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ENZYMATIC HYDROLYSIS OF STEAM EXPLODED STRAW WITH THE ADDITION OF ACETIC ACID

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ABSTRACT

The effect of steam explosion on the enzymatic hydrolysis of straw was investigated in the presence of 5, 10, 15 and 20% wt. addition of acetic acid. Analysis was performed at temperatures of 160, 170, 180, 190, 200 and 210°C. The concentration of monosaccharides obtained after enzymatic hydrolysis was considered the main indicator of the increased availability of cellulose due to their release into the solution. The results indicate that the addition of acetic acid increases the concentration of monosaccharides, but only at lower temperatures. The temperature of 180°C corresponded to the most effective pretreatment by steam explosion in the presence of acetic acid with the highest concentration of 10%, which corresponds to the conversion of polysaccharides to monosaccharides of 74.78%. At high temperatures above 200°C, the addition of acetic acid results in a decrease in the concentration of monosaccharides due to the high severity factor in the range of 3.94 – 4.24.

KEYWORDS:Steam explosion, acetic acid, enzymatic hydrolysis, straw, liquid chromatography, polysaccharides, monosaccharides.

INTRODUCTION

Steam explosion is one of the most used methods for biomass preprocessing (Jiang and Guo 2016, Russ et al. 2016, Pažitný et al. 2019a,b). This is a more effective method than similar steam extrusion (Gigac et al. 2017, Stankovská et al. 2018), or twin-screw extrusion (Thian Hong 2013) or mechanical milling (Jiang et al. 2017). Straw or biomass residues are most often used (Robak and Balcerek 2018, Lübke et al. 2014, Ihnát et al. 2015). Wood

dendromass is also processed (Tengborg et al. 2001, Zhu and Pan 2010, Pažitný et al. 2019c, 2020a, Tengborg et al. 2001), which is morphologically and also specific in its chemical composition (Ihnát et al. 2021). The basic principle is the heating of water-impregnated biomass to a temperature far exceeding 100ºC at high pressure and subsequent decompression of the mixture, whereby the lignocellulosic complex is mechanically disrupted, which leads to a significant improvement in the accessibility of cellulose and hemicelluloses to hydrolytic enzymes (Yang et al. 2011). The temperature during a steam explosion is usually around 200ºC (Pažitný et al. 2020b, 2022b). At higher temperatures, inhibitors of enzymatic hydrolysis such as carboxylic acids, hydroxymethylfurfural and furfural are formed to a greater extent (Russ et al. 2016). The decomposition of acetyl groups leads to the formation of acetic acid, which lowers the pH of the water and under these conditions further hydrolysis of hemicelluloses occurs (Pažitný et al. 2022a).

The addition of dilute acids has a catalytic effect on the steam explosion process, thanks to which it is possible to reduce the retention time and temperature, thereby reducing energy costs and the formation of inhibitors, and at the same time it is possible to almost completely remove hemicelluloses. A common steam explosion procedure uses the addition of 1% sulfuric acid, which increases the total yields of monosaccharides at a lower reaction temperature (Ballesteros et al. 2006, Chundawat et al. 2010). Unlike sulfuric acid, acetic acid is less harmful and, moreover, it is volatile, so it can also be separated by distillation.

The aim of the presented work was to follow up on our previous work (Russ et al. 2016) and evaluate the addition of acetic acid in the higher concentrations during the impregnation of straw with water before steam explosion. The results were evaluated based on the yields of monosaccharides after enzymatic hydrolysis.

MATERIAL AND METHODS

Material and methods

The straw was obtained from the Bratislava region. Ready-to-use stabilized enzyme complex CTec3 from Novozymes A/S (Bagsværd, Denmark) was used. Enzyme activity was measured as 1700 BHU (biomass hydrolysis units)/g product. The straw was pre-processed by mechanical dry grinding to a fraction of 0.7 mm in a Brabender mill (Brabender®, GmbH& Co. KG, Germany). The straw was impregnated with water so that the final relative humidity of the samples during the steam explosion was 85% by weight. Acetic acid was added to the water in volumes of 5, 10, 15 and 20% by weight. The samples were finally pretreated in a 2 L stainless steel batch reactor for steam explosion pretreatment (Amar EquipmentsPvt. Ltd., India). The reactor was set to a temperature of 160, 170, 180, 190, 200 and 210°C. The retention time was 10 min. The samples after the steam explosion were divided into unwashed and washed. The washed samples were rinsed in 2 l of water.

