# EFFECT OF ALKALINE EXTRUSION PRETREATMENT OF WHEAT STRAW ON FILTRATE COMPOSITION AND ENZYMATIC HYDROLYSIS

MONIKA STANKOVSKÁ, MÁRIA FIŠEROVÁ, JURAJ GIGAC and ANDREJ PAŽITNÝ

*Pulp and Paper Research Institute, Dúbravská cesta 14,*
*841 04 Bratislava, Slovak Republic*
✉ *Corresponding author: Mária Fišerová, fiserova@vupc.sk*

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Thermo-mechano-chemical pretreatment of wheat straw with NaOH concentrations ranging from 1% to 6% w/w in a single screw extruder was investigated in terms of filtrate composition and sugar yields after enzymatic hydrolysis. The filtrates contained lignin, xylose and its oligomers, glucose, arabinose, acetic and formic acid. The yields of all the substances increased with rising NaOH concentration. After extrusion of the wheat straw with 6% NaOH, 53% of lignin, 34% of arabinan, 9% of xylan and 2% of glucan were transferred into the filtrate. Alkaline extruded wheat straw was subsequently extracted at 80 °C for 30, 60, 120 and 180 minutes in order to increase the efficiency of enzymatic hydrolysis. After extracting the alkaline extruded wheat straw for 180 minutes, 70% of lignin, 50% of arabinan, 15% of xylan and 3% of glucan were removed. The results of polysaccharides conversion showed that alkaline extrusion pretreatment increased the accessibility of hydrolytic enzymes to the wheat straw structure. With increasing concentration of NaOH, the conversion of glucan increased from 40.5 to 77.0%, that of xylan – from 27.5 to 72.1% and that of total polysaccharides – from 34.8 to 74.6%. The addition of the alkaline extrusion pretreatment only slightly increased the conversion of glucan from 78.5 to 81.2%, that of xylan – from 74.0 to 77.7% and that of total polysaccharides from 75.6 to 79.0%.

***Keywords***: extrusion, wheat straw, NaOH, extraction, filtrate, enzymatic hydrolysis, monosaccharides

## INTRODUCTION

Lignocellulosic biomass is widely available and contains large amounts of cellulose and hemicelluloses, for all that, it is suitable for renewable energy production. The conversion of lignocellulosic biomass to monosaccharides is usually performed by enzymes. However, the complex structure and recalcitrant nature of lignocellulosic biomass necessitates a pretreatment stage, when compared to ethanol production from corn[1] and short waste fibres from pulp and waste paper.[2] The purposes of biomass pretreatment are to open up the structure, increase the accessible surface area, reduce the cellulose crystallinity and increase the porosity, pore size and pore volume. These factors affect the enzymatic hydrolysis of cellulose and hemicelluloses.[3,4] Several pretreatment approaches have been investigated on different varieties of lignocellulosic biomass and they have shown varying results based on the raw material used for fermentation.[5-9] An effective pretreatment should meet the following criteria: reduce costs and minimize energy requirements, preserve hemicellulose fractions (mainly pentoses), avoid the degradation of monosaccharides and minimize the formation of inhibitors for a further fermentation stage, as well as recover lignin for valuable products.[8] One of the possibilities of lignocellulosic biomass pretreatment, steam explosion of wheat straw, was investigated. When steam explosion was used alone, the optimum temperature for effective hydrolysis of wheat straw was found around 200 °C and these conditions were sufficient to make the lignocellulose structure accessible to enzymes and less inhibitors were created.[10] Water prehydrolysis as the first stage in a two-stage pretreatment of wheat straw, in combination with steam explosion, was used before enzymatic hydrolysis.[11] The optimal conditions of water prehydrolysis were determined. The steam explosion technique seems to be very good for increasing the efficiency of enzyme hydrolysis as well, but the disadvantage of steam explosion, in comparison with the extrusion method, is the discontinued treatment and thus, a smaller amount of biomass can be processed.

