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EFFECT OF STEAM EXPLOSION TEMPERATURE ON WHEAT STRAW ENZYMATIC HYDROLYSIS

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ABSTRACT

Wheat straw is an abundant and readily available lignocellulosic material potentially suitable for the second generation bioethanol production. Steam explosion was investigated as a suitable pretreatment method. Effect of steam explosion temperature on wheat straw enzymatic hydrolysis was investigated. Optimum steam explosion temperature at around 200°C was determined based on concentration of monosaccharides in hydrolysates, conversion of cellulose and xylan and yield of monosaccharides from wheat straw. This corresponds to creating conditions resulting in sufficient damage to the lignocellulose structure which leads to higher enzyme accessibility. Lower temperature does not enable sufficient enzyme accessibility while excessively high temperature results in significant breakdown of monosaccharides and lignin and creation of inhibitors. The amount of originated inhibitors was also determined for each studied steam explosion temperature.

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

INTRODUCTION

Bioconversion of lignocellulosic biomass to ethanol is one of the most important alternatives to petroleum based liqued fuels. Lignocellulosic biomass includes agriculturel and forestry residues, portions of municipal solid waste, and herbaceous and woody crops. These raw materials are highly abundant, have high energy potential and low cost for ethanol production. Furthermore, lignocellulosic biomass can be produced in many regions of the world that do not have much petroleum.

Wheat straw is one of the most abundant crop residues, and it seems to be one of the cheepest and the most useful raw material for ethanol production (Tabka et al. 2006). Wheat straw is composed of a mixture of cellulose and hemicelluloses (45 and 30 %, respectively) that are bond to lignin (approx. 20 %) by hydrogen and covalent bonds. The lignocellulosic nature of wheat straw makes the pretreatment an essential step of bioethanol production technology because physical and chemical barriers caused by the close association of main components greatly limits the saccharification of cellulose and hemicellulose.

There are several possibilities to increase efficiency of cellulose and hemicellulose conversion to monosaccharides, i.e. better pretreatment method (Yang and Wyman 2008, Alvira et al. 2010), optimal enzyme complex composition and hydrolysis conditions (Ferreira et al. 2009, Zhou et al. 2009, Zhang et al. 2010, Banerjee et al. 2010, Alvira et al. 2011) or addition of proteins, surfactants and other chemicals (Sipos et al. 2011).

A number of pretreatment methods have been developed and evaluated for bioconversion of wheat straw (Talebnia et al. 2010). Among pretreatment processes of lignocellulosic biomass, steam explosion has been extensively studied and considered as one of the most successful method for fractionating biomass and enhancing the accessibility of cellulose to enzyme. Steam explosion has been proved to be effective in a great variety of lignocellulosic biomass (Negro et al. 2003, Emmel at al. 2003). Steam explosion can be carried out at shot resident time and relative lower reaction temperature with or without the addition of acid catalysts such as dilute H_2SO_4 or SO_2 (Linde et al. 2008, Őhren et al. 2007, Chandra et al. 2007, Schell et al. 1991).

In order to achieve efficient hydrolysis of hemicelluloses, and improving the accessibility of cellulose by enzymes steam explosion conditions have been optimized. Most previous investigations of wheat straw by steam explosion were carried out in batch reactors (Ballesteros et al. 2006, Linde et al. 2008). Afterwards a continuous steam explosion device was developed.

The aim of this study was to determine the optimum steam explosion temperature for effective hydrolysis of wheat straw and to compare yield of monosaccharides from original and washed samples without hemicelluloses and inhibitors which formed during steam explosion.

MATERIAL AND METHODS

Wheat straw of winter wheat (*Triticum aestivum*) variety Evina grown in the region Senec, Slovak Republic. Wheat straw contains: cellulose (as glucose) 46.5 %, hemicellulose (as xylose) 26.6 %, acid insoluble lignin 14.8 %, acid soluble lignin 2.45 %, extractives 5.1 % and ash 4.3 %.