Enzymatic hydrolysis

After pretreatment, the samples were subjected to enzymatic hydrolysis. Enzymatic hydrolysis was carried out using the cellulase complex Cellic® CTec3 (Novozymes) with a recommended dosage of 15% by weight of enzyme suspension (15 g Cellic® CTec3/100 g

suspension). All enzymatic hydrolysis experiments were performed in citrate buffer (0.05 M), the pH of which was adjusted with NaOH solution (10% wt.) to pH = 5.0 at 50°C. After 48 h, the Erlenmeyer flask (250 ml) with the suspension was removed from the incubator. Using plastic pipettes, a volume of 2 ml was taken into plastic cuvettes for a Unipan centrifuge (high-speed centrifuge, type 310). Cuvettes were boiled for 10 min in a water bath to stop enzymatic degradation. The suspension was centrifuged at 5000 rpm for 10 min. High-performance liquid chromatography (HPLC) with a Rezex ROA H+ column was used to determine the content of monosaccharides in the hydrolysates (Sluiter et al. 2008). The mobile phase consisted of a 0.0025 M solution of sulfuric acid in deionized water (Hodge et al. 2008). Samples of neutralized hydrolysates were filtered by pressing through a disposable Teflon filter (MS PTFE, 0.22 μm pore size) using a syringe. After filtration, a volume of 0.5 ml was taken from each sample and diluted with deionized water 20 times to a final volume of 10 ml. The sample prepared in this way was dosed into the column using a 20 μl dosing loop. Analyzes were performed at 30°C and a mobile phase flow rate of 0.5 ml/min. A Shodex RI-101 refractometric detector was used to detect analytes, and the Clarity program from Data Apex was used to collect and evaluate chromatographic records.

Calculation of consistency during enzymatic hydrolysis

Calculations were performed according to Pedersen et al. (2010), Viell et al. (2013) and Chen et al. (2007). The suspension consistency in all experiments was expressed according to the following relationship:

$$w\ (hm.\%) = \frac{m_{LCS\ a.s.}}{m_{LCS\ a.s.}+m_{LCS\ H_2O}+m_{TR}+m_E} \times 100 \qquad (1)$$

where: m_{LCS} as is the mass of lignocellulosic raw material without water content (g); m_{LCSH20} is the mass of water in the lignocellulosic raw material (-); m_{TR} is the mass of added buffer solution (g); m_E is the mass of added enzyme (g).

Calculation of the conversion of polysaccharides to monosaccharides

The conversion of cellulose, xylan or polysaccharides to glucose, xylose or all monosaccharides of the ratios and to the content of cellulose, xylan, polysaccharides (holocellulose) in LCM a.s., denoted Y (wt. %) was expressed according to the equation:

$$Y = \frac{c \times V_H \times 0{,}9}{m_{Pa.s}} = \frac{c \times V_H \times 0{,}9}{m_{LCSa.s} \times OP} \times 100\ \% \qquad (2)$$

where: c is the concentration of monosaccharides determined by HPLC (g.L-1), V_H is the volume of liquid during hydrolysis (L), 0.9 is the factor (anhydro correction), mPa.s. is the mass of cellulose, xylan or polysaccharides (holocellulose) in a.s. lignocellulosic material (g), $m_{LCSa.s.}$ is the mass of a.s. lignocellulosic raw material (g), OP is the content of cellulose, xylan or polysaccharides (holocellulose) in absolutely dry lignocellulosic material (Stankovská et al. 2018).

Severity factor

The severity factor computation for corresponding temperatures and constant retention time was based on the integral form according to Hashemi et al. (2019) and Batista et al. (2019).The dependence of the severity factor value on the temperature at which the experiment was performed is shown in Tab. 1. The experiment used an optimized retention time of 10 min (Ballesteros et al. 2006, Russ et al. 2016, Pažitný et al. 2019a,b).

(3)

$$R_O = \int_0^t exp\left[\frac{T_r - T_b}{14.75}\right] dt$$

where: R_O–is the severity factor, T_r - reaction temperature (°C), T_b base temperature (100°C),t - retention time in biomass hydrothermal reaction (min), 14.75 is an empirical temperature value based on the conventional activation energy assuming first order kinetics ($kJ \cdot mol^{-1}$).

Tab. 1: Dependence of the severity factor values on temperature.

