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ENZYMATIC HYDROLYSIS OF EXTRUDED WHEAT STRAW WITH ADDITION OF SODIUM HYDROXIDE AND CALCIUM HYDROXIDE

Juraj Gigac, Mária Fišerová, Monika Stankovská, Andrej Pažitný
Pulp and Paper Research Institute
Bratislava, Slovak Republic

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ABSTRACT

Extrusion pretreatment of wheat straw in a single screw extruder was investigated in terms of effectiveness of enzymatic hydrolysis and the formation of fermentation inhibitors. The effect of sodium hydroxide in extrusion pretreatment was compared with calcium hydroxide. The accessibility of wheat straw structure to hydrolytic enzymes increased with NaOH and $Ca(OH)_2$ loadings, whereby more with the addition of NaOH. With 6% w/w of NaOH loading, the conversion of polysaccharides was 76.1% and with the same $Ca(OH)_2$ loading it was 47.3%. The conversion of polysaccharides with 12% w/w of $Ca(OH)_2$ loading was 66.6%. Without alkali in extrusion pretreatment, the conversion of polysaccharides was only 36.7%. The polysaccharides conversion of original extruded wheat straw was about 1.5 to 3.3% higher in comparison to washed extruded wheat straw. Fermentation inhibitors such as lignin, acetic and formic acid are primarily formed in the presence of alkali during extrusion pretreatment, most of which was lignin. Alkaline extrusion is a suitable method for pretreatment of lignocellulosic biomass.

KEYWORDS: Extrusion pretreatment, sodium hydroxide, calcium hydroxide, wheat straw, enzymatic hydrolysis, inhibitors, bioethanol.

INTRODUCTION

Lignocellulosic biomass, including agricultural residues, energy crops, forestry wastes and a part of municipal waste, are a renewable materials for bioethanol production. The conversion efficiency of lignocellulosic biomass is limited by the biomass recalcitrance, which refers to the natural resistance of plants to enzymatic hydrolysis (Himmel et al. 2007). The biomass recalcitrance is caused by the spatial structure and chemical composition of lignocellulose, including its crystalline cellulose content, degree of polymerization, accessibility and lignin protection, and all these properties cause its low hydrolysis efficiency (Zhang and Lynd 2004).

To enhance the lignocellulose hydrolysis process and achieve profitable bioethanol production, pretreatment is required to change the chemical and physical structure of lignocellulose and overcome the biomass recalcitrance. Many chemical, mechanical, thermo-chemical and biological pretreatment methods have been studied and are still in the development with varying levels of success, including acid hydrolysis, alkali hydrolysis, the organosolv process, steam explosion, ammonia fibre explosion (AFEX), hot water treatment, and microorganism treatment (Corredor et al. 2008, Galbe et al. 2007, Galbe and Zacchi 2007, Sun and Cheng 2002). Many of pretreatment methods can be adopted to reduce the recalcitrance of lignocellulose, enhance enzyme accessibility and increase the fermentation efficiency. However, currently available pretreatment methods can hardly meet the requirements of commercial application due to long processing times, chemical recycle difficulty, or higher operational cost (Galbe et al. 2007, Agbor et al. 2011). The success of lignocellulosic bioethanol production will depend on the development of simple pretreatment methods that effectively delignify different types of lignocellulosic biomass feedstocks. Reducing the enzyme cost while enhancing cellulose hydrolysis efficiency is another important consideration when developing suitable pretreatment methods (El-Naggar et al. 2014, Wi et al. 2013).