Cellic CTec2 is an enzyme complex supplied by Novozymes A/S (Bagsvaerd, Denmark) for degradation of lignocellulosics to fermentable sugars. The enzyme activity was measured to be 122 FPU/g in our laboratory.

Steam explosion pretreatment

Wheat straw was grinded in a blender and sieved to obtain a particle size of around 5-7 mm. Before pretreatment, straw particles (50 g o.d.) were impregnated in water (5 L) for 60 min. The pretreatment was carried out in a 2 L stainless steel batch reactor (AMAR EQUIPMENT PVT. Ltd., India), in which the impregnated straw was loaded at the top and heated to the the required temperature (175, 195, 215 a 230°C). When the pre-set residence time concluded (10 min), the steam-treated biomass was released from reactor by rapid depressurization of vessel. The treatment results in substantial breakdown of lignocellulosic structure, hydrolysis of the hemicellulosic fraction, depolymerization of the lignin components and defibrations (Cara et al. 2006). After pretreatment, the pretreated wheat straw was labelled as original sample (O) and part of pretreated wheat straw was washed with warm water (65°C), separated by filtration as washed samples (W) without hemicelluloses and inhibitors. Original (O) and washed (W) samples of pretreated wheat straw was stored in a freezer before enzymatic hydrolysis.

Enzymatic hydrolysis

Enzymatic hydrolysis of original sample (O) and washed sample (W) with Cellic CTec2 dose of 15 % w/w (g-Cellic CTec2 /100g cellulose) took place at 50°C, pH 5 and 12.5 % of total solid loading. The pH of original samples (O) were adjusted with NaOH before hydrolysis and pH of washed samples (W) were adjusted to 5 with 0.05 M citrate buffer. The hydrolysate samples were sampled after 6, 24, 48, 72 and 96 hours.

Analytical methods

Chemical composition of wheat straw was estimated using the procedure of National Renewable Energy Laboratory (NREAL 2008). Monosaccharides (glucose and xylose) and inhibitors (formic acid, acetic acid, levulic acid, furfural and hydroxymethylfurfural) were determined in hydrolysates by HPLC with Rezex ROA H^+ column. The mobile phase was 0.005 N H_2SO_4 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 concentration of inhibitors was determined in hydrolysates of original samples (O) after 96 h of enzymatic hydrolysis. The amount of total phenolic compounds in hydrolysates after 96 h was determined according to a modification of the Folin-Ciocalteu method (Nurmi et al. 1996). Every test was conducted in duplicate.

RESULTS AND DISCUSSION

Wheat straw is a suitable alternative for industrial production of bioethanol based on their high polysaccharides content and availability. Enzymatic hydrolysis is the key factor in this process of bioethanol production and remains a major obstacle of the process mainly due to high enzyme cost. In order to achieve sufficient enzyme accessibility to wheat straw, proper pretreatment method is required for disruption of the lignin-carbohydrate linkages while avoiding degradation of monosaccharides to furan compounds and carboxylic acids. In this study, relationship between the steam explosion temperature and yield of monosaccharides from original (O) and washed (W) wheat straw samples has been investigated. In this way conditions were simulated which are used in technological process of bioethanol production. Either the whole sample or just the solid part of the sample undergoes enzymatic hydrolysis. In the latter case the filtrate can be used for production of valuable products or be added to the hydrolysate of solid part for additional hydrolysis. The wheat straw samples impregnated with water were being treated with steam explosion at a range of temperatures between 175 and 230°C before subjected to enzymatic hydrolysis.

Kinetics of hydrolysis

Glucose concentration determined in hydrolysates of original (O) and washed (W) pretreated wheat straw is illustrated in Fig. 1. Dependence of xylose concentration and hydrolysis time is illustrated in Fig. 2. This dependance is analogical to that of glucose concentration.