Temperature T (□)	Retention time t (min)	Severity factor (-)
160	10 min	2.77
170		3.06
180		3.36
190		3.65
200		3.94
210		4.24

RESULTS AND DISCUSSION

Generally, LCM are composed of cellulose (35-50%), hemicelluloses (20-35%), lignin (15-20%), ash, and other components (15-20%) (Mood et al. 2013). Cellulose and hemicelluloses in the LCM create a complex that is an obstacle to enzyme access under natural conditions. Moreover, the cellulose-hemicellulose complex is encapsulated by lignin, which increases the biomass hydrolysis barrier. In order to obtain fermentable sugars, pretreatment of LCM before enzymatic hydrolysis is needed to decompose the lignin-cellulose-hemicellulose complex in macroscopic, submicroscopic, and microscopic structure (Chundawat 2011, Meng et al. 2014).

Biomass pretreatment and cellulose release are accompanied by physical and chemical processes that bring both desirable and undesirable effects on this process (Palmqvist et al. 1996). While the efficiency of biomass pretreatment increases with the degree of disruption of the lignocellulose complex and the release of cellulose and hemicellulose fibers to hydrolytic enzymes, excessively “harsh” conditions cause significant formation of inhibitors, decomposition of less thermally stable hemicelluloses and, in extreme cases cellulose may also begin to decompose itself, which in the end reduces the amount of polysaccharides that can be depolymerized into soluble and fermentable monosaccharides (Kim et al. 2011). In our study, we focused on the pretreatment of straw by steam explosion using water and aqueous acetic acid solutions in the temperature range from 160°C to 210°C. The results of the concentration of monosaccharides in the hydrolysates of unwashed and washed samples after

steam explosion depending on the concentration of acetic acid and temperature are summarized in Tab. 2.

Tab. 2: Concentration of monosaccharides (g/l) from enzymatic hydrolysis of straw after steam explosion with the addition of acetic acid. The table shows mean values.

	unwashed (g/l)					**washed (g/l)**				
	0%	**5%**	**10%**	**15%**	**20%**	**0%**	**5%**	**10%**	**15%**	**20%**
	Enzymatic hydrolysis 48 hod									
no STEX	29,9 g/l									
	(3,1)									
160□	38.9	50.4	54.8	60.7	62,3	47.1	63.1	71.6	81.6	93.1
	(3.2)	(4.1)	(3.9)	(4.0)	(5.1)	(3.7)	(4.5)	(4.0)	(4.1)	(4.2)
170□	53.8	62.3	66.1	64.1	63.4	61.8	77.5	85.3	94.8	94
	(3.8)	(4.9)	(4.8)	(5.1)	(4.9)	(3.9)	5,0()	(4.9)	(5.2)	(4.6)
180□	59.4	73.6	76.7	65.1	61.5	68.1	88.5	98.6	101.4	97.9
	(4.2)	(4.6)	(5.2)	(5.1)	(4.3)	(4.2)	(5.6)	(5.2)	(5.3)	(5.1)
190□	64.5	72.7	74.2	64.4	60.6	84.5	96	100.9	94	85.5
	(3.9)	(4.2)	(5.1)	(4.7)	(4.4)	4,1()	(4.8)	(5.7)	(5.1)	(4.9)
200□	68	60.3	56	48.4	44.4	82.8	85.1	86.8	68.9	47.8
	()	(4,5)	(4.4)	(5.1)	(4.8)	(4.2)	(4.6)	(4.9)	(4.7)	(4.4)
210□	79.9	43.2	27.1	22.6	16.3	85	72.5	55.4	43.1	30.4
	(3.9)	(4.4)	(4.7)	(4.8)	(4.7)	(3.9)	(4.2)	(4.8)	(4.4)	(4.5)

*Standard deviations are given in parentheses (%).

The results showed that at lower steam explosion temperatures, in the presence of acetic acid significantly greater amount of monosaccharides was released into the solution than without its addition. While acetic acid at lower concentrations increased the efficiency of the lignocellulose structure disruption, we also conducted an experiment where 50% acetic acid already had a destructive effect on the entire lignocellulose complex, and the sample burned on the walls of the container. The highest value achieved of 76.7 g/l corresponds to the addition of 10% wt. acetic acid. This concentration was achieved at 180□. The highest monosaccharide concentration in the case of unwashed monosaccharides of 101.4 g/l was obtained at 180°C at a concentration of 15% acetic acid. In the case of washed samples, the monosaccharide concentration values were significantly higher. With further increase in temperature, the monosaccharide concentration decreased. Compared to the concentrations achieved in the steam explosion of straw impregnated with water only (Russ et al. 2016), the optimal pretreatment temperature is reduced by 20□.