Extrusion pretreatment is a novel method in which biomass is processed by means of heat, compression and shear forces, leading to physical disruption and chemical modifications of biomass during the passage through the extruder. Compared with traditional physical pretreatment method such as chipping, grinding or milling, extrusion can not only reduce size of the particle, but it also provides a continuous thermo-mechanical treatment that will cause a further expansion of biomass fibril structure (Chen et al. 2014, Kratky and Jirout 2011). Therefore, extruded lignocellulosic materials achieve a lower bulk density, higher water-holding capacity and better biodegradability. Although extrusion can effectively enhance the enzymatic hydrolysis efficiency, the effect is still limited because it only changes the structure of biomass. The chemical composition such as lignin content is also an important factor of the biomass recalcitrance. Various types of extrusion processes have been studied for pretreatment of biomass and the extrusion pretreatment is considered as a promising technology for biomass conversion to ethanol production in recent studies (Kadam et al. 2009, Alvira et al. 2010, Lee at al. 2010, Karunanithy and Muthukumarappan 2010). However, there are still some disadvantages in certain cases, such as the low treatment rate, low biomass/liquid ratios or relatively high temperature (Senturk-Ozer et al. 2011).

Alkaline pretreatment is an efficient method to change the chemical composition, especially through lignin removal (Krishania et al. 2013). The main mechanisms of alkaline pretreatment are the degradation of ester bonds and cleavage of glycosidic linkages in lignocellulosic wall matrix, which lead to the alteration of the structure of lignin, the reduction of the lignin-hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose (Bobleter 1994, Sun and Cheng 2002, Fan et al. 1987). During the alkali pretreatment, solvation and saponification reactions take place rapidly (Park and Kim 2012). This will result in a greater swelling of the biomass and decomposition of the polysaccharides, which increases the enzyme accessibility and decrease the biomass recalcitrance (Hendriks and Zeeman 2009, Krishania et al. 2013). Studies of $Ca(OH)_2$ pretreatment have been carried out with different lignocellulosic biomass, such as wheat straw, corn stover, switchgrass, bagasse and rice hull, and successfull increase of monosaccharides yield in the enzymatic hydrolysis has been shown (Chang et al. 1997, Chang et al.1998, Saha and Cotta 2008, Kim and Holtzapple 2003).

The pretreatment of lignocellulosic biomass only in extruder has some limitations. For further improvement of enzymatic hydrolysis efficiency of extruded biomass, alkali pretreatment is a good option. The usual alkaline compounds are hydroxides of sodium, potassium, calcium, and ammonium. NaOH and $Ca(OH)_2$ are two alkalis widely used in lignocellulosic biomass pretreatment. When compared to other sources of alkali, $Ca(OH)_2$ is a relatively inexpensive and easily handled reagent. However, $Ca(OH)_2$ has very limited solubility in water (Oates 2007). The combination of both extrusion and alkaline pretreatment has been studied more recently (Zhang et al. 2012, Karunanithy and Muthukumarappan 2011, Duque et al. 2013). Overview of this type of thermo-mechanical pretreatment, including the mechanism influencing extruder and feedstock parameters and evaluation of pretreatment efficiency has already been published (Kurananithy and Muthukumarappan 2013). Alkaline twin-screw extrusion pretreatment in the presence of an alkali solution was used to improve the enzymatic hydrolysis efficiency of corn stover for the production of fermentable monosaccharides (Liu et al. 2013). A delignification of 71% and total monosaccharides yield of 78% could be achieved with NaOH loading of 0.06 g/g biomass and solid-to-liquid ratio of 1:2 at 99°C. The synergic effect of alkaline NaOH soaking and screw press pretreatment of wheat straw was evaluated based on polysaccharides conversion and energy efficiency (Yan et al. 2016). It was found that delignification was more significant for higher monosaccharides yield than the increase of the substrate surface area. An improved enzymatic hydrolysis could be achieved by longer interaction of biomass and NaOH in a screw reactor, furthered by higher concentration of NaOH. $Ca(OH)_2$ pretreatment has already been used to enhance the enzymatic hydrolysis and biogas production of extruded rice straw (Gu et al. 2015). The optimal $Ca(OH)_2$ loading was 8%, when efficient hydrolysis as well as high biogas production were achieved.

The aim of this study was to compare the effect of NaOH and $Ca(OH)_2$ in extrusion pretreatment of wheat straw on conversion of polysaccharides to monosaccharides and on formation of fermentation inhibitors.