Concentration of both glucose and xylose reached high values after the first 24 hours of hydrolysis and concentration of these monosaccharides only slightly increased with additional hydrolysis. In our experiments high dosage of Cellic CTec2 was used in order to determine the maximum enzyme accessibility after lignocellulosic material pretreatment. Due to this reason, high initial monosaccharide concentration was achieved after a short time of hydrolysis. After 48 hours the concentration of monosaccharides reaches values which are only slightly different than values obtained after 96 hours of hydrolysis. The same trend was observed for both original (O) and washed (W) samples pretreated with steam explosion at all used temperatures.

There is a steady increasing trend of glucose concentration in hydrolysates with increasing steam explosion temperature (Fig. 1). In general, glucose concentration in hydrolysates of original samples (O) was higher than that of washed samples (W). Exception was in case of samples pretreated with steam explosion at the highest temperature of 230°C. Interestingly, in this case concentration of glucose in hydrolysates of washed samples (73.5 g/L) was higher than in that of original samples (67.4 g/L).

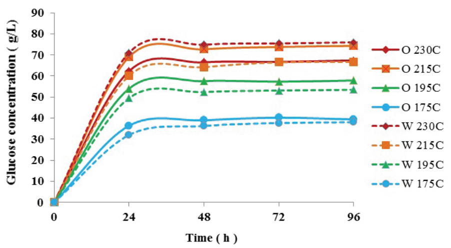

Fig. 1: Effect of steam explosion temperature on glucose concentration in hydrolysates of original (O) and washed (W) wheat straw samples.

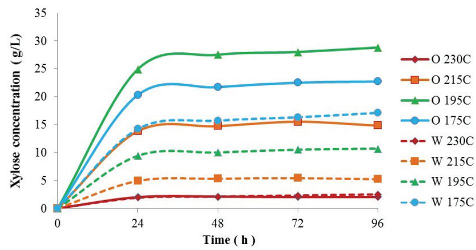

Fig. 2: Effect of steam explosion temperature on xylose concentration in hydrolysates of original (O) and washed (W) wheat straw samples.

As in case of glucose, in general concentration of xylose in hydrolysates (Fig. 2) of original samples (O) is higher than that in washed samples (W). An exception is again in case of samples pretreated at 230°C where there is barely any difference in xylose concentration. Overall, the differences in xylose concentration between original (O) and washed (W) samples are greater than those in glucose concentration (Fig. 1). Unlike glucose, xylose concentration in hydrolysates of original and washed samples posesses a different trend based on different temperature of steam explosion. Between 175 and 195°C xylose concentration rises and reaches maximum values 28.8 g/L in case of original samples (O) and 10.7 g/L in case of washed samples (W) whereas between 195 and 230°C xylose concentration decreases significantly to concentration values of only 2.5 g/L.

In general, increasing steam explosion temperature during pretreatment resulted in increased glucose concentration in hydrolysates. Glucose concentration was higher in hydrolysates of original samples as compared to those of washed samples except for samples pretreated at the highest temperature. Xylose concentration in hydrolysates of original samples was significantly higher than in those of washed samples. In general xylose concentration in hydrolysates of both original and washed samples decreased with increasing steam explosion temperature. In case of the highest steam explosion temperature xylose concentration in hydrolysates of both original and washed samples was very low.

From the fermentation aspect it is important to determine the total content of fermentable monosaccharides. In case of wheat straw, the total content consisted of summary of glucose and xylose and is illustrated in Fig. 3. The total concentration of fermentable monosaccharides is also influenced by concentration of xylose, especially in case of wheat straw pretreated at lower temperature of steam explosion. After sufficient hydrolysis time (the differences in total monosaccharide concentration does not change much after 48 h), the values are the lowest for 175°C at 62 g/L. The highest concentration of monosaccharides in hydrolysates of original samples (O) pretreated at 195 and 215°C were 85-90 g/L. These temperatures are sufficiently high to alter lignocellulosic material which enables sufficient accessibility. The highest concentration of monosaccharides in washed samples (W) was reached at 230°C (76.0 g/L).