The trend of the effect of added acetic acid on the pretreatment efficiency based on the concentration of glucose and xylose is shown in Fig. 1 for an unwashed straw sample. Fig.1a shows a significant increase in glucose concentration in the case of the addition of acetic acid by approximately 10% in the temperature range of 160-190°C. The maximum glucose concentration is reached at 5-10% acetic acid and a temperature of 180-190°C, where the curves corresponding to these concentrations of added acetic acid reach a maximum. Above 190°C, the glucose concentration decreases in case of all concentrations of added acetic acid, with the rate of decline increasing with the concentration of added acetic acid. In case of pure water, the glucose concentration continues to increase and reaches its maximum at 210°C of 66.2 g/l.

In the case of xylose concentration in Fig. 1b the situation is different. While at 160°C the xylose concentration increases along with the concentration of added acetic acid, at higher temperatures the xylose concentration begins to decrease at 15% to 20% acetic acid, with the decrease in xylose being more moderate at 15%. The 5-10% addition of acetic acid at the results in an increase in xylose concentration up to approximately 175°C, where maximum values are reached and with further increase in temperature there is a sharp decrease in concentration. At 210°C the destructive effect of acetic acid is becoming so intense that the xylose values in all cases become almost identical.

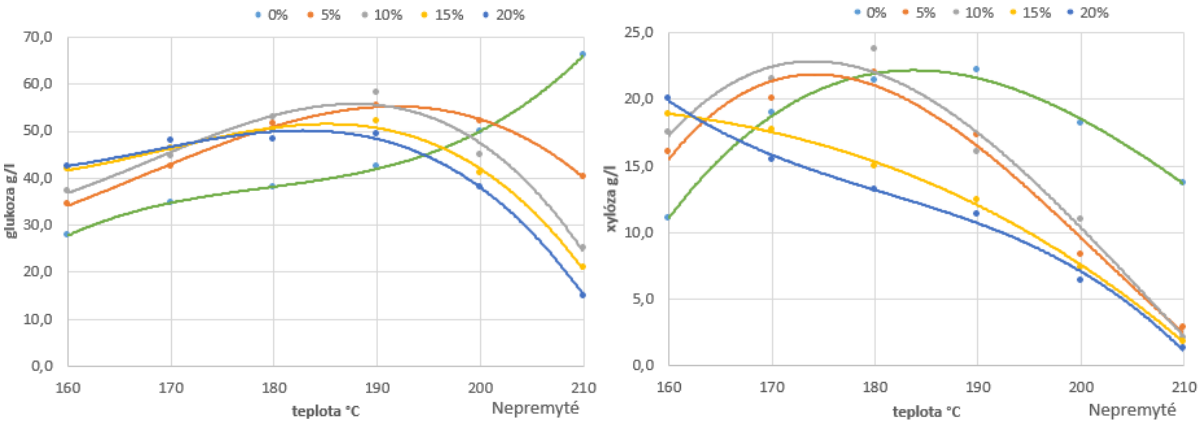

Fig. 1: Concentration of glucose (a; g/l) and xylose (b; g/l) from enzymatic hydrolysis of an unwashed straw sample after steam explosion with the addition of acetic acid.

In the case of steam explosion in pure water, the maximum value of the xylose concentration is reached at approximately 185°C. With increasing temperature, the xylose concentration in water decreases, however the decline is much slower than in the case of added acetic acid. Fig. 1 shows that with increasing temperature, acetic acid becomes destructive to polysaccharides in lignocellulosic material at higher concentrations, but due to the higher thermal stability of cellulose compared to hemicelluloses, the loss of xylose at higher acetic acid concentrations is more pronounced compared to the loss of glucose.In the case of washed samples, the concentration of glucose and xylose as a function of temperature and the concentration of added acetic acid is shown in Fig. 2.

