

EFFECT OF PREHYDROLYSIS ON ENZYMATIC HYDROLYSIS OF TWO-STAGE PRETREATED WHEAT STRAW

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ABSTRACT

The effect of water prehydrolysis conditions, which was used as the first stage of wheat straw pretreatment, on the composition of filtrates was analysed. Many of the substances that are present in the prehydrolysis filtrates are broadly used and thus they contribute to improvements in the efficiency of bioethanol production. Prehydrolysis was carried out at temperatures of 140, 160 and 180°C and times of 30, 60 and 120 min. Xylose and its oligomers were the most represented in prehydrolysis filtrates, their yields increased up to 12.1 % with increasing weight of wheat straw removed up to 28.6 %. Besides xylose and glucose, acetic acid, formic acid and the phenolic compounds were also present in the prehydrolysis filtrates. At 160 and 180°C, levulinic acid and furfural and hydroxymethylfurfural, respectively, were also present. The solid fractions of wheat straw were subjected to steam explosion under the same conditions at 200°C for 2 min. Two-stage pretreated wheat straw was subsequently enzymatically hydrolysed. The conditions of water prehydrolysis combined with steam explosion pretreatment had a significant effect on the results of enzymatic hydrolysis of wheat straw. The conversion of cellulose increased with increasing prehydrolysis temperature and time from 64.8 to 91.7 %. Similar results were observed for the conversion of xylan from 73.7 to 92.4 %. With increasing of prehydrolysis temperature and time, the amount of inhibitors in hydrolysates decreased. The recommended conditions for water prehydrolysis at two-stage pretreatment of wheat straw correspond to temperature of 160°C and time of 60 min.

KEYWORDS: Wheat straw, water prehydrolysis, prehydrolysis filtrate, steam explosion, enzymatic hydrolysis, glucose, xylose, inhibitors.

INTRODUCTION

Lignocellulosic biomass, as an important source for production of bioethanol and bioproducts has aroused worldwide interest. It can continuously provide low cost feedstock (Cardona and Sánchez 2007) without disturbing the food supply - a problem associated with conventional biofuels. On the other hand, production of biofuels from lignocellulosic biomass is not yet commercially feasible, due to the low conversion and high feedstock logistics costs. Economics of the process of bioethanol production can be improved by simultaneous utilization of cellulose and hemicellulose (Chandra et al. 2007).

The utilization of all lignocellulose components requires the changes of existing conversion processes, especially the initial pretreatment stage. Previously, the pretreatment process was focused on effective cellulose recovery and a variety of pretreatment methods have been developed including physical, chemical, physicochemical and biological methods and variety of their combinations (Kumar et al. 2009). Unfortunately, none of these methods lead to a high utilization of hemicelluloses (Mosier et al. 2005).

Pretreatment is the most expensive step throughout the conversion process, making up to 40 % of the total processing cost (Eggeman and Elander 2005; Zhang et al. 2009). Since pretreatment is the first stage of the conversion process, it strongly affects energy demand and cost of the downstream stages (Wyman et al. 2005).

To achieve the maximum yield of monosaccharides it has been proposed to divide the pretreatment into separate stages (Nguyen et al. 2000; Kim 2005). The severe pretreatment conditions greatly affect the hydrolysis of lignocellulose components, especially hemicelluloses (Galbe and Zacchi 2007). These severe conditions would cause significant degradation of hemicellulose monosaccharides into inhibitors, while a relatively high degree of severity is still desirable to enhance the enzymatic digestibility of cellulose.

Degradation products of monosaccharides and lignin could present certain levels of inhibitory effects on the fermentation microorganisms (Palmqvist and Hahn-Hägerdal 2000; Klinke et al. 2004). These products counteract the benefit of monosaccharides uptake for ethanol production; thereby they impact the ethanol production rate or reduce the final ethanol yield.

Currently, the choice of a pretreatment technology for a particular biomass depends on the biomass composition and target products (Hu et al. 2008). However, for most biomass types, cellulose, hemicelluloses and lignin account for the major part (Kumar et al. 2009), and it is therefore economic to recover all three components (Hinman et al. 1989). Hence, it is necessary to develop a pretreatment technology with efficient recovery of all major components, and with negligible quantity of inhibitory by-products.