MATERIAL AND METHODS

Material

Wheat straw of winter wheat (*Triticum aestivum* L.) grown in the Senec region, Slovak Republic, the moisture content was 10.1%. Wheat straw had the following composition (in % w/w): cellulose (as glucose) 39.1%, xylan (as xylose) 24.7%, arabinan (as arabinose) 2.6%, acid insoluble lignin 15.1%, acid soluble lignin 1.4%, extractives 13.8% and ash 4.3%.

Cellic CTec3 is a cellulase and hemicellulase enzyme complex supplied by Novozymes A/S (Bagsvaerd, Denmark) for degradation of lignocellulosic biomass to fermentable monosaccharides. Cellic CTec3 contained a minimum of 1.700 BHU (Biomass Hydrolysis Units)/g product.

Methods

Impregnation

Wheat straw was grinded in a blender and sieved to obtain a particle size of around 2-5 mm. Prior to extrusion of wheat straw, it was treated with 6% w/w of NaOH loading, 6% w/w and 12% w/w of $Ca(OH)_2$ loadings based on oven dry (o.d.) wheat straw. NaOH and $Ca(OH)_2$ were dissolved in an amount of distilled water to achieve a ratio of wheat straw/water 2:3 (w/w). The solution of alkali was mixed with wheat straw manually 12 hours before extrusion. To evaluate the effect of NaOH and $Ca(OH)_2$, the wheat straw was also treated with distilled water to obtain the same moisture content as with alkali and the sample was used as the reference for extrusion pretreatment.

Extrusion pretreatment

After water or alkali impregnation, wheat straw was fed manually into the laboratory single screw extruder with a screw length of 400 mm and the die cap diameter of 6 mm. The pretreatments were carried out at screw speed of 50 rpm and at barrel temperature of 155°C. Under these conditions, the residence time of the wheat straw inside the extruder was about 3 min.

Washing

Post-extrusion washing was conducted by mixing the extruded wheat straw into distilled water at wheat straw to water ratio of 1:7 (w/w) for 15 min at temperature of 65°C. The mixture was filtered with a 100 mesh sieve to separate the solid extrudate and filtrate. The extrudate was used for enzymatic hydrolysis trial (washed) and the liquid filtrate was analysed.

Enzymatic hydrolysis

Enzymatic hydrolysis of original and washed extruded wheat straw samples with Cellic CTec3 dose of 15% w/w (g Cellic CTec3/100g cellulose) took place at 50°C, pH 5, 72 hours and 12.5 % w/w of total solids loading. The pH of samples was adjusted to 5 with 0.05 M citrate buffer containing 0.02% of sodium azide to prevent microbial activity during hydrolysis.

Analytical methods

Chemical composition of wheat straw was estimated using the procedure of National Renewable Energy Laboratory (Sluiter et al. 2011). After extrusion of wheat straw, the filtrates were subjected to a hydrolysis with 4% w/w H_2SO_4 for 1 hour at 121°C to convert oligomers into monomers. Monosaccharides (glucose, xylose, arabinose) in filtrates, and hydrolysates were determined after 72 hours of enzymatic hydrolysis by HPLC with Rezex ROA H^+ column. Formic acid and acetic acid in the filtrates and extracts were also determined by HPLC. The mobile phase was 0.005 N H_2SO_4 at a flow rate of 0.7 $ml \cdot min^{-1}$ and 30°C. The samples were passed through a 0.22 μm syringe filter before testing. The concentration of lignin in hydrolysates and filtrates were determined using UV-visible spectrometer Helios Beta. Lignin concentration was calculated from the Beer-Lambert law with extinction coefficient for wheat straw lignin at 280 nm.

RESULTS AND DISCUSSION

Wheat straw is one of the most abundant crop residues in European countries, and it also seems to be one of the cheapest ones and it is the most useful raw material for ethanol production, too. Enzymatic hydrolysis is the key factor of this process and remains a major obstacle mainly due to high enzyme cost. In order to achieve sufficient enzyme accessibility to wheat straw, an effective pretreatment method is required for disruption of the complex structure and recalcitrant nature of lignocellulosic biomass while avoiding the formation of inhibitors.

