroscopy to model alkaline pretreatment and enzymatic saccharification of six lignocellulosic biomasses," *Biotechnology and Bioengineering* 109(4), 894-903. DOI: 10.1002/bit.24376

Sivers, M., and Zacchi, G. (1995). "A techno-economical comparison of three processes for the production of ethanol from pine," *Bioresource Technology* 51(1), 43-52. DOI: 10.1016/0960-8524(94)00094-H

Stachowiak-Wencek, A., Zborowska, M., Waliszewska, H., and Waliszewska, B. (2018). "Zmiany struktury ligniny osadków kukurydzy pod wpływem fermentacji metanowej," ["Changes in the structure of lignin of corncobs under the influence of anaerobic digestion"], *Przemysł Chemiczny* 97(12), 2162-2165. DOI: 10.15199/62.2018.12.30

Sun, Y., and Cheng, J. J. (2005). "Dilute acid pretreatment of rye straw and bermudagrass for ethanol production," *Bioresource Technology* 96(14), 1599-1606. DOI: 10.1016/j.biortech.2004.12.022

TAPPI T204 cm-07 (2007). "Solvent extractives of wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T207 cm-99 (2008). "Water solubility of wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T 211om – 07: "Ash in wood, pulp, paper, and paperboard: Combustion at 525 °C.

TAPPI T9 wd-75 (2015). "Holocellulose in wood," TAPPI Press, Atlanta, GA.

TAPPI T212 om-02 (2007). "One percent sodium hydroxide solubility of wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T222 om-06 (2006). "Acid insoluble lignin in wood and pulp," TAPPI Press, Atlanta, GA.

TAPPI T223 cm-01 (2001). "Pentosans in wood and pulp," TAPPI Press, Atlanta, GA.

Theuretzbacher, F., Lizasoain, J., Lefever, C., Saylor, M. K., Enguidanos, R., Weran, N., Gronauer, A., and Bauer, A. (2015). "Steam explosion pretreatment of wheat straw to improve methane yields: Investigation of the degradation kinetics of structural compounds during anaerobic digestion," *Bioresource Technology* 179, 299-305. DOI: 10.1016/j.biortech.2014.12.008

Todorciuc, T., Căpraru, A.-M., Kratochvílová, I., and Popa, V. I. (2009). "Characterization of non-wood lignin and its hydoxymethylated derivatives by spectroscopy and self-assembling investigations," *Cellulose Chemistry and Technology* 43(9-10), 399-408.

Waliszewska, H., Zborowska, M., Stachowiak-Wencek, A., Waliszewska, B., and Czekała, W. (2019). "Lignin transformation of one-year-old plants during anaerobic digestion (AD)," *Polymers* 11(5), 835. DOI: 10.3390/polym11050835

Waliszewska, H., Zborowska, M., Waliszewska, B., Borysiak, S., Antczak, A., and Czekała, W. (2018). "Tr Witaszek 25(2), 1207-1216. DOI:10.1007/s10570-017-1622-1

Witaszek, K., Pilarska, A. A., and Pilarski, K. (2015). "Wybrane metody wstępnej obróbki surowców roślinnych stosowanych do produkcji biogazu [Selected methods of pre-treatment of plant raw materials used for biogas production]," *Ekonomia i Środowisko* [*Economics and Environment*] 2(53), 130-144.

Yokoi, H., Nakase, T., Goto, K., Ishida, Y., Ohtani, H., Tsuge, S., Sonoda, T., and Ona, T. (2003). "Rapid characterization of wood extractives in wood by thermal desorption-gas chromatography in the presence of tetramethylammonium acetate," *Journal of Analytical and Applied Pyrolysis* 67(1), 191-200. DOI: 10.1016/S0165-2370(02)00061-X

Zheng, M., Li, X., Li, L., Yang, X., and He, Y. (2009a). "Enhancing anaerobic biogasification of corn stover through wet state NaOH pretreatment," *Bioresource Technology* 100(21), 5140-5145. DOI: 10.1016/j.biortech.2009.05.045

Zheng, Y., Pan, Z., and Zhang, R. (2009b). "Overview of biomass pretreatment for cellulosic ethanol production," *International Journal of Agricultural and Biological Engineering* 2(3), 51-68. DOI: 10.3965/j.issn.1934-6344.2009.03.051-068

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