During the pretreatment of biomass, the substances passing into the liquid fraction can be used in various industries. In the case of water prehydrolysis, mainly hemicelluloses, water soluble lignin and extractive substances pass into a liquid fraction. From the hemicelluloses, xylan is the most abundant, which is further depolymerised to the oligosaccharides and monosaccharides and the result of its deacetylation is acetic acid. Formic acid occurs to a lesser extent by the splitting off the formyl functional groups, and greater extend as by-product of hexoses dehydration.

At two-stage pretreatment, the separate first-stage was conducted at low severity for efficient hemicellulose hydrolysis, and another stage under more severe conditions was followed to treat the remaining residue (Taherzadeh and Karimi 2008). The presumption is that each stage of pretreatment has varied preference to treat certain specific components. Many pretreatment methods have been employed including dilute acid hydrolysis, steam explosion and hot water treatment in the acid stage succeeded by ammonia, alkaline peroxide treatment and Organosolv process in the alkaline stage (Pan et al. 2005; Sun et al. 2005; Kim and Lee 2006; Brosse et

al. 2009). Many of them demonstrated significantly improved yields of both cellulose and hemicellulose monosaccharides, and required fewer enzymes for hydrolysis than process of single stage pretreatments. The results showed the effect of pretreatment conditions on the production of important hydrolysis products, but the overall performance is still not well known. In order to understand pretreatment mechanism and enable further improvements of the two-stage pretreatment process further informations are needed.

The goal of this study was to evaluate the effect of prehydrolysis conditions on the composition of wheat straw prehydrolysis filtrates and the enzymatic hydrolysis results of solid fraction, pretreated in the second stage with steam explosion.

MATERIAL AND METHODS

Wheat straw of winter wheat (*Triticum aestivum*) grown in the region Senec, Slovak Republic. Wheat straw contains: cellulose (as glucose) 46.5 %, xylan (as xylose) 26.6 %, arabinan (as arabinose) 1.2 %, acid insoluble lignin 14.8 %, acid soluble lignin 2.4 %, extractives 4.2 % and ash 4.3 %.

Cellic CTec3 is a cellulase and hemicellulase enzyme complex supplied by Novozymes A/S (Bagsvaerd, Denmark) for degradation of lignocellulosic materials to fermentable monosaccharides. Cellic CTec3 contained a minimum of 1,700 BHU (Biomass Hydrolysis Units)/g product.

The first stage pretreatment– water prehydrolysis

Wheat straw (moisture content 10.1 %) was grinded in a blender and sieved to obtain a particle size of around 5-7 mm. The water prehydrolysis experiments were performed in a series of six laboratory autoclaves, each of volume 0.75 l. The autoclaves were filled with 20 g o.d. (oven dry) of wheat straw. The liquor-to-wood ratio was 5:1. The prehydrolysis temperatures were 140, 160 and 180°C and the dwell times at these temperatures were 30, 60 and 120 min. After prehydrolysis, the residual solid fractions and filtrates of wheat straw were separated on a 200 mesh nylon filter.

The prehydrolysis filtrates were collected and stored at 4°C for further analysis, while the residual solid fractions of wheat straw were thoroughly washed with tap water and air-dried for determination of wheat straw weight losses. After determination of solid fraction weight and the solids content, the prehydrolysis yield (%) on raw wheat straw was calculated. The difference between weight of raw and prehydrolysed wheat straw represents the weight loss (Tab.1).

The second stage pretreatment – steam explosion

Steam explosion of prehydrolysed wheat straw (15 % total solids loading) was carried out in a 2 l stainless steel batch reactor (AMAR EQUIPMENT PVT. Ltd., India), in which prehydrolysed wheat straw was loaded at the top and heated to the temperature 200°C. When the pre-set residence time concluded (2 min), the steam-treated wheat straw was released from the reactor by rapid depressurization of vessel. The solids content was determined and the sample of pretreated wheat straw was used for the enzymatic hydrolysis.