Among the pretreatments that are currently studied and further developed, extrusion stands out for its ability to provide high shear, rapid heat transfer, as well as effective and rapid mixing.[12] Other advantages of this method are the feasibility of continuous operation and its versatility in adopting different configurations. The extrusion parameters, such as compression ratio, screw speed and barrel temperature, have a significant effect on the conversion of polysaccharides.[13] The physical and chemical structure of the material will be disturbed and altered during its passage through the extruder barrel, resulting in a large specific area with increased accessibility of cellulose to the enzymatic action.[14] Different types of extruders, such as single-screw extruders and twin-screw extruders, have been widely examined for different lignocellulosic biomass, resulting in subsequent high enzymatic hydrolysis rates. An extruder can accommodate a wide range of feedstock sizes, especially larger sizes, when compared to other pretreatment methods; thereby, extrusion leads to remarkable savings, in terms of size reduction, depending on the construction of the extruder. The thermo-mechanical extrusion pretreatment process for bioconversion of lignocellulosic biomass to ethanol can be conducted in a large number of systems, with or without the addition of chemicals.

Lignocellulosic biomass can be treated with chemical solutions, such as acid and alkali, during the extrusion process,[15,16] however, the acid would cause corrosion of the extruder parts. Thus, acid resistant stainless steel alloys would be required for extruder screws and barrel fabrication. Alkali pretreatment is the preferred method due its delignification effect and lesser degradation of monosaccharides. Among the different alkalis employed on lignocellulosic biomass, such as sodium, potassium, calcium and ammonium hydroxides, Morrison[17,18] found that sodium hydroxide is the most commonly used alkali in lignocellulosic biomass pretreatment as it is able to cleave ester linkages and dissolve some hemicelluloses and lignin.

Alkaline pretreatment can be conducted by soaking the biomass in a sodium hydroxide solution at room temperature or by adding alkali to the biomass before extrusion, using a volumetric pump. Alkaline pretreatment can be performed at lower temperature and pressure, compared to other chemical pretreatment methods. Also, the process in the extruder does not lead to extensive degradation of monosaccharides.[19] Corn stover was mixed with alkaline solutions in a mechanical mixer and then pretreated in a screw extruder under different conditions.[20] The optimum glucose and xylose recovery was of 86.8% and 50.5%, respectively, at a NaOH concentration of 0.04 g/g biomass and a screw speed of 80 rpm. In the study by Liu *et al*.,[21] alkaline extrusion of corn stover before enzymatic hydrolysis was performed with a biomass/liquid ratio of ½ (w/w) at a temperature of 99 °C. They used a NaOH concentration of 0.06 g/g biomass and converted 83% of glucan and 89% of xylan, respectively. The combined effect of alkali soaking and extrusion on big bluestem, using a single screw extruder, was evaluated.[22] Big bluestem was soaked in different alkali solutions at room temperature for 30 min and then extruded under different conditions. Consequently, 90.1%, 81.5% and 89.9% of glucose, xylose and total monosaccharides were recovered, respectively, at a barrel temperature of 90 °C, screw speed of 155 rpm, 2.0% alkali concentration, and 4 mm particle size. The effect of two alkalies, NaOH and $Ca(OH)_2$, in extrusion pretreatment of wheat straw was compared.[23] At the same loading of alkali, the accessibility of wheat straw to hydrolytic enzymes increased more significantly with NaOH and resulted in higher conversion of polysaccharides to monomers. After enzymatic hydrolysis of unwashed wheat straw, extruded with 6% w/w of NaOH, the glucan conversion was by about 13.3% higher than that obtained with 12% w/w of $Ca(OH)_2$, while the concentration of lignin was similar. The conversion of polysaccharides in unwashed extruded wheat straw was higher, when compared to that in washed extruded wheat straw. An economic, feasible assessment of the extrusion pretreatment method should be carried out in order to design an industrial bioethanol production plant. However, a comparison of the economic feasibility of each pretreatment is very difficult to make because of different underlying assumptions.[24]

The goal of this study was to evaluate the effect of extrusion pretreatment of wheat straw with varying NaOH concentrations, as well as its combination with extraction, on filtrate composition and enzymatic hydrolysis efficiency.

## EXPERIMENTAL

### Materials

The wheat straw (*Triticum aestivum* L.) used in the study was grown in the Senec region, Slovak Republic, and had 10.1% of moisture content. The composition of the wheat straw (in % w/w) was the following: 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), which was used 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 treated with NaOH concentrations from 1% to 6% w/w based on oven dry (o.d.) wheat straw. NaOH was dissolved in distilled water to achieve a ratio of wheat straw/water of 2:3 (w/w). The solution was mixed with wheat straw manually 12 h before extrusion. The next step was extrusion. For a blank experiment, the wheat straw was treated with distilled water to obtain the same moisture content as with NaOH and the sample was used as the reference for extrusion pretreatment.

