VARIOUS LIGNOCELLULOSIC RAW MATERIALS PRETREATMENT PROCESSES UTILIZABLE FOR INCREASING HOLOCELLULOSE ACCESSIBILITY FOR HYDROLYTIC ENZYMES PART II. EFFECT OF STEAM EXPLOSION TEMPERATURE ON BEECH ENZYMATIC HYDROLYSIS

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ABSTRACT

Beech wood is one of the most abundant species and the most harvested hardwood in Slovak Republic. The structure and chemical composition predetermines beech wood for the second generation bioethanol production. Steam explosion of beech wood from industrial treatment was investigated as a suitable pretreatment method. The effect of steam explosion temperature on beech sawdust enzymatic hydrolysis was investigated. Optimum steam explosion temperature at around 180°C was determined based on concentration of monosaccharides in hydrolysates and concentration of enzymatic hydrolysis inhibitors such as formic acid and acetic acid from beech sawdust. This corresponds to creating conditions resulting in good disintegration to the lignocellulosic structure which leads to increased cellulose accessibility. Non-treated beech sawdust does not enable sufficient cellulose accessibility while excessively high temperature

results in significant breakdown of monosaccharides and lignin and formation of inhibitors. The concentration of inhibitors was also determined for each studied steam explosion temperature. Based on steam explosion of beech sawdust, the effect of severity factors was investigated to find the optimum conditions of steam explosion pretreatment on cellulose and xylan recovery of beech wood. The obtained optimum steam explosion temperature corresponds to severity factor R_0 = 3.36 (180°C, 10 minutes).

KEYWORDS: Beech wood, biofuels industry, biomass, steam explosion, pretreatment processes, severity factor

INTRODUCTION

The European beech or common beech (*Fagus sylvatica* L.) is one of the most abundant species and the most harvested hardwood in Slovak Republic. It is used to produce sawn wooden materials with a mediocre yield of 43-50 % (De Martini et al. 2015, Monavari et al. 2009, Simangunsong et al. 2018). As a result, a significant amount of beech wood residues is produced which might constitute a potential source of biopolymers for biofuel production (Brethauer et al. 2017) and/or bioenergy. Xylans are the main hemicellulosic components of hardwoods, thus also beech wood (Aspinall et al. 1954). They account for approximately 30 % of the woody cell wall (Awano et al. 2001). They constitute a valuable potential source for the production of high added value additives for functional foods (xylo-oligosaccharides and/or xylitol from xylose) according to the published information (Simangunsong et al. 2018). However, beech wood among other hardwoods shows a higher lignin content and is more compact than typical herbaceous residues, which make it more recalcitrant to enzymatic monosaccharides release (Zhu and Pan 2010). Correspondingly, harsher pretreatment conditions are required for beech wood, leading to an increased consumption of thermal energy and chemicals and to a higher formation of toxic enzymatic hydrolysis and fermentation inhibitors (Kim et al. 2011). However, steam explosion is the hopeful pretreatment method for disintegration of the compact structure of beech wood and thrifty to hydrolysable components. The steam explosion process was developed by Mason in 1924 and patented in 1926. The explosive disintegration by sudden pressure reduction is realized in the specially designed process and equipment for disintegration of wood (Mason 1926). The original idea of steam explosion utilization included treatment of wood particles for the production of chipboard panels (Jacquet et al. 2015). The improved steam explosion processes for lignocellulosic materials decomposition are used in the production of chemimechanical and chemithermomechanical pulps (Kokta and Ahmed 1998), which extends the use of patent technology that includes manufacture of paper and board pulp and the like (Mason 1926). The main equipments of the process are a steam generator, a high-pressure reactor and a discharge tank to receive the material after steam explosion (Akinlabi et al. 2017). The progressive types of devices include a high-pressure reactor in which steam is produced and a discharge tank which need not to be tightly connected to the reactor (Pažitný et al. 2019). During the steam explosion process, that uses high pressure steam for certain residence time, the biomass fibers open up. A rupture of the structure of lignocellulosic fibers and lower bulk density of biomass is caused by a sudden pressure drop. Steam explosion is a thermo-mechanico-chemical pretreatment which induces a breakdown of lignocellulosic material by chemical modification from in situ generated acids which principally cause the depolymerization of hemicelluloses and by mechanical action due to high shearing forces applied to the lignocellulosic material during the explosive release.