Enzymatic hydrolysis

Enzymatic hydrolysis of two-stage pretreated wheat straw samples with Cellic CTec3 dose of 15 % w/w (g Cellic CTec3/100 g cellulose) took place at 50°C, pH 5 and 12.5 % 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 (NREL 2008). The prehydrolysis filtrates were hydrolysed with 4 % H₂SO₄ at 121°C in an autoclave. Monosaccharides (glucose, xylose and arabinose) and inhibitors (formic acid, acetic acid, levulinic acid, furfural and hydroxymethylfurfural) in original and hydrolysed prehydrolysis filtrates, and hydrolysates after enzymatic hydrolysis were determined by HPLC with Rezex ROA H+ column. The mobile phase was 0.005 N H₂SO₄ at a flow rate of 0.7 ml.min⁻¹ at 30°C. The samples were passed through a 22 µm filter before testing. The total phenolic compounds in prehydrolysis filtrates and hydrolysates after 96 hours enzymatic hydrolysis were determined according to a modification of the Folin-Ciocalteu method (Nurmi et al. 1996).

RESULTS AND DISCUSSION

Characterization of wheat straw prehydrolysis filtrates

Water prehydrolysis was used as the first stage at two-stage pretreatment of wheat straw in combination with steam explosion before enzymatic hydrolysis. In the first stage, which is carried out at 140, 160 and 180°C and times of 30, 60 and 120 min, hemicelluloses are hydrolysed and dissolved, in particular to the oligomers and/or monomers. Depending on the temperature and time, certain reactions take place in which acetic acid, formic acid, levulinic acid, furfural, hydroxymethylfurfural (HMF) and phenolic compounds (hydroxybenzaldehyde, vanillin, syringaldehyde, coumaric acid, ferulic acid) can also be formed from monosaccharides and lignin. These compounds inhibit enzymatic hydrolysis, in particular the fermentation in bioethanol production. For this reason, the prehydrolysis filtrates are usually treated separately.

The aim of this part of the study was to evaluate the effect of temperature and time of prehydrolysis on the composition of filtrates in term of use of these compounds as by-products at bioethanol production. In Tab.1, the weight of wheat straw removed in the first stage of pretreatment – water prehydrolysis, are listed. With increasing temperature and time of prehydrolysis, the amount of substances that have passed into the filtrates has significantly increased. Depending upon temperature and time of prehydrolysis, 9.3-37.1 % of wheat straw weight was removed.

Tab.1: Wheat straw weight removed in the first stage pretreatment – water prehydrolysis.

Temperature	Time (min)	Weight removed (%)
140°C	30	9.3
	60	10.6
	120	12.5
160°C	30	18.8
	60	23.6
	120	27.4
180°C	30	28.6
	60	34.8
	120	37.1

The xylose and glucose yields in the original and hydrolysed prehydrolysis filtrates of wheat straw prepared under different conditions were compared (Figs. 1 and 2). The xylose and glucose

yields in original prehydrolysis filtrates prepared at 140, 160 and 180°C were lower than in prehydrolysis filtrates after total hydrolysis with sulfuric acid. Xylose yields were significantly higher than glucose yields.

With increasing temperature at the prehydrolysis time of 30 min the yield of both glucose and xylose increased. The highest yields of xylose (12.1 %) and glucose (1.7 %) in hydrolysed prehydrolysis filtrates were achieved at 180°C. After 60 and 120 min of prehydrolysis, the maximum xylose and glucose yield was achieved at 160°C. Similar glucose yields were achieved at 60 min (1.3 %) and 120 min (1.4 %). At 180°C and times of 60 and 120 min, the xylose and glucose yield decreased, while at the same time the yield of by-products increased, because the monosaccharides in prehydrolysis filtrates were subjected to degradation at high temperatures. The yield of glucose and its oligomers after 120 min of prehydrolysis at 180°C was 0.8 %, which is similar to the yields for 30 and 60 min prehydrolysis at 140°C.

With increasing weight of removed wheat straw (Tab. 1), the xylose and its oligomers yield increased until the removal of about 28 % weight (160°C, 120 min and 180°C, 30 min). The yield of glucose and its oligomers changed analogously depending on the removed weight of wheat straw. However, the yield of glucose and its oligomers was approximately 10 times lower than the yield of xylose and its oligomers (Figs. 1 and 2).