In order to determine the optimum temperature of steam explosion, dependence of steam explosion temperature on concentration of monosaccharides in hydrolysates of original (O) and washed (W) wheat straw samples after 96 h hydrolysis time was made (Fig. 4). Increasing temperature of steam explosion results in higher concentration of monosaccharides. Concentration of monosaccharides in hydrolysates of original samples reaches its maximum at around 200°C. These dependencies suggest that the optimum temperature of steam explosion pretreatment of wheat straw is around 200°C where after hydrolysis of original samples the highest resulting monosaccharide concentration occurs. While accessibility of lignocellulosic material after pretreatment with steam explosion at this temperature is very high, monosaccharide breakdown to a series of inhibitors is still sufficiently low in these conditions. Unlike hydrolysis of original samples, in case of hydrolysis of washed samples (W), concentration of monosaccharides rises with increasing temperature and reaches the maximum at 230°C. Although concentration of monosaccharides in hydrolysates of washed samples continues to rise throughout the interval of steam explosion temperature, its maximum value is still significantly lower than that obtained for 195°C and 215°C in case of original samples.

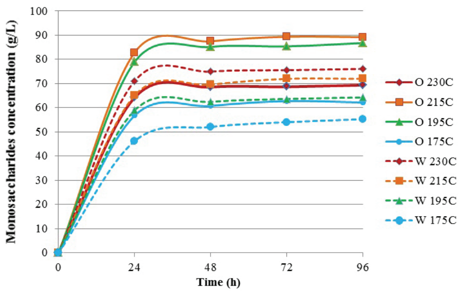

Fig. 3: Effect of steam explosion temperature on monosaccharides concentration in hydrolysates of original (O) and washed (W) wheat straw samples.

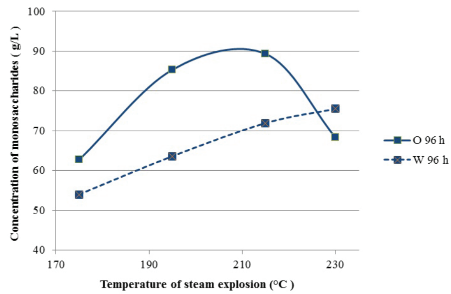

Fig. 4: Dependence of monosaccharides concentration in hydrolysates of original (O) and washed (W) wheat straw samples on steam explosion temperature after 96 h hydrolysis time.

Conversion of cellulose and xylan

Concentration of glucose and xylose provides information on hydrolysate composition but does not sufficiently clarify the effectivity of pretreatment on hydrolysis. Polysaccharides conversion directly represents effectivity of lignocellulosic material hydrolysis based on its pretreatment and accessibility to enzymes. In ideal conditions all polysaccharides in lignocellulosic material are hydrolyzed into monosaccharides. Usually not all polysaccharides can be quantitatively hydrolyzed into monosaccharides and some polysaccharide chains, especially the least accessible ones, remain. At higher steam explosion temperature, part of polysaccharides is already reduced to monosaccharides but in these harsh conditions monosaccharides decompose, lowering the overall conversion. Dependence of cellulose, xylan and polysaccharides conversion on temperature of steam explosion is illustrated in Fig. 5.

The results suggest that accessibility of polysaccharides to cellulase complex was higher at higher temperatures. Conversion of cellulose was somewhat higher in case of original samples but both original (O) and washed (W) samples exhibited rising trends of cellulose conversion with increasing temperature of steam explosion. The highest cellulose conversion of 97 % was reached in case of original samples (O) pretreated by steam explosion at 215°C. Further increase of steam explosion temperature to 230°C results in partial decomposition of newly formed monosaccharides which corresponds to increased levels of inhibitors. To the contrary the highest cellulose conversion (also 97 %) was achieved for washed samples at 230°C.