### *Extrusion pretreatment*

Wheat straw was fed manually into a laboratory single screw extruder with a screw length of 400 mm and a die cap diameter of 6 mm. The conditions of extrusion were as follows: screw speed of 50 rpm and barrel temperature of 155 °C. The residence time of the wheat straw inside the extruder was about 3 min.

### *Washing*

The extruded wheat straw was mixed with distilled water at a wheat straw to water ratio of 1:7 (w/w) for 15 min at a temperature of 65 °C and was subsequently filtered on a 100 mesh sieve to separate the solid extrudate and filtrate. The extrudate was used for enzymatic hydrolysis and the liquid filtrate was analysed.

### *Extraction*

The wheat straw extruded with NaOH concentration of 6% w/w based on o.d. wheat straw was then extracted in glass laboratory bottles in an incubator shaker (ES-20/60 Biosan) at 160 rpm for 30, 60, 120 and 180 min, a temperature of 80 °C and 12.5% w/w of total solids loading. The concentration of NaOH was 0.86% w/w. The mixture was filtered with a 100 mesh sieve to separate the solid extrudate and filtrate. The extrudate was used for enzymatic hydrolysis and the liquid filtrate was analysed.

### *Enzymatic hydrolysis*

The two-stage pretreated wheat straw samples were subjected to enzymatic hydrolysis with a Cellic CTec3 dose of 15% w/w (g Cellic CTec3/100 g cellulose) under the following conditions: 50 °C, pH 5, 72 h and 12.5% w/w of total solids loading. The pH of the samples was adjusted to 5 with 0.05 M citrate buffer containing 0.02% sodium azide to prevent microbial activity during the hydrolysis. At the end of the hydrolysis, each sample was incubated in a water bath at 100 °C for 10 min to inactivate the enzymes, and was then centrifuged (IEC CL 30 Thermo Scientific) at room temperature and 5,000 rpm for 10 min. The supernatant was used for determining the content of monosaccharides.

### *Analytical methods*

The chemical composition of the wheat straw was estimated using the procedure of National Renewable Energy Laboratory.[25] After extrusion and extraction of the wheat straw, the filtrates were subjected to hydrolysis with 4% w/w $H_2SO_4$ for 1 h at 121 °C to convert oligomers into monomers. The monosaccharides (glucose, xylose, arabinose) in the filtrates, extracts and hydrolysates, as well as the formic acid and acetic acid in the filtrates and extracts, after 72 hours of enzymatic hydrolysis, were determined by HPLC with a Rezex ROA $H^+$ column. The mobile phase was 0.005 N $H_2SO_4$ at a flow rate of 0.7 $mL.min^{-1}$ and 30 °C. The samples were filtered through a 22 µm filter. The concentration of lignin in the filtrates and extracts were determined using a Helios β UV-VIS spectrometer. Lignin concentration was calculated in accordance with the Beer-Lambert law, with the extinction coefficient for wheat straw lignin of 280 nm.

The conversion of glucan to glucose in enzymatic hydrolysis was determined by the ratio of glucose concentration that was released during enzymatic hydrolysis to the glucose in the wheat straw and was calculated using the following formula:

$$\text{Glucan conversion (\%)} = \frac{\text{glucose} \times \text{V} \times 0.9}{\text{glucan content} \times m} \times 100 \qquad (1)$$

where glucose is glucose concentration in enzymatic hydrolysis liquor (g/L); V is the volume of enzymatic hydrolysis liquor (L); glucan content in wheat straw, *m* is mass of o.d. wheat straw (g), and 0.9 is the conversion factor for glucose to glucan. The conversion of xylan and arabinan was calculated analogously, using the conversion factor of 0.88 for xylan to xylose and arabinan to arabinose.