The extrusion pretreatment leads to physical disruption and chemical modification of wheat straw during the passage through extruder. However, sufficient accessibility for enzymes was not achieved. Therefore, the effect of addition NaOH and $Ca(OH)_2$ on the extrusion pretreatment efficiency was tested. $Ca(OH)_2$ is much cheaper reagent compared to NaOH, and Ca^{2+} has no inhibitory effect on anaerobic digestion (Chen et al. 2008).

The enzymatic hydrolysis of original and washed extruded wheat straw prepared without alkali and with Na(OH) and $Ca(OH)_2$ was performed under the same conditions. In Fig. 1 the concentration of glucose, xylose and all monosaccharides in hydrolysates reached after 72 h of enzymatic hydrolysis of original and washed extruded wheat straw are presented. The purpose of the washing stage was to remove part of the alkali and inhibitors that might hinder enzymatic hydrolysis and microbial fermentation (Larsson et al. 1999, Mes-Hartree and Saddler 1983).

The presence of alkali in pretreatment of wheat straw by extrusion was manifested by a higher concentration of monosaccharides in the hydrolysates. Based on our previous work (Stankovská et al. 2018), the alkali loading of 6% w/w on oven dry wheat straw was used in laboratory experiments. The concentration of monosaccharides in hydrolysates at this loading of $Ca(OH)_2$ were significantly lower than with NaOH. Even with 12% w/w of $Ca(OH)_2$ loading, the lower monosaccharides concentration was achieved than with 6% w/w of NaOH loading in extrusion pretreatment. Concentration of monosaccharides in hydrolysates of original extruded wheat straw was higher in comparison to hydrolysates of washed extruded wheat straw. The glucose concentration was lower by about 1.5 to 2.1%, xylose concentration was lower by about 0.8 to 1.1% and concentration of all monosaccharides was about 2.0 to 3.9% lower in hydrolysates of washed extruded wheat straw than in hydrolysates of original extruded wheat straw.

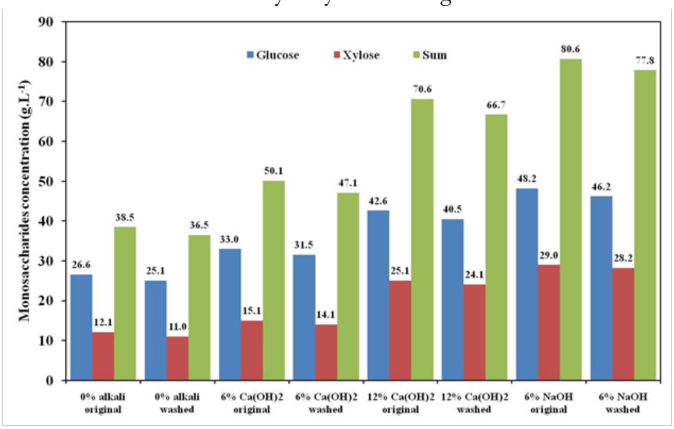

Fig. 1: Effect of NaOH and $Ca(OH)_2$ loading in extrusion pretreatment on monosaccharides concentration in hydrolysates of original and washed extruded wheat straw after 72 h of enzymatic hydrolysis.