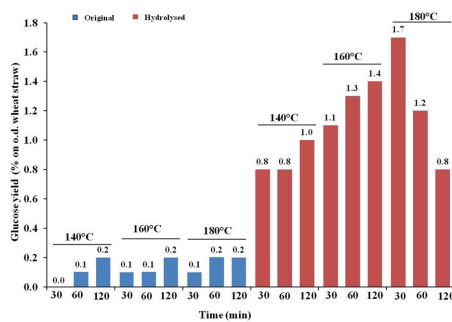
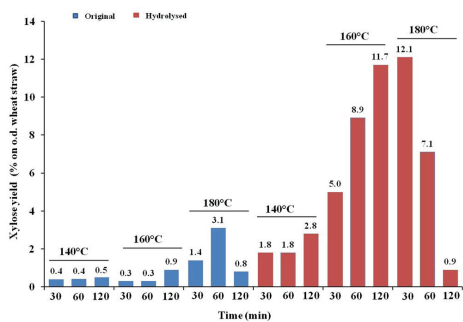


Fig. 1: Comparison of xylose yields in the original and hydrolysed wheat straw filtrates prepared at different temperatures and times of prehydrolysis.

Fig. 2: Comparison of glucose yields in the original and hydrolysed wheat straw filtrates prepared at different temperatures and times of prehydrolysis.

Xylan from the prehydrolysis filtrates of wheat straw can be used for increasing the paper strength properties or in the form of a xylooligosaccharides for various prebiotic applications. Xylose from hydrolysed prehydrolysis filtrates can also be used for bioethanol production, but it is necessary to remove inhibitors.

In Fig. 3 the yields of compounds determined in water prehydrolysis filtrates prepared at temperature of 140°C and times of 30, 60 and 120 min are compared. After total hydrolysis of prehydrolysis filtrates, xylose yields ranged from 1.8 to 2.8 % and glucose yields from 0.8 to 1.0 %. In the prehydrolysis filtrates, acetic acid, formic acid, and phenolic compounds also were present, and phenolic compounds yield was the highest. With prolongation of prehydrolysis time, yields of all compounds increased with highest increase observed for xylose. The yields of formic acid are in the range from 0.1 to 0.2 %, acetic acid from 0.4 to 0.6 %, and the total phenolics from 0.8 to 0.9 %.

In prehydrolysis filtrates of wheat straw, which were prepared at 160°C (Fig. 4), as compared to the prehydrolysis at 140°C (Fig. 3), the levulinic acid was also determined (0.1-0.2 %). Glucose degradation at higher temperature results in HMF formation, which can eventually degrade to

formic acid and levulinic acid. The part of xylose degrades to furfural and thereafter to formic acid. With prolongation of the prehydrolysis time, the yields of all compounds increased, especially xylose (from 5 to 11.7 %). Glucose yield ranged from 1.1 to 1.4 %, formic acid yield from 0.2 to 0.5 %, acetic acid yield from 0.5 to 1.1 % and the yield of total phenolics from 1.5 to 1.8 %.

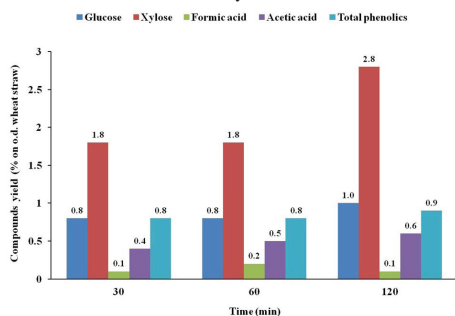


Fig. 3: Comparison of glucose, xylose, formic acid, acetic acid and total phenolics yields in wheat straw prehydrolysis filtrates prepared at temperature of 140°C and times of 30, 60 and 120 min.

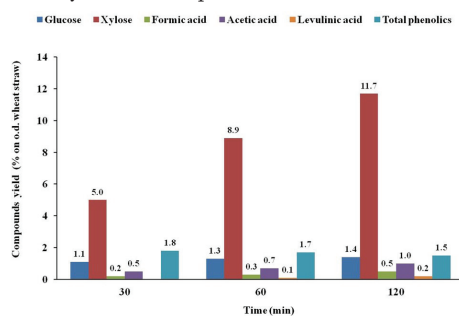


Fig. 4: Comparison of glucose, xylose, formic acid, acetic acid and total phenolics yields in wheat straw prehydrolysis filtrates prepared at temperature of 160°C and times of 30, 60 and 120 min.