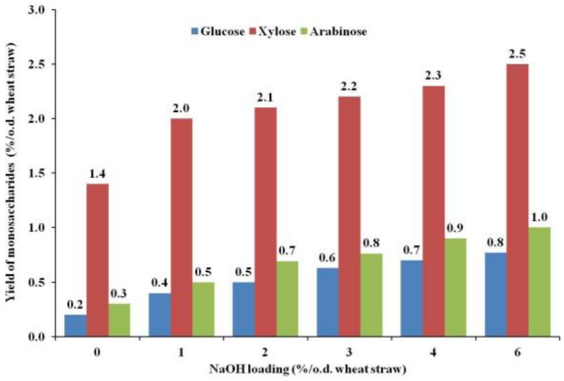


Figure 1: Effect of NaOH concentration in extrusion pretreatment of wheat straw on the yield of monosaccharides in the filtrates after total hydrolysis

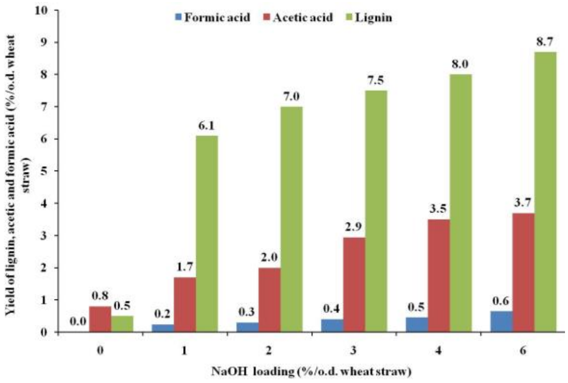


Figure 2: Effect of NaOH concentration in extrusion pretreatment of wheat straw on lignin, formic and acetic acid yields in the filtrates

## RESULTS AND DISCUSSION

### Characterization of filtrates after alkaline extrusion and subsequent extraction

The purpose of wheat straw pretreatment is to remove lignin and hemicelluloses, reduce cellulose crystallinity and increase the porosity of wheat straw. Therefore, the selection of appropriate pretreatment methods plays a significant role in increasing the efficiency of enzymatic hydrolysis.

The aim of this part of the study was to evaluate the influence of NaOH concentration during extrusion of wheat straw on filtrate composition, considering the possibility of using these compounds as by-products from bioethanol production. The filtrates contained a small amount of monosaccharides and, therefore, total hydrolysis of the oligomers was performed. The yield of monosaccharides in the filtrates after total hydrolysis with sulfuric acid is presented in Figure 1. The yields of xylose, glucose and arabinose increased with the increase of NaOH concentration in extrusion. The yield of xylose in the filtrate after extrusion of wheat straw (0% of NaOH concentration) was 1.4%, of arabinose – 0.3% and glucose – 0.2%. The highest yields of xylose (2.5%), arabinose (1.0%) and glucose (0.8%) in the hydrolysed filtrates were achieved with a NaOH concentration of 6% w/w. Xylose yields were significantly higher than those of arabinose and glucose.

Simultaneously with the extraction of xylose, arabinose and glucose oligomers, alkaline extrusion pretreatment of wheat straw resulted in the extraction of lignin. The hydrolysis of the acetyl group present in the hemicelluloses is responsible for acetic acid formation. However, formic acid also forms during this process. These substances inhibit the fermentation process. In Figure 2, the yields of lignin, acetic and formic acid in the filtrates after alkaline extrusion pretreatment of wheat straw are presented. With increasing concentration of NaOH, the yields of all the substances increased. The yields of lignin ranged from 0.5 to 8.7%, those of acetic acid – from 0.8 to 3.7%, and of formic acid – from 0 to 0.6%.

The analysis of the filtrates showed that the addition of NaOH before the extrusion pretreatment of wheat straw significantly increased the solubility of lignin. At a concentration of 6% w/w NaOH, about 53% lignin, 34% arabinan, 9% xylan and 2% glucan were removed. Considering the varied content of lignin during the extrusion pretreatment, alkali concentration has a considerable impact on the lignin solubilization. Compared to acid and hydrothermal processes, mild alkaline pretreatments lead to less solubilization of hemicelluloses and less formation of inhibitory compounds, and can be operated at lower temperatures.[7,10]

The effect of the extraction of alkaline extruded wheat straw, at a concentration of NaOH of 6% w/w, on the filtrate composition was determined. The yield of monosaccharides in the filtrates after total hydrolysis with sulfuric acids is presented in Figure 3. Prolonged extraction times increased slightly the yields of xylose, glucose and arabinose in the hydrolysed filtrates. The yields of xylose increased from 2.5 to 3.8%, the yields of arabinose – from 1.0 to 1.3%, and those of glucose – from 0.8 to 1.1%. A small amount of polysaccharides removed during the extraction resulted in their higher content in the pretreated wheat straw before enzymatic hydrolysis.

