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Effects of soaking aqueous ammonia pretreatment on chemical composition and enzymatic hydrolysis of corn stover

FLORENTYNA AKUS-SZYLBERG, ANDRZEJ ANTCZAK, JANUSZ ZAWADZKI

Department of Wood Science and Wood Preservation, Institute of Wood Sciences and Furniture, Warsaw University of Life Sciences WULS-SGGW, Poland

Abstract: *Effects of soaking aqueous ammonia pretreatment on chemical composition and enzymatic hydrolysis of corn stover.* The aim of this research was to investigate the effect of applying two different temperatures of the soaking aqueous ammonia treatment on the chemical composition and enzymatic hydrolysis yield of the corn stover. Native corn stover as well as solid fractions after 20 h of alkali pretreatment performed at 15% ammonia solution and at 50 °C or 90 °C were analysed in terms of cellulose, holocellulose, lignin and extractives content. Both untreated and treated samples were subjected to the enzymatic hydrolysis and hydrolysates were examined with a high performance liquid chromatography (HPLC). Results indicated a significant development of enzymatic digestibility of the SAA treated biomass. Furthermore, a 38.7% and a 68.9% delignification levels in the biomass treated with ammonia at respectively 50 °C and 90 °C process comparing to the raw material were achieved.

Keywords: soaking aqueous ammonia, chemical composition, enzymatic hydrolysis, corn stover, bioethanol

INTRODUCTION

Bioethanol is a principle substitute for traditional petrol transportation fuel with constantly growing production (Kumar and Murthy 2011). It can be produced from agricultural feedstocks through direct fermentation processes (sugarcane, beetroot) or hydrolysis and subsequent fermentation processes (potatoes, corn grain). However, using food-competing crops such as corn or wheat affect global food prices, therefore other substrates that cannot be utilized for human consumption are of great interest. Such so-called second generation biofuels from nonedible sources are more effective economically and generate lower carbon dioxide emission. Consequently, corn stover, which is an abundant lignocellulosic agricultural residue presents itself as a prominent substrate for enzymatically-based cellulosic ethanol bioconversion. As it is stated by Zhao et al. (2018) corn stover contains on average 36.2% ± 3.2% cellulose and 22.7% ± 4.2% hemicelluloses, which are polysaccharides that could be easily hydrolysed into fermentable simple sugars.

As a typical lignocellulosic feedstock, corn stover consists of lignin, cellulose and hemicelluloses, collectively constituting a lignin carbohydrate complex (LCC) which is a rigid and compact structure restricting the polysaccharides accessibility to the enzymes used during hydrolysis (Zhao et al. 2012). Therefore adding an additional pretreatment step to the overall bioconversion process is required to overcome the innate biomass recalcitrance. Enhancing the susceptibility of lignocellulosic material to the enzymes may be achieved by removal of lignin and hemicelluloses, breaking the crystalline structure of cellulose as well as increasing the surface area and porosity (Hu et al. 2020). Generally many pretreatment methods have been developed and investigated since it is necessary to adopt a suitable technologies based on the particular raw feedstock properties.

Chemical pretreatment called soaking aqueous ammonia (SAA) is praised as a remarkable delignification method, which allows to retain almost all of cellulose for the subsequent reactions and increases the accessible surface area (Kim et al. 2009; Meng and Ragauskas 2014). For instance, Kim et al. (2008) reported ethanol yields as high as 89.4% of the theoretical ethanol yield from barley hull pretreated with the SAA method in a simultaneous saccharification and fermentation (SSF) process. Furthermore, the ammonia soaking procedure is one of the few pretreatment methods that allow to retain both glucan and xylan in the solids, which presents opportunity for potential utilization of xylose during subsequent fermentation process. During the alkali treatment, the ester linkages in hemicelluloses and lignin are easily broken down, as well as significant removal of acetyl groups and uronic acid substitutions on hemicelluloses are triggered, thus despite high efficiency in carbohydrate preservation still part of xylan is solubilized together with lignin (Kim et al. 2009; Sun et al. 2016). Additionally, high xylose recovery achieved at low temperature applied during the SAA procedure (up to 90 °C) is reflected in lower amount of inhibitory compounds released form sugar degradation (Alvira et al. 2010). Another advantage of the aforementioned pretreatment method is that ammonia used in solutions may be recycled from the effluent and reused (Gao et al. 2016). Nevertheless long time, high concentration of the base and formation of residual salts in pretreated biomass are some of the disadvantages of the SAA process (Verardi et al. 2012).