The process enhances the recovery of monosaccharides and makes biopolymers more accessible for subsequent processes such as enzymatic hydrolysis, fermentation or densification. It can be utilized as a pretreatment process to produce solid biofuel pellets, to increase the calorific value, to improve the pelletizing properties of the lignocellulosic materials or to produce cellulose which would be more accessible to enzymatic agents for purposes of conversion to fermentable simple saccharides (Boháček et al. 2014, Simangunsong et al. 2018). The steam explosion process has been extensively studied to improve the enzymatic hydrolysis of the different cellulosic pulp types (pine wood, corn stover, poplar wood, wheat bran and wheat straw) that are produced to a large extent (Grous et al. 1986, Silva et al. 2017, Ballesteros et al. 2000, Kumar et al. 2011, Liu et al. 2013, Jiang and Guo 2016, Boháček et al. 2014, Russ et al. 2016, Pažitný and Šutý 2018).

Only cheap biomass such as beech wood composed of cellulose, hemicelluloses and lignin, which is readily available all over the world, is suitable for the production of the second-generation bioethanol. In addition, beech wood has excellent year-around availability. One of the reasons for using beech wood particles for bioethanol production is its relatively high content of hydrolysable polysaccharides – cellulose and hemicelluloses (Kleindienst et al. 2017). Additionally, activation energy value of beech wood is higher than in case of some other species due to the higher hemicellulose content for beech (Chien et al. 2018). Regarding to the higher hemicellulose content, the accessibility of hydrolysable polysaccharides consisting of cellulose and hemicelluloses is a very important factor for successful enzymatic hydrolysis (Letko et al. 2015, Pažitný et al. 2019). As described in the previous part, enzymatic agents (Reese 1956, Reese and Mandels 1959, Sandhu and Kalra 1985) are useful in the natural cellulose or hemicelluloses conversion to monosaccharides such as glucose or pentoses (xylose, arabinose etc.). Although the mechanism of enzymatic hydrolysis of cellulose is not yet fully explained, the basic procedure of cellulose decomposition is well described (Whistler and Smart 1953) by following chemical equations scheme 1:

$$natural\ cellulose \rightarrow linear\ cellulose \rightarrow cellobiose \rightarrow glucose \tag{1}$$
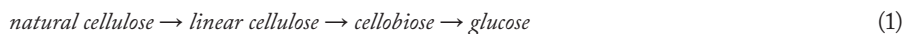
The first step includes linear cellulose formation by the first type cellulase (endoglucanase) action and the second step includes cellobiose formation by the second type cellulase (exoglucanase) action. The reaction is finalized with bonding cleavage by adding of β-glucosidase (Sandhu and Kalra 1985). Cellulose terminal units and cellulose macromolecule converted to oligosaccharides – cello-oligosaccharides (Kuba et al. 1990) are converted to glucose monomers overall. In addition, recent enzymatic agents are able to convert hemicelluloses to pentoses (Gong et al. 1981) by analogical manner and obtained pentoses can be conveniently converted into a common organic intermediate which is useful in organic synthesis (Bercier et al. 2007). Enzymatic hydrolysis is an indirect method for cellulose accessibility control when compared to Simons' analytical procedure which is a direct method for determination of cellulose accessibility (Boháček et al. 2014, Esteghlalian et al. 2001). The great advantage of enzymatic hydrolysis performance is getting information about hydrolysate compositions which can vary significantly depending on biomass species and enzymatic hydrolysis conditions (Gigac et al. 2017).