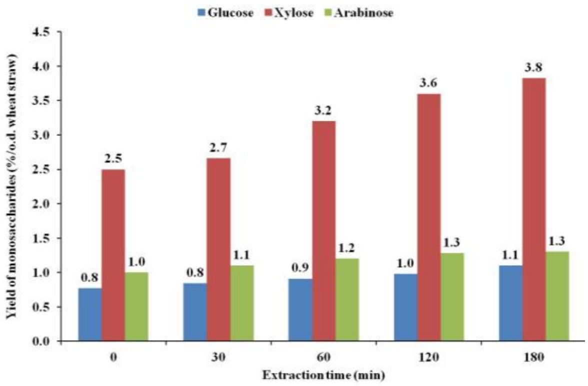


Figure 3: Effect of extraction time of wheat straw extruded with 6% w/w NaOH on the yield of monosaccharides in the filtrates after total hydrolysis

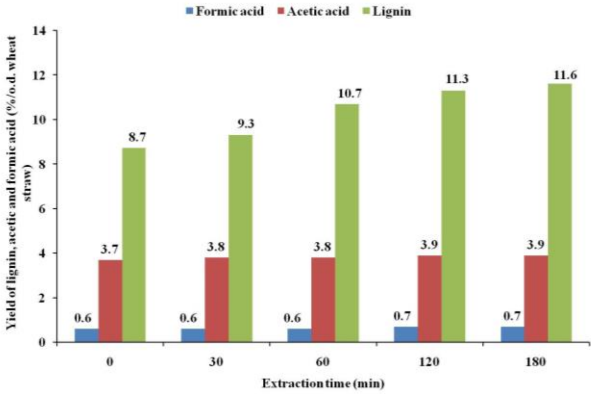


Figure 4: Effect of extraction time of wheat straw extruded with 6% w/w NaOH on lignin, formic and acetic acid yields in the filtrates

In Figure 4, the yields of lignin, acetic and formic acid in the filtrates after extraction of alkaline extruded wheat straw are presented. The yields of all the substances increased slightly with extraction time, but the most notably that of lignin. The yields of lignin ranged from 8.7 to 11.6%, those of acetic acid – from 3.7 to 3.9%, and those of formic acid – from 0.6 to 0.7%. After 180 min of extraction of alkaline extruded wheat straw, 70% lignin, 50% arabinan, 15% xylan and 3% glucan were removed. The extraction of extruded wheat straw increased the yield of xylose by 52%, that of glucose by 37%, of lignin by 33%, of arabinose by 30%, of formic acid by 17%, and of acetic acid by 5.5%.

Under alkaline conditions, the polysaccharides are better preserved than at low pH values, but some degradation also occurs, leading to the formation of carboxylic acids. The yield of lignin increased by 75%, while those of xylose decreased by 80% and of glucose – by 45%, when compared with the composition of the filtrate after the steam explosion of wheat straw at 195 °C.[10] Furfural and hydroxymethyl furfural, which are fermentation inhibitors, were not detected in the filtrates.

Lignin as a by-product in the production of bioethanol can be used in many applications. Its structure, as well as quantity, can be affected by the method of lignocellulosic biomass pretreatment and the method of lignin isolation. The future lignin application is predetermined by its structure and composition. After alkaline extrusion, the filtrates can be recovered to alkali lignin or combusted, resulting in recovering the chemicals and energy using well-developed industrial technology (*i.e.* black liquor evaporation) existing in pulp mills.[26]

The xylan from the wheat straw filtrates can be used for increasing paper strength properties or in the form of xylooligosaccharides for various prebiotic applications. The monosaccharides from the hydrolysed filtrates can also be used for bioethanol production. Acetic acid is an important chemical, used primarily in the production of cellulose acetate for photographic film, polyvinyl acetate for wood glue, and synthetic fibres and fabrics.