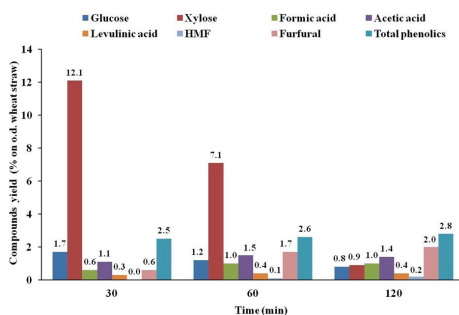


Fig. 5: Comparison of glucose, xylose, formic acid, acetic acid, levulinic acid, furfural, HMF and total phenolics yields in wheat straw prehydrolysis filtrates prepared at temperature of 180°C and times of 30, 60 and 120 min.

The yields of glucose, xylose, formic acid, acetic acid, levulinic acid, furfural, HMF, and total phenolics in the prehydrolysis filtrates of wheat straw prepared at 180°C are compared in Fig. 5. At this temperature, formation of HMF and furfural also result in significantly lower yield - mainly of xylose from 12.1 to 0.9 % and of glucose from 1.7 to 0.8 %. Therefore, in the term of the yield of xylose and its oligomers, the optimal conditions of prehydrolysis are 180°C and time 30 min. The prolongation of prehydrolysis time increased the yield of formic acid from 0.6 to 1 %, acetic acid yield from 1.1 to 1.5 %, levulinic acid yield from 0.3 to 0.4 %, furfural yield from 0.6 to 2 %, HMF yield from 0.1 to 0.2 % and the total phenolics yield from 2.5 to 2.8 %.

Acetic acid can be used directly in the pretreatment of lignocellulosic materials to increase efficiency or in organic synthesis with various applications. This is the case for levulinic acid. Furfural is used as an organic solvent at the production of furfuryl alcohol, methylfuran, tetrahydrofurfuryl alcohol, tetrahydrofuran, 2-furoic acid, drugs, gums, food additives, etc.

HMF is a key intermediate in the synthesis of 2,5-dimethylfuran biofuels as an alternative to bioethanol. Levulinic acid can be used as photosensitizer for photodynamic oncology and phenolic compounds in the production of phenol-formaldehyde or phenol-furan resins.

The highest yields of xylose (12.1 %) and glucose (1.7 %) in the prehydrolysis filtrates were achieved at 180°C and time 30 min with the removal of 28.6 % wheat straw weight. A similar yields were also obtained at 160°C and a time 120 min, the yield of xylose and glucose was 11.7 and 1.4 % respectively, with the removal of 27.4 % wheat straw weight. At 140°C, the prehydrolysis filtrates only contained formic acid, acetic acid and phenolic compounds, besides glucose and xylose and their oligomers. The presence of levulinic acid was observed at a higher temperature of prehydrolysis 160°C, while the furfural and HMF were only formed at 180°C.

Enzymatic hydrolysis of two-stage pretreated wheat straw

The goal of this part of the study was to evaluate the effects of prehydrolysis conditions on hydrolysability of wheat straw pretreated in two-stage process. After separation of the prehydrolysis filtrates and washing, the solid fractions of wheat straw were subjected to steam explosion under the same conditions. Chemical composition of the solid fractions after prehydrolysis varied depending upon the prehydrolysis conditions (Tab. 2). With increasing temperature and time of prehydrolysis, the Klason lignin and cellulose content in solid fractions increased, while xylan content decreased. Subsequently, the two-stage pretreated wheat straw samples were hydrolysed with commercial enzyme agent Cellic CTec3.

Tab. 2: Chemical composition of wheat straw solid fraction after the first stage pretreatment – water prehydrolysis.

Temperature	Time (min)	Ash (%)	*Klason lignin (%)	Acid soluble lignin (%)	Total lignin (%)	Cellulose as glucose (%)	Xylan as xylose (%)
140°C	30	2.4	17.9	1.8	19.7	44.6	25.0
	60	2.2	18.2	1.7	19.9	44.7	24.8
	120	2.4	19.0	1.6	20.6	44.9	24.5
160°C	30	3.5	19.6	1.5	21.1	46.0	23.6
	60	2.7	22.6	1.2	23.8	49.0	16.6
	120	2.5	25.5	1.1	26.6	53.5	9.1
180°C	30	1.7	25.4	1.0	26.4	54.3	11.3
	60	3.0	29.5	1.2	30.7	55.7	6.4
	120	1.8	34.3	1.4	35.7	56.0	4.0

* Klason lignin corrected for ash content.