MATERIAL AND METHODS

Materials

Beech wood of common beech (*Fagus sylvatica* L.) as a source of holocellulose was obtained from Bukóza Píla JSC, Slovak Republic. The raw material was recovered as waste from sawing

beech wood. Cellic CTec3 was used as a ready-to-use stabilized enzyme complex supplied by Novozymes A/S (Bagsværd, Denmark) for degradation of lignocellulosic raw material to fermentable monosaccharides. The enzyme activity was measured to be 1.700 BHU (Biomass Hydrolysis Units)/g product in our laboratory.

Methods

Beech wood was pretreated mechanically by dry milling in Brabender mill (Brabender®, GmbH & Co. KG, Germany). A bottom sieve was used to obtain a finer fraction, however, a 0.7 mm mesh screen was finally used for the laboratory experiment. The 0.7 mm beech particles were impregnated with fresh water so that the final moisture content of the samples during the steam explosion was at least 85 % w/w. That is the amount of water in which the sample was soaked prior to pretreatment was calculated to be 15 % w/w of the material. The samples were soaked in water at 20°C for at least 1 hour prior to steam explosion pretreatment. The quick determination of dry matter in samples was performed on moisture analyser Denver IR35 that uses infrared sample heating. Samples (50 g o.d.) were finally pretreated in a 2 L stainless steel batch reactor for steam explosion (Amar Equipments Pvt. Ltd., India). The reactor was used for steam reactions at different temperatures (180°C, 220°C). The retention time of each thermo-hydro-mechanical experiment with beech wood (steam explosion) was 10 minutes.

Enzymatic hydrolysis of the original and pretreated beech wood particles with Cellic Ctec3 at a dose of 15 % w/w (g Cellic Ctec3 / 100 g cellulose) was carried out at 50 °C, pH = 5.0 for 72 hours and 12.5 % w/w of the samples. The pH was adjusted continuously during the process using 0.1 N sulphuric acid or 0.1 N sodium hydroxide. The hydrolysate samples were sampled after 24, 48 and 72 hours.

Concentration of monosaccharides and inhibitors was determined using the procedure of National Renewable Energy Laboratory (NREL 2008, Sluiter et al. 2011). Monosaccharides (glucose and xylose) and inhibitors (formic acid, acetic acid and residual inhibitors) were determined in hydrolysates by HPLC method with Rezex ROA (organic acid) H⁺ column. The mobile phase was 0.005 N sulphuric acid at a flow of 0.5 mL·min⁻¹ and temperature set to 30°C.

RESULTS AND DISCUSSION

Beech wood as a perspective substrate in 2G bioethanol production

Beech wood is one of the most commonly used hardwoods in European countries, and waste obtained from treatment of this hardwood is promising raw material for production of 2G bioethanol (Budzinski and Nitzsche 2016). Biorefinery is able to convert 400,000 dry metric tons of beech wood into the primary products such as polymer-grade ethylene, organosolv lignin and biofuels. The most significant efforts are in the field of conversion of cellulose II included in hardwood to bioethanol (Nagarajan et al. 2017).

Steam explosion of beech wood as a pretreatment method and its evaluation

Selected methods of pretreatment and their evaluation were described in our previous papers (Boháček et al. 2014, Letko et al. 2015, Pažitný and Šutý 2018). In this study we tested the steam explosion of beech wood particles at 180°C, 200°C and 220°C. The samples were firstly mechanically treated by dry milling in Brabender mill which is common procedure to treat another biomass species for laboratory purposes (Vizitiu et al. 2012). The selected particle size was 0.7 mm, however, the size optimum of 1.0 mm was recorded in scientific literature

(Simangunsong et al. 2018). The low differences in optimum particle size can be caused by the different input moisture immediately before the steam explosion. Taking into account the moisture content of the samples (at least 85 % w/w), it can be stated that optimum particle size entering the steam explosion is in good accordance with the literature.

The used steam explosion experimental conditions were as follows: temperature from 180°C to 220°C, constant retention time 10 minutes, beech wood sawdust size of 0.7 mm. The corresponding severity factors are listed in Tab. 1.

Tab. 1: Effect of pretreatment process temperature on severity factor values at constant retention time of 10 minutes.