Conversion of polysaccharides to monosaccharides directly represents the hydrolysis yield of lignocellulosic biomass, based on pretreatment efficiency and accessibility to enzymes. Under ideal conditions, all polysaccharides in lignocellulosic biomass are hydrolysed into monosaccharides. Usually, not all polysaccharides are quantitatively hydrolysed to monosaccharides, and some polysaccharide chains, especially those least accessible ones remain unhydrolysed. Conversions of glucan, xylan and all polysaccharides after 72 h of enzymatic hydrolysis of wheat straw extruded without alkali and with NaOH and $Ca(OH)_2$ are illustrated in Fig. 2. The results indicate that accessibility of polysaccharides to hydrolytic enzymes increased in the presence of alkali in extrusion pretreatment. The conversion of glucan after enzymatic hydrolysis of original extruded wheat straw (0% alkali) was 42.9%, conversion of xylan was 29.8% while for all polysaccharides it was 36.3%. The highest conversion of glucan (77.7%), xylan (72.3%) and polysaccharides (76.1%) was achieved after enzymatic hydrolysis of original extruded wheat straw with 6% w/w of NaOH loading. After enzymatic hydrolysis of original extruded wheat straw with 12% w/w of $Ca(OH)_2$ loading, 68.6% conversion of glucan, 62.6% conversion of xylan and 66.6% conversion of all polysaccharides were achieved. The polysaccharides conversion of original extruded wheat straw was higher in comparison to washed extruded wheat straw. The glucan conversion was about 2.4 to 3.3% lower for washed extruded wheat straw than for original extruded wheat straw, for xylan conversion the value was about 2.0 to 2.5% and for all polysaccharides the conversion was about 1.5 to 3.3% lower.

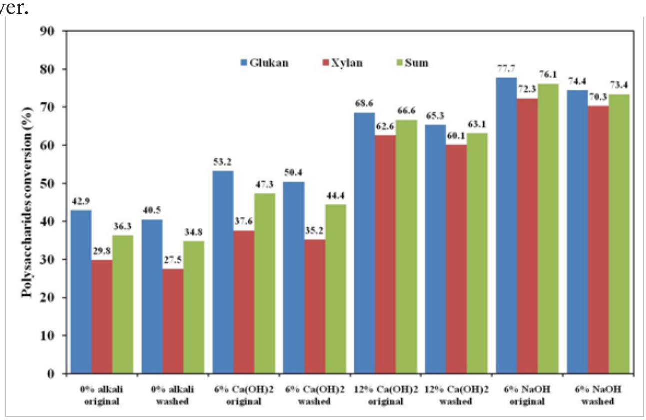

Fig. 2: Effect of NaOH and $Ca(OH)_2$ loading in extrusion pretreatment on polysaccharides conversion of original and washed extruded wheat straw after 72 h of enzymatic hydrolysis.

Yields of monosaccharides of raw material also express the efficiency of extrusion pretreatment. They are also dependent on enzymatic hydrolysis conditions and chemical composition of raw material. For bioethanol production, monosaccharides yield is an important factor for comparing different raw materials. Effect of alkali in extrusion pretreatment of wheat straw on monosaccharides yields is presented in Fig. 3.

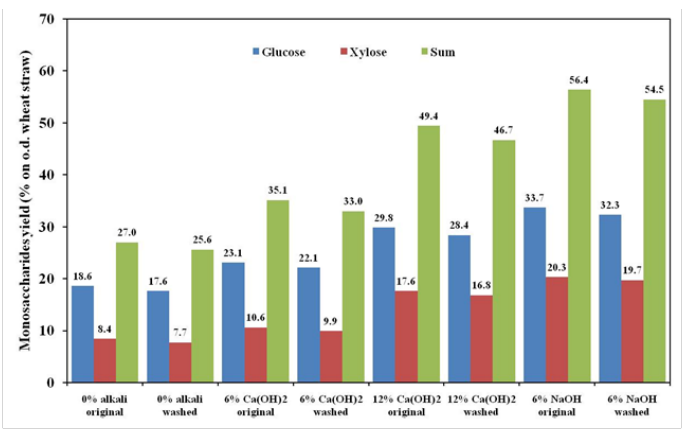

Fig. 3: Effect of NaOH and $Ca(OH)_2$ loading in extrusion pretreatment on monosaccharides yield of original and washed extruded wheat straw after 72 h of enzymatic hydrolysis.