The influence of prehydrolysis temperature and time on concentration of monosaccharides in hydrolysates of two-stage pretreated wheat straw after 96 hours of enzymatic hydrolysis is presented in Fig. 6. The monosaccharides concentrations in hydrolysates increased with the prehydrolysis temperature from 72.9 to 93.2 g.l⁻¹. This increase was related to the fact that after prehydrolysis at high temperature, the solid fraction contained more cellulose (Tab. 2). The monosaccharides concentration increased with prolongation of prehydrolysis time only at temperatures of 140 and 160°C. At 180°C, the monosaccharides concentration decreased with prolonging of prehydrolysis time. The highest monosaccharides concentration in the hydrolysates of two-stage pretreated wheat straw was achieved after 30 min (93.2 g.l⁻¹), after 60 min the monosaccharides concentration decreased to 90.6 g.l⁻¹, and after 120 min to 87.5 g.l⁻¹, as a result of extending the prehydrolysis time, xylan content was significantly reduced in the solid fractions.

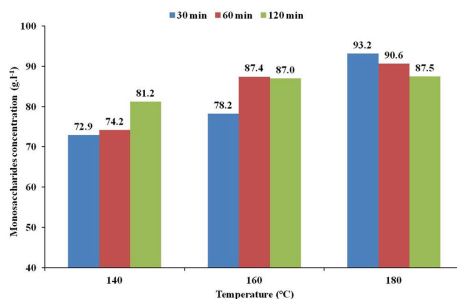


Fig. 6: Monosaccharides concentration in hydrolysates after 96 h of enzymatic hydrolysis of two-stage pretreated wheat straw (prehydrolysis at 140°C, 160°C, 180°C and time of 30, 60 and 120 min – and steam explosion).

Concentration of glucose and xylose provides information about the hydrolysate composition but does not sufficiently clarify the effectiveness of pretreatment. Polysaccharides conversion into monosaccharides directly represents the hydrolysis yield of lignocellulosic raw material, based on pretreatment efficiency and accessibility to enzymes. Not all polysaccharides can usually be quantitatively hydrolysed into monosaccharides and some polysaccharide chains, especially the least accessible ones, remain non-hydrolysed. Under severe pretreatment conditions, a portion of monosaccharides decomposes, resulting in the reduction of the overall conversion.

Based on cellulose and xylan conversions, the impact of prehydrolysis conditions on the accessibility of cellulose and xylan of two-stage pretreated wheat straw to enzymes was evaluated. The conversion of cellulose to glucose increased by increasing the prehydrolysis temperature and time (Fig. 7). At 140°C temperature of prehydrolysis, the conversion of cellulose ranged from 65 to 73.8 %, at 160°C from 73.5 to 84.3 %, and at 180°C from 90.7 to 91.7 %. With the prolongation of prehydrolysis time, the cellulose conversion increased only at temperatures of 140 and 160°C.

Similarly to the conversion of cellulose, xylan conversion to xylose also increased with the temperature and time of prehydrolysis (Fig. 8). At 140°C of prehydrolysis, the conversion of xylan ranged from 73.7 to 74.4 %, at 160°C from 59.5 to 87.9 %, and at 180°C from 81.8 to 92.4 %. With the prolongation of the prehydrolysis time, the xylan conversion increased only at temperatures of 160 and 180°C. With increasing temperature and time of prehydrolysis, the accessibility of wheat straw to enzymes increased as a result of the increases in the amount of removed hemicelluloses (Tab. 1).

The monosaccharides yield calculated on lignocellulosic material also reflects the efficiency of pretreatment but it is also dependent upon enzymatic hydrolysis conditions and lignocellulosic material composition. The monosaccharides yield on prehydrolysed wheat straw increased with rising prehydrolysis temperature from 51 to 65.2 % (Fig. 9).

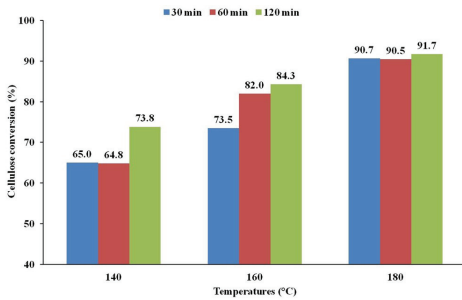


Fig. 7: Cellulose conversion after 96 h of enzymatic hydrolysis of two-stage pretreated wheat straw (prehydrolysis at 140°C, 160°C, 180°C and time of 30, 60 and 120 min and – steam explosion).