**Enzymatic hydrolysis**

Wheat straw is a suitable alternative for industrial production of bioethanol based on its high content of polysaccharides and availability. Enzymatic hydrolysis is the key factor in the process of bioethanol production and remains a major obstacle of the process mainly because of the high costs of enzymes. In order to achieve sufficient enzyme accessibility to wheat straw, a proper pretreatment method is required for disrupting the lignin-carbohydrate linkages, while avoiding the degradation of monosaccharides to furan compounds and carboxylic acids.

Alkali conditions induce swelling of lignocellulosic biomass, leading to an increase of the internal surface area, the disruption of the lignin structure (lignin removal), the reduction of cellulose crystallinity, resulting in improved accessibility of cellulose and hemicelluloses in enzymatic hydrolysis. High compression and shear forces during extrusion cause an increase of the surface area, shortening of fibres and their fibrillation, enabling to obtain homogeneous and clean fibres.[27]

The purpose of alkaline extrusion pretreatment was to remove as much lignin as possible, and partially, hemicelluloses, in order to increase the cellulose content and porosity of wheat straw, which is important for enzymatic hydrolysis. Delignification results in improved enzymatic hydrolysis of lignocellulosic biomass, as lignin removal can significantly enhance the exposure of cellulose to enzymes.[28,29] For optimal ethanol production, the goal is to maximize the release of monosaccharides in enzymatic hydrolysis and their subsequent fermentation to ethanol. Washing alkaline extruded wheat straw with water leads to the removal of a large amount of dissolved lignin and to the complete removal of formic and acetic acids,[23] as they could inhibit the enzymatic hydrolysis and fermentation.

The conversion of polysaccharides represents the effectiveness of lignocellulosic biomass hydrolysis based on its pretreatment and accessibility to enzymes. Under ideal conditions, all the polysaccharides in lignocellulosic biomass are hydrolysed into monosaccharides. Usually, not all polysaccharides can be quantitatively hydrolyzed into monosaccharides. Some polysaccharide chains remain, especially, the least accessible ones. The conversion of glucan, xylan and of total polysaccharides during enzymatic hydrolysis of wheat straw extruded with different NaOH concentration is illustrated in Figure 5. The results suggest that the accessibility of polysaccharides to hydrolytic enzymes increased with increasing NaOH concentration in extrusion pretreatment. The conversion of glucan during enzymatic hydrolysis of extruded wheat straw (0% NaOH) was 40.5%, that of xylan – 27.5% and that of total polysaccharides – 34.8%. The highest conversion of glucan (77.0%), xylan (72.1%) and total polysaccharides (74.6%) was achieved with 6% w/w NaOH concentration. These results are comparable with those obtained in the work of Gigac *et al.* for the conversion of polysaccharides at a NaOH concentration of 6% w/w without washing and after washing extruded what straw.[23] The increase in the recovery of polysaccharides with an increase of NaOH concentration was possible due to high delignification.

The yields of monosaccharides from the raw material also express the efficiency of alkaline extrusion pretreatment. They are also dependent on the enzymatic hydrolysis conditions and the chemical composition of the raw material. In the production of bioethanol, the yield of monosaccharides is an important factor in comparing different raw materials. The effect of NaOH concentration in the extrusion pretreatment of wheat straw on the yield of monosaccharides is presented in Figure 6. With increasing NaOH concentration, the yields of glucose increased from 17.6 to 35.5%, the yields of xylose – from 7.7 to 20.3% and those of total monosaccharides – from 25.8 to 55.4%.

The effect of extraction time of wheat straw extruded with 6% w/w NaOH on the conversion of polysaccharides was determined (Fig. 7). Prolonged extraction time increased the conversion of glucan from 77.0 to 81.2%, that of xylan – from 72.1 to 77.7% and that of total polysaccharides – from 74.6 to 79.0%.

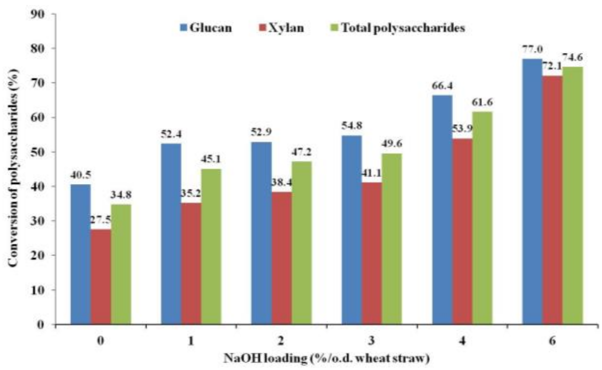


Figure 5: Effect of NaOH concentration in extrusion pretreatment of wheat straw on the conversion of polysaccharides