Xylan conversion to xylose was much lover in general mostly because of hydrolysis with cellulase comples which contains only low hemicellulase suitable for hydrolysis of xylan. In case of xylan conversion of original samples (O) conversion rises between 175 and 195°C and reaches a maximum conversion of 67.1 %. As the steam explosion temperature increases above 195°C, xylan conversion values decrease and reach only about 5 %, in consequence of xylose decomposition. In case of washed wheat straw samples (W), xylan conversion is much lower than in case of original samples (O) and decreases exhibits a steady decreasing trend with increasing temperature. At 175°C xylan conversion of washed wheat straw samples was 40 % and decreases to about 5 % at 230°C. Xylan conversion was much lower in washed samples (W) because of removal of water-soluble hemicelluloses prior to hydrolysis. Decrease of xylan conversion at higher steam explosion temperature is also related to relatively low thermal stability of xylan as compared to cellulose.

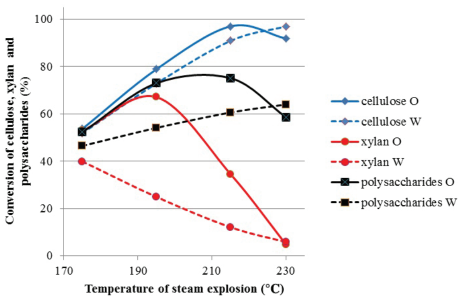

Fig. 5: Effect of steam explosion temperature on conversion of cellulose, xylan and polysaccharides of original (O) and washed (W) wheat straw samples.

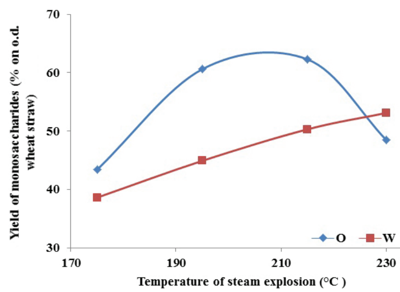

Fig. 6: Effect of steam explosion temperature on monosaccharides yield from original (O) and washed (W) wheat straw samples.

Yield of monosaccharides

Yield of monosaccharides of raw material also expresses the effectivity of pretreatment with steam explosion but is also dependent on enzymatic hydrolysis and chemical composition of lignocellulosic raw material. In this study yield of monosaccharides from original (O) and washed (W) wheat straw samples has been compared at various temperatures of steam explosion pretreatment. The results are illustrated in Fig. 6.

Yield of monosaccharides of raw material from original wheat straw samples (O) increases with increasing temperature of steam explosion and reaches maximum values between 195 and 215 while at 230°C it decreases. Therefore the optimum conditions are achieved at around 200°C with yield of about 63 %. During steam explosion pretreatment at this temperature, structural breakdown and accessibility gain is sufficient while conditions at this temperature are not yet severe enough to significantly decompose monosaccharides. Beyond 215°C severe conditions become more pronounced and although high accessibility is retained, thermal decomposition of monosasaccharides rises to a greater extent. Yield of monosaccharides from washed wheat straw samples (W) increases with temperature of steam explosion pretreatment. Yield of monosaccharides from original wheat straw samples is higher than that from washed samples except the highest temperature of steam explosion.

For bioethanol production, monosaccharide yield is an important factor. The highest yield of monosaccharides was obtained at around 200°C which was found to be the optimum steam explosion temperature leading to high enzyme accessibility and minor production of inhibitors.

Inhibitors in hydrolysates

Monitoring concentration of selected inhibitors is vital for detailed understanding of thermodynamics of the pretreatment and hydrolysis processes. Steam explosion pretreatment at higher temperatures not only liberates fibers from lignocellulosic material and exposes them to enzymes during hydrolysis. In sufficiently high temperature autohydrolysis of polysaccharides occurs and decomposition of newly produced monosaccharides and lignin into inhibitors occurs. These inhibitors have a major effect on fermentation but presence of these compounds may also have an effect on hydrolysis itself. Selected inhibitors were analyzed in hydrolysates of wheat straw original sample after pretreatment at various steam explosion temperature. The results are illustrated in Fig. 7.