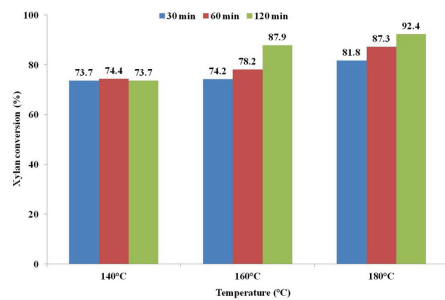


Fig. 8: Xylan conversion after 96 h of enzymatic hydrolysis of two-stage pretreated wheat straw (prehydrolysis at 140°C, 160°C, 180°C and time of 30, 60 and 120 min – and steam explosion).

In terms of bioethanol production effectiveness, the monosaccharides yield from the raw wheat straw is very important (Fig. 10). The highest monosaccharides yields were achieved after prehydrolysis of wheat straw at 140°C (46.3 – 49.9 %), because at this temperature the smallest amount of hemicelluloses was removed. With increasing temperature of prehydrolysis the amount of removed hemicelluloses increased while monosaccharides yields decreased. At prehydrolysis temperature of 160°C, the monosaccharides yields ranged from 44.5 to 46.8 % and at 180°C they ranged from 38.5 to 46.5 %. The monosaccharides yields were lower by 12.5 to 15.5 % than the monosaccharides yields calculated on prehydrolysed wheat straw, depending upon the weight of wheat straw removed during prehydrolysis (Tab. 1).

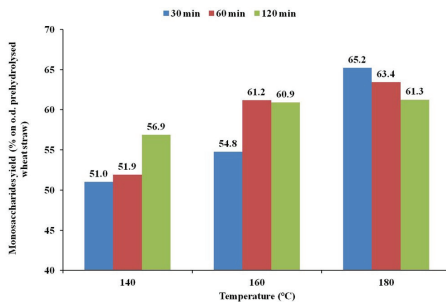


Fig. 9: Monosaccharides yield on prehydrolysed wheat straw after 96 h of enzymatic hydrolysis of two-stage pretreated wheat straw (prehydrolysis at 140°C, 160°C, 180°C and time of 30, 60 and 120 min – and steam explosion).

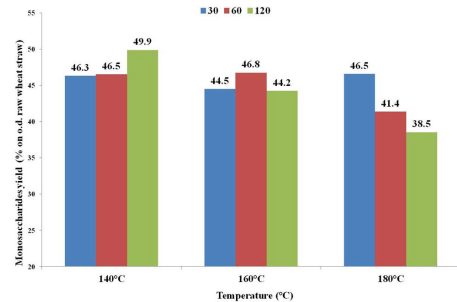


Fig. 10: Monosaccharides yield on raw wheat straw after 96 h of enzymatic hydrolysis of two-stage pretreated wheat straw (prehydrolysis at 140, 160, 180°C and time of 30, 60 and 120 min – and steam explosion).

Prehydrolysed wheat straw was subsequently pretreated by steam explosion at which the partial autohydrolysis of polysaccharides takes place. Subsequently, the decomposition of monosaccharides and lignin resulting in the formation of inhibitors, similarly as in the first stage of pretreatment, i.e. in water prehydrolysis.

The concentration of inhibitors such as acetic acid, formic acid, levulinic acid, furfural and total phenolics was determined in hydrolysates (Fig. 11). The hydrolysates contained mainly

phenolic compounds, the concentration of which ranged from 1.9 to 2.8 g.l⁻¹. The concentration of acetic acid varied from 0.6 to 1.1 g.l⁻¹, for formic acid from 0.2 to 0.8 g.l⁻¹, for furfural from 0.05 to 0.4 g.l⁻¹ and for levulinic acid from 0.03 to 0.2 g.l⁻¹. The concentration of these inhibitors was approximately half of those observed in hydrolysates of wheat straw from steam explosion pretreatment (Russ et al. 2016). This reflects the fact that in the first pretreatment stage (water prehydrolysis) deacetylation of hemicelluloses, removal of easily hydrolysable hemicelluloses and part of lignin occurred. The concentration of phenolics and acetic acid decreased with increasing temperature and time of prehydrolysis.