Steam explosion temperature (°C)	Retention time (minutes)	Severity factor
180	10	3.36
200	10	3.94
220	10	4.53

The severity factor calculation for corresponding temperatures and retention time was based on the following integral form of equation for severity factor computing (Hashemi et al. 2019, Batista et al. 2019):

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

where: R_O - severity factor,
T_r - temperature of reaction (°C),
T_b - base temperature (100°C),
t - retention time in biomass hydrothermal reaction (minutes),
14.75 - empirical temperature value based on the conventional activation energy when expecting first order kinetics (kJ·mol^{-1}).

In the previous equation for calculating the severity factor R_O quantifies reaction coordinate and the equation can be written in simplified form as follows:

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

Fig. 1 and Fig. 2 give respectively the total monosaccharides concentrations, the glucose monomer and the xylose monomer released in the liquid phase during the enzymatic hydrolysis of beech samples with particle size of 0.7 mm pretreated by the steam explosion process and subsequently hydrolysed using the above method of enzymatic hydrolysis. As expected, the sugar concentration recovered in the liquid phase is very low for non-pretreated beech sample and grow by leaps and bounds for pretreated beech samples.
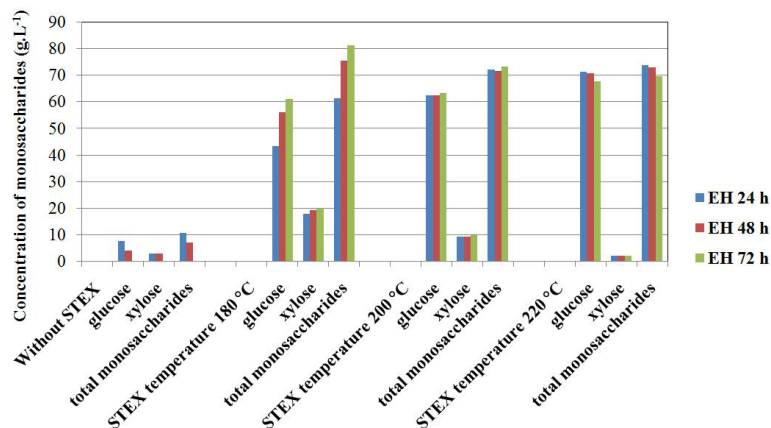
Fig. 1: Effect of steam explosion temperature on monosaccharides (glucose and xylose) concentration in hydrolysates of pretreated beech wood samples.

By comparing the results of the HPLC analysis, it was found that the highest monosaccharide concentrations are in case of the beech particles hydrolysed after pretreatment at a steam explosion temperature of 180°C (total monosaccharides of 81.1 g·L^{-1}). For the 10 minute retention time chosen for the steam explosion pretreatment at 180 °C and the severity factor value is R_0 = 3.36. Severity factor values for other steam explosion pretreatment processes were higher. The temperature of 200°C corresponds to the severity factor value of R_0 = 3.94 (200°C, retention time of 10 minutes) and 220°C corresponds to the severity factor value of R_0 = 4.53 (220°C, retention time of 10 minutes). The severity factor values are in agreement with previous studies performed on hardwood (Simangunsong et al. 2018) or another plant species (Damay et al. 2018). Also, from the recorded severity factor values and the attribution of monosaccharides concentrations based on beech to the mentioned processes of steam explosion, the optimum conditions are obtained in case of the pretreatment by steam explosion already at a temperature of 180°C. From the Fig. 1 the growth of monosaccharides concentrations can be seen as early as 48 hours after the start of enzymatic hydrolysis. However, in 72 hours from initialization of the enzymatic hydrolysis process, the increase of monosaccharide concentrations is only modest compared to 48 hours. The values of severity factor being a function of retention time and pretreatment temperature on which the sugar concentration at enzymatic hydrolysis process depends, are chosen for steam explosion pretreatment temperatures ranging from 3.36 to 4.53 (Tab. 1). It is evident that temperature dependence of the severity factor values is non-linear – logarithmic.