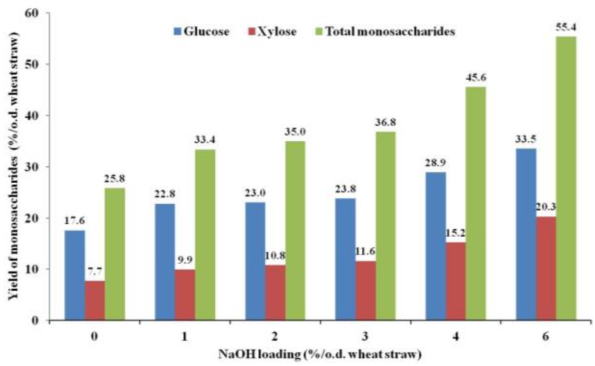


Figure 6: Effect of NaOH concentration in extrusion pretreatment of wheat straw on the yield of monosaccharides

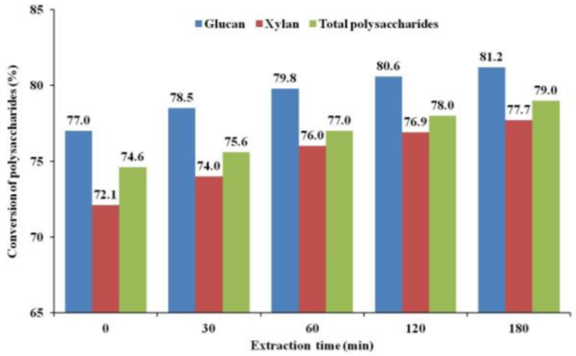


Figure 7: Effect of extraction time of wheat straw extruded with 6% w/w NaOH concentration on the conversion of polysaccharides

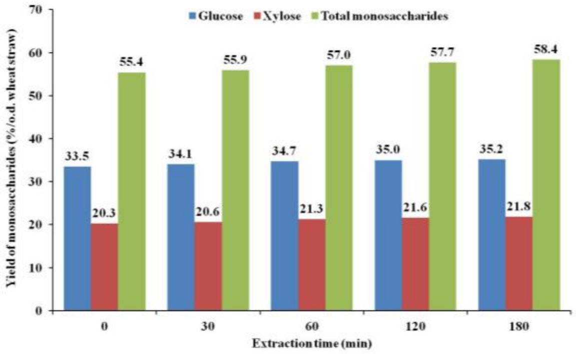


Figure 8: Effect of extraction time of wheat straw extruded with 6% w/w NaOH concentration on the yield of monosaccharides

Figure 8 shows the effect of extraction time after alkaline extrusion on the yield of monosaccharides calculated on oven dry wheat straw. Prolonged extraction time increased the yield of glucose from 33.5 to 35.2%, that of xylose – from 20.3 to 21.8%, and that of total monosaccharides – from 55.4 to 55.8%.

## CONCLUSION

Wheat straw is an abundant and readily available lignocellulosic biomass potentially suitable for the bioethanol production. Alkaline extrusion was investigated as a possibly convenient pretreatment method. When increasing the NaOH concentration in extrusion pretreatment and the extraction time for extruded wheat straw, the yields of lignin, xylose oligomers, arabinose, glucose, acetic and formic acid in the filtrates enhanced. Many of the substances detected in the filtrates are broadly used and thus they contribute to improving the efficiency of bioethanol production. With higher NaOH concentration and extraction time, the lignin removal particularly increased. Under alkaline conditions, polysaccharides are better preserved than under acidic conditions, therefore, neither furfural nor hydroxymethylfurfural was detected in the filtrates.

The NaOH concentration in extrusion pretreatment of wheat straw had a significant impact on the conversion of polysaccharides during enzymatic hydrolysis. Xylan conversion increased more significantly than glucan conversion with increasing NaOH concentration. The conversion of polysaccharides increased only slightly when the alkaline extruded wheat straw was subsequently extracted. With increasing extraction time, the conversion of glucan and xylan increased approximately equally. The results of enzymatic hydrolysis confirmed that the removal of lignin and hemicelluloses has a significant impact on the conversion of polysaccharides.

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