The yield of glucose after enzymatic hydrolysis of original extruded wheat straw (0% alkali) was found to be 18.6%, while yield of xylose was 8.4% and yield of all monosaccharides 27.0%. The highest yield of glucose (33.7%), xylose (20.3%) and all monosaccharides (56.4%) was achieved after enzymatic hydrolysis of original extruded wheat straw with 6% w/w of NaOH loading. After enzymatic hydrolysis of original extruded wheat straw with 12% w/w of $Ca(OH)_2$ loading, 29.8% yield of glucose, 17.6% yield of xylose and 49.4% yield of all monosaccharides were achieved. The yield of monosaccharides of original extruded wheat straw was higher in comparison to washed extruded wheat straw. The yield of glucose was about 1.0 to 1.4%, xylose about 0.6 to 1.0% and yield of all monosaccharides was about 1.4 to 2.7% lower from washed extruded wheat straw than from original extruded wheat straw.

Fermentation inhibitors such lignin, acetic and formic acids are formed particularly in the presence of alkali during extrusion pretreatment. Hydrolysis of the hemicelluloses acetyl group is responsible for acetic acid formation. As a result of this pretreatment, a small amount of formic acid was formed. In Fig. 4, the concentrations of lignin, acetic and formic acids in hydrolysates of original and washed extruded wheat straw are presented. The results suggest that the presence of alkali in the pretreatment had a significant impact especially on the concentration of lignin. The hydrolysates of washed extruded wheat straw only contained lignin.

Concentration of acetic acid in hydrolysate of original extruded wheat straw (0% alkali) was 0.9 $g \cdot L^{-1}$ and concentration of lignin was 1.4 $g \cdot L^{-1}$. The highest concentration of lignin (17.2 $g \cdot L^{-1}$), acetic acid (5.4 $g \cdot L^{-1}$) and formic acid (0.8 $g \cdot L^{-1}$) was found to be in the hydrolysate of original extruded wheat straw with 6% w/w of NaOH loading. In the hydrolysate of original extruded wheat straw with 12% w/w of $Ca(OH)_2$ loading, the concentration of lignin was 17 $g \cdot L^{-1}$, the value for acetic acid was 4.1 $g \cdot L^{-1}$ and concentration of formic acid 0.8 $g \cdot L^{-1}$. The hydrolysates of washed extruded wheat straw contained only lignin, whose concentration was approximately 3.5 times lower than in hydrolysates of original extruded wheat straw. In washing stage, acetic acid, formic acid and substantial portion of lignin, sodium and calcium ions were removed.

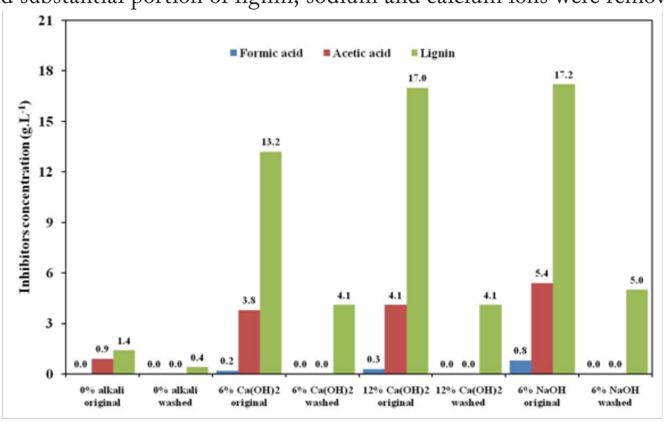

Fig. 4: Effect of NaOH and $Ca(OH)_2$ loading in extrusion pretreatment on concentration of formic acid, acetic acid and lignin in hydrolysates of original and washed extruded wheat straw after 72 h of enzymatic hydrolysis.

After extrusion pretreatment, the filtrates only contained a small amount of monosaccharides but more their oligomers, therefore total hydrolysis of oligomers with sulfuric acids was performed. In addition, inhibitors were also present in the filtrates. The yields of monosaccharides, formic acid, acetic acid and lignin in the filtrates are shown in Fig. 5. The yield of monosaccharides in filtrate of extruded wheat straw (0% alkali) was 1.8%, for acetic acid and lignin yields, the values were 1.0% and 1.3% respectively. The highest yield of monosaccharides (3.5%), formic acid (0.6%), acetic acid (3.7%) and lignin (8.7%) was observed in filtrate of extruded wheat straw with

6% w/w of NaOH loading. In the filtrate of extruded wheat straw with 12% w/w of $Ca(OH)_2$ loading, yield of monosaccharides was 3.0%, formic acid 0.2%, acetic acid 2.8% and yield of lignin was 8.6%.