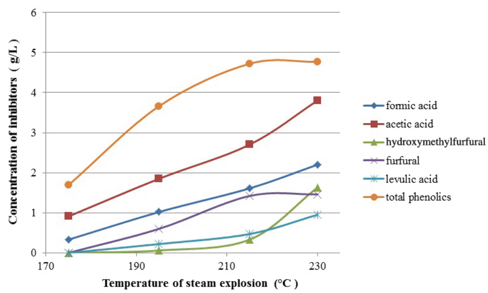

Fig. 7: Concentration of inhibitors in hydrolysates of wheat straw original samples based on steam explosion temperature.

The studied inhibitors included carboxylic acids (formic, acetic, levulic), hydroxymethylfurfural, furfural and the total phenolic compounds (hydroxybenzaldehyde, vanillin, syringaldehyde, coumaric acid, ferulic acid) that are produced by depolymerization of lignin during steam explosion. Steam explosion temperature of 175°C corresponded to relatively mild conditions. Acetic acid and total phenolics were determined in low concentrations whereas other inhibitors were not found at all in hydrolysates. At higher steam explosion temperature, concentration of all inhibitors was eventually present and steadily rose with increasing temperature. The highest values of inhibitor concentrations were obtained at steam explosion temperature of 230°C. This temperature corresponded to conditions which were no longer suitable for steam explosion pretreatment due to increase monosaccharide decomposition which resulted in lower monosaccharides overall concentration and monosaccharide yield.

Determination of selected inhibitors in hydrolysates of original wheat straw samples (O) enabled to understand decomposition of monosaccharides and severity of conditions associated with steam explosion temperature. Hydrolysis and fermentation inhibitors are products of decomposition of monosaccharides and lignin in severe conditions such as excessively high temperature of pretreatment. Lower steam explosion temperature of 175°C resulted in low concentration of acetic acid and minor presence of total phenolics. With increasing temperature of steam explosion other studied inhibitors also became present in hydrolysates and their concentration increased to highest concentrations at 230°C. Increased severity conditions associated with steam explosion temperature of 230°C lead to greater amount of decomposition of monosaccharides and lignin which leads to lower monosaccharide yield and higher concentration of undesirable inhibitors. Optimum steam explosion temperature was determined around 200°C which was sufficiently high temperature for lignocellulosic structure to be highly accessible to enzymes during subsequent enzymatic hydrolysis while undesirable thermal decomposition of monosaccharides resulting in lower yields and greater formation of inhibitors was still low.

CONCLUSIONS

The main goal of this study was to determine effectivity of steam explosion pretreatment of wheat straw for hydrolysis and fermentation and to determine the effect of steam explosion temperature on enzyme accessibility. For hydrolysis commercial cellulase enzyme product Cellic CTec2 was used. In order to modify the technological process of bioethanol production both original and washed solid samples were hydrolyzed and analyzed.

Kinetics of enzymatic hydrolysis proved to be the fastest during the first 24 hours during which high glucose and xylose concentration was reached. The optimum temperature of steam explosion was determined at around 200°C. This determination was based on results obtained at 195 and 215°C. At this temperature the pretreatment conditions were sufficient for making the lignocellulosic structure accessible to enzymes and not severe enough to decompose monosaccharides along the process. Total monosaccharide concentration, cellulose and xylan conversion as well as monosaccharide yield was the highest between 195 and 215°C.

Increasing temperature of steam explosion resulted in steady increase of selected inhibitors which corresponded to monosaccharide and lignin breakdown at this temperature. Higher temperature of steam explosion and the resulting increased concentration of inhibitors in hydrolysates corresponded to higher severity factor describing the process of monosaccharide and lignin decomposition.

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