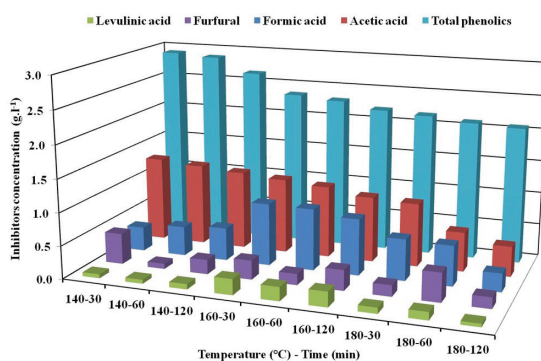


Fig. 11: Inhibitors concentration in hydrolysates after 96 h of enzymatic hydrolysis of two-stage pretreated wheat straw (prehydrolysis at 140, 160, 180°C and time of 30, 60 and 120 min – and steam explosion).

The total amount of inhibitors in hydrolysates of two-stage pretreated wheat straw was reduced with the increase in temperature and time of prehydrolysis, because a large part of xylan and easily degradable lignin were removed in the first stage – water prehydrolysis.

For the evaluation process of enzymatic hydrolysis, the monosaccharides yield calculated on raw wheat straw, the inhibitors concentration in hydrolysates as well as the composition of the prehydrolysis filtrates are very important. At the prehydrolysis temperature of 140°C, the highest monosaccharides yields (46.3 - 49.9 %) were achieved, but the prehydrolysis filtrates after total hydrolysis contained only 1.8 and 2.8 % xylose. At the prehydrolysis temperature of 160°C, the monosaccharides yields were somewhat lower (44.5 - 46.8 %), but the prehydrolysis filtrates after total hydrolysis contained 5.0 to 11.8 % of xylose.

Based on the enzymatic hydrolysis results and the amount of inhibitors in hydrolysates of wheat straw, the optimal prehydrolysis conditions were 160°C and time of 60 min, when the monosaccharides yield was 46.8 %.

CONCLUSIONS

In the first pretreatment stage, specific conditions were used, in which particularly easily extractable hemicelluloses passed to the prehydrolysis filtrate, while in the second stage, the solid fraction of wheat straw was subjected to steam explosion at severe conditions to obtain a material with a good accessibility to the enzymes.

The impact of water prehydrolysis conditions on the composition of the prehydrolysis filtrates and on the results of enzymatic hydrolysis of wheat straw after a two-stage pre-treatment was evaluated. Depending upon the prehydrolysis temperature (140, 160 and 180°C) and time

(30, 60 and 120 min), from 9.3 to 37.1 % weight of wheat straw, especially xylan, passed into the prehydrolysis filtrates. With increasing weight of wheat straw removed in the prehydrolysis, the xylose and its oligomers yield increased up to removal of 28.6 % wheat straw weight. At the prehydrolysis temperature of 140°C, formic acid, acetic acid and phenolics compounds in addition to glucose and xylose were present in the prehydrolysis filtrates. Levulinic acid was determined in the prehydrolysis filtrates at 160°C, while furfural and HMF were created only at 180°C.

The water prehydrolysis conditions, combined with steam explosion pretreatment had a significant effect on the results of wheat straw enzymatic hydrolysis. The monosaccharides concentration in hydrolysates of two-stage pretreated wheat straw increased with prehydrolysis temperature from 72.9 to 93.2 g.l⁻¹, but with increasing of prehydrolysis time, these concentrations only increased at 140 and 160°C. The cellulose conversion increased with increasing of prehydrolysis temperature and time from 64.8 to 91.7 %, the xylan conversion also increased from 73.7 to 92.4 %. The highest monosaccharides yields on raw wheat straw were obtained at prehydrolysis temperature of 140°C (46.3 - 49.9 %), and then at 160°C (44.5 - 46.8 %). The inhibitors concentration in hydrolysates of two-stage pretreated wheat straw decreased with increasing prehydrolysis temperature and time. The recommended conditions for prehydrolysis in the two-stage pretreatment of wheat straw are 160°C and 60 min, when the monosaccharides yield was 46.8 %.

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