It is also important for the industrial practice producing biofuels based on cellulose to know the proportion of inhibitors in the hydrolysates obtained (Fig. 2) as they slow down the enzymatic hydrolysis itself. It can be seen from Fig. 2 that there were significant concentrations of acetic acid and formic acid in the obtained hydrolysates and possibly other inhibitors.
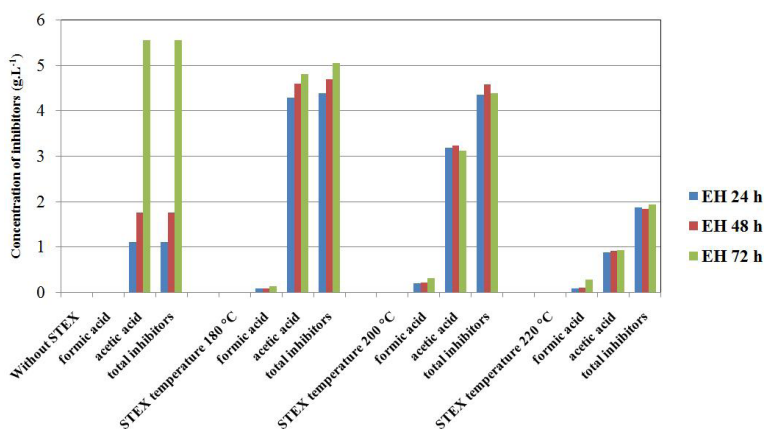
Fig. 2: Effect of steam explosion temperature on inhibitors (formic acid and acetic acid) concentration in hydrolysates of pretreated beech wood samples.

The increase in enzymatic hydrolysis time also shows a rapid or slight enhancement in the concentration of total inhibitors which is significant for the enzymatic hydrolysis of non-pretreated beech particles or pretreated at a steam explosion temperature of 180°C, respectively. However, for higher steam explosion temperatures the kinetics changes are not significant. Regarding the effect of the pretreatment temperature on concentration of inhibitors in the enzymatic hydrolysis process of 0.7 mm pretreated beech particles, the trend is decreasing, thus the highest concentration of inhibitors (about 5.0 $g \cdot L^{-1}$) was found at the steam explosion temperature of 180°C, consistent with repeated experiments discussed in our previous studies performed on wheat straw (Pažitný et al. 2019). The obtained results prove that formic acid is being released during steam explosion at each temperature (Elliston et al. 2015) and not during enzymatic hydrolysis at all as the kinetics determination by HPLC method did not identify presence of formic acid in non-pretreated hydrolysed beech sample. However, acetic acid is formed mainly during enzymatic digestion with culmination at pretreatment temperature of 180°C (Fig. 2). The complete removal of formic acid and acetic acid can be performed by effective washing as they could inhibit the fermentation which is one of the immediately following processes in biofuel production (Stankovská et al. 2018).

CONCLUSIONS

The basic goal of this partial study was to evaluate holocellulose accessibility of pretreated beech particles obtained by dry milling in Brabender mill. A 0.7 mm mesh screen was eventually used for laboratory experiments and the holocellulose accessibility was evaluated indirectly.

It was proved that steam explosion improves accessibility of holocellulose as the monosaccharides concentration in pretreated beech samples increased significantly during enzymatic hydrolysis compared to non-pretreated beech sample. The highest monosaccharide concentration was obtained at a steam explosion temperature of 180°C with the severity factor value of $R_0 = 3.36$ as the retention time for steam explosion pretreatment was 10 minutes.

Another interesting piece of knowledge obtained from experimental results was the rapid enhancement or slight increase in the concentration of total inhibitors with increasing enzymatic hydrolysis time which is significant for the enzymatic hydrolysis of non-pretreated beech particles or pretreated at a steam explosion temperature of 180 °C, respectively. However, for higher steam explosion temperatures the kinetics changes are not significant. Among the studied inhibitors, acetic acid is formed mainly during enzymatic digestion with culmination at the pretreatment temperature of 180 °C while formic acid is being released during steam explosion at each temperature but not during enzymatic hydrolysis.

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