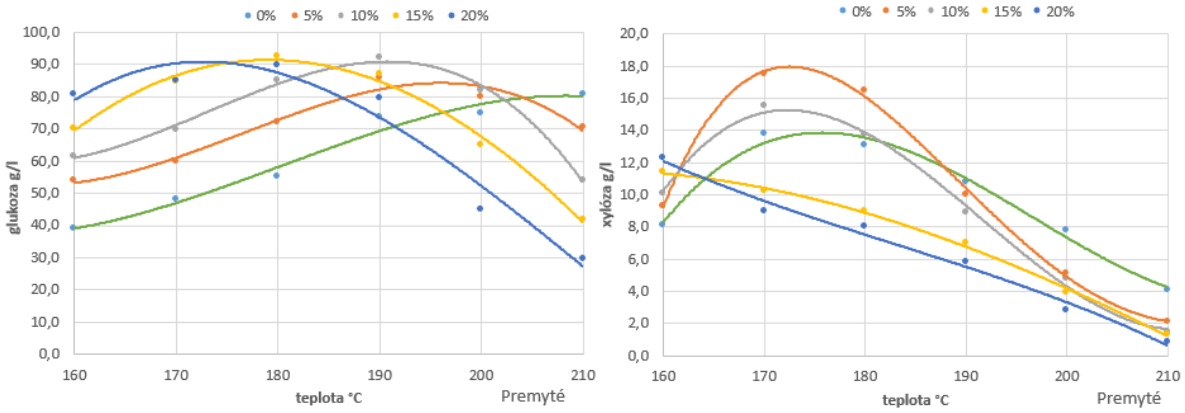

Fig. 2: Concentration of glucose (a; g/l) and xylose (b; g/l) from enzymatic hydrolysis of washed straw sample after steam explosion with the addition of acetic acid.

The glucose concentration in hydrolysates depends on the addition of acetic acid more of washed samples than in the hydrolysates of unwashed samples. At 160°C, the lowest glucose concentration corresponds to steam explosion in pure water at the level of 39 g/l and gradually rises to above 81 g/l at a concentration of 20% acetic acid. With increasing temperature, the glucose concentration values initially increase uniformly at all glucose concentrations, but eventually reach a maximum and with further increase in temperature, the glucose concentration values record a gradual decrease. The maximum glucose concentrations are reached most quickly in the case of the highest addition of acetic acid and the maxima shift to higher temperatures with decreasing acetic acid concentration as follows. Steam explosion hydrolysates in 20% acetic acid solution reach maximum glucose concentration at 170°C, in 15% solution at 180°C, in 10% solution at 190°C, in 5% solution at 195°C and in pure water at 210°C. The highest glucose values in the maxima exceed 90 g/l with additions of 10-20% acetic acid, while at higher temperatures in 5% acetic acid and in pure water these values are lower, at the level of 80-85 g/l. In the case of xylose, similar dependences were achieved for washed samples as for unwashed samples. At 160°C, xylose concentrations in hydrolysates increased with increasing acetic acid concentration. With increasing temperature, the xylose concentration began to decrease rapidly at 15% and 20% acetic acid, while at 0-10% acetic acid it continued to increase and reached a maximum at approximately 175°C. At temperatures higher than 175°C, the xylose concentration decreased at all acetic acid concentrations, but most slowly in the case of pure water. The highest xylose concentration in the hydrolysates of washed samples was recorded during steam explosion in an environment of 5% acetic acid, at a level of 17.5 g/l.

Conversion of polysaccharides to monosaccharides

Conversion of polysaccharides to monosaccharides was calculated according to Eq. 2. The highest conversion of 74.78% of polysaccharides to monosaccharides in unwashed samples was achieved at180□ in 10% acetic acid. In washed samples, high conversions of 98.87% and 98.38% were achieved under conditions of 180□ (15%) and 190□ (10%), respectively. The highest achieved conversion to monosaccharides greatly exceeds the conversion under given conditions without the presence of acetic acid (Russ et al. 2016). On the other hand, it should be noted that higher conversions that can be achieved at higher temperatures in the absence of acetic acid are not possible with its presence.

CONCLUSIONS

The addition of acetic acid had a positive effect on the efficiency of wheat straw pretreatment by steam explosion at a temperature of 180°C. At the lowest determined temperature, the efficiency of pretreatment was the highest at 10% acetic acid addition, corresponding to the highest concentrations of glucose and xylose, while at increasing temperature, the destructive effect of higher concentration of acetic acid became quite significant, which implies that higher additions at standard steam explosion temperatures are inappropriate. The optimal pretreatment temperature was therefore reduced by 20°C as

a result of adding acetic acid as compared to the results by Russ et al. (2016). The most significant effect on the increased efficiency of wheat straw pretreatment was shown in case of washed samples, where at lower temperature the glucose concentration in hydrolysates with higher addition of acetic acid was approximately twice than that in pure water. The results show that lower concentrations of acetic acid significantly reduce the steam explosion temperature of wheat straw pretreatment while achieving the same pretreatment efficiency, while the use of acetic acid for steam explosion at higher temperatures quickly acquires a significantly adverse effect.

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