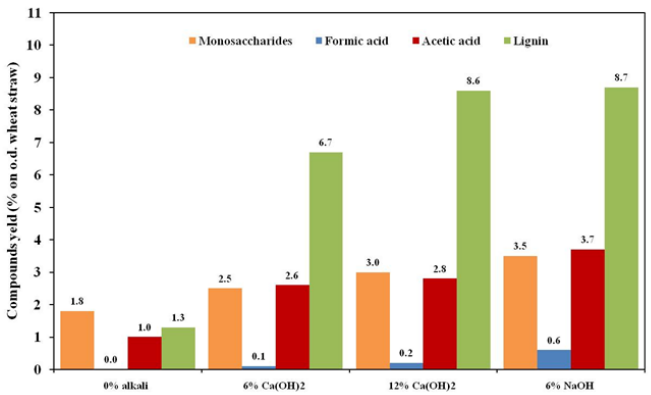

Fig. 5: Effect of NaOH and $Ca(OH)_2$ loading in extrusion pretreatment of wheat straw on yield of monosaccharides, formic acid, acetic acid and lignin in filtrates.

The polysaccharides are better preserved under alkaline conditions than at low pH values, but some degradation also occurs - leading to the formation of carboxylic acids. In comparison with the composition of the filtrate after the steam explosion of wheat straw at 195°C, the yield of lignin increased by 75%, while the yield of xylose decreased by 80% and the yield of glucose decreased by 45% (Russ et al. 2016). It is important to point out that neither furfural nor hydroxymethyl furfural were detected in filtrates.

Many substances obtained from filtrates can be used in various industries. The separation of lignin as a by-product in the production of bioethanol is especially desirable from an economic standpoint. Structure and amount of lignin are affected by method of lignocellulosic biomass pretreatment and method of lignin isolation. The structure and the composition of lignin restrict the subsequent applications. The filtrates from alkaline extrusion pretreatment can be easily recovered to alkali lignin or combusted to recover the chemicals and energy using existing industrial technology (Su et al. 2004). Acetic acid is an important chemical reagent and industrial chemical, used primarily in the production of cellulose acetate for photographic film, polyvinyl acetate for wood glue, and synthetic fibres and fabrics.

CONCLUSIONS

The thermo-mechanical effect of the flow restricting elements of the screw extruder ensures physical disintegration of the wheat straw by separating the fibre bundles. The presence of sodium hydroxide or calcium hydroxide ensures additional chemical destruction by solubilization of organic matter, this is the case especially for lignin, depending on the amount used. In addition to lignin, the fermentation inhibitors - acetic acid and formic acid are formed.

The accessibility of polysaccharides to hydrolytic enzyme also increased in the presence of alkali in extrusion pretreatment. At the same loading of alkali, the accessibility increased more with NaOH than with $Ca(OH)_2$. The conversion of glucan after enzymatic hydrolysis of original extruded wheat straw (0% alkali) was 42.9%, conversion of xylan 29.8% and conversion of all polysaccharides was 36.3%. With 6% w/w of NaOH loading in extrusion, the conversion of glucan was 77.7%, while it was 72.3% for xylan and 76.1% for all polysaccharides, whereas with the same loading of $Ca(OH)_2$, the conversion of glucan was only 53.2%, xylan 37.6% and all polysaccharides 47.3%. The polysaccharides conversion of original extruded wheat straw was higher when compared to washed extruded wheat straw.

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Juraj Gigac, Mária Fišerová*, Monika Stankovská, Andrej Paźitný
Pulp and Paper Research Institute
Dúbravská cesta 14
841 04 Bratislava
Slovak Republic
Corresponding author: fiserova@vupc.sk