Thus, the objective of this research was to investigate the effect of applying the SAA pretreatment method at different temperatures on the corn stover intended for further bioethanol production via the enzymatic hydrolysis process, as the reaction temperature is proven to influence compositional changes and enzymatic digestibility of treated biomass (Yoo et al. 2013). Since suitability of the particular lignocellulosic feedstock for bioconversion depends on its chemical composition it was investigated in this research, however final assessment of the SAA pretreatment conditions was based on its subsequent enzymatic hydrolysis yield.

MATERIALS AND METHODS

Raw material

Corn stover from commercial fields was subject to this research. The stover was ground into particles with dimensions of 0.43–1.02 mm. It was air dried and stored at the room temperature, while at equilibrium the moisture content of ground corn stover was approximately 6%. Furthermore, Cellic CTec2 (Novozymes, Denmark), which is a mixture of cellulases, β-glucosidases and hemicellulases with the activity of 151 FPU/mL measured with Whatman No.1 filter paper according to the National Renewable Energy Laboratory (NREL) method was used as a catalyst (Adney and Baker 1996). Chemicals used in this experiment were of analytical grade purchased from Chempur (Poland).

SAA pretreatment

The pretreatment process was carried out following the procedure characterized by Akus-Szylberg et al. (2021) in a cylindrical reactor made of stainless steel with the capacity of 250 mL and a thermometer sensor planted inside the tube routed through allowing to control the temperature inside. About 20 g of the air-dried intended fraction of raw material was quantitatively placed in a reactor and respective amount of 15% (w/w) ammonia solution was added in order to apply a solid : liquid ratio of 1:12.5. Then the reactor was placed in oil bath that was pre-set and subsequently maintained at 50 °C or 90 °C with each treatment lasting for 20 h after the load inside the reactor reached the set temperature and then cooled rapidly to end the reaction. The solid fraction was separated by filtration with a Büchner funnel, washed with distilled water until pH reached 7.0 and then stored at 6 °C until performing enzymatic hydrolysis and compositional analysis. The applied conditions of the SAA method chosen for this study were based on previous literature review and were selected for their preferable delignification ability (Kim et al. 2008; Ko et al. 2009). Two experiments were carried out at each given condition and the obtained yield was mixed.

Chemical composition analysis

Subsequently, the solid samples, which included treated or untreated corn stover, were subject to compositional analysis after drying for 6 h at 105 °C. The extractives content was determined by extraction in mixture of chloroform and ethanol $93{:}7_{w/w}$ following the procedure developed by Antczak et al. (2006). Kürschner-Hoffer method (Kürschner and Hoffer 1929; Krutul 2002) was performed for cellulose isolation and its content determination. Both lignin and holocellulose content was determined according to PN-92/P50092 (1992) standard. Each sample was run in triplicate and single standard deviations were calculated.

Enzymatic hydrolysis

Finally, to assess the enzyme digestibility of the treated corn stover the 72 h enzymatic hydrolysis was carried out in triplicates for each feedstock. Both the hydrolysis procedure and high-performance liquid chromatography (HPLC) analysis of glucose and xylose content in the hydrolysates were executed according to the method described by Akus-Szylberg et al. (2020).

RESULTS AND DISSCUSION

The aqueous ammnia pretreatment changed the chemical composition of the corn stover noticeably as shown in the Table 1. Generally, the SAA process demonstrated to be a very efficient delignification method. It triggered a 38.7% and a 68.9% lignin content decrease in the biomass treated with ammonia at respectively 50 °C and 90 °C process comparing to the raw material. For comparison, Kim et al. (2009) achieved 73.6% delignification in the corn stover treated with the SAA procedure performed at 60 °C and 15% ammonia concentration for 24 hours.

During treatment in alkaline environment lignin degradation advances in three stages: initial, bulk and residual according to several entities of lignin components dissolving at different rates (Gao et al. 2016). Lignin bonds are destroyed, especially aryl-ether bonds which are the most labile, however as a result of lignin degradation different inhibitory compounds to the enzymatic hydrolysis process may be formed, such as phenolic compounds or aromatic acids (Balan et al. 2012).

Table 1. The chemical composition of untreated and SAA pretreated corn stover.

Component [%]	Untreated corn stover	Temperature [°C]	
		50 °C	90 °C
Extractives	5.0 ± 0.1	2.0 ± 0.7	3.3 ± 0.1
Cellulose	38.3 ± 03	62.4 ± 0.2	69.0 ± 0.7
Lignin	22.5 ± 0.2	13.8 ± 0.1	7.0 ± 1.7
Holocellulose	65.7 ± 0.1	81.9 ± 0.9	80.8 ± 1.5
Hemicelluloses*	27.4	19.5	11.8
Mass loss	-	26.0	27.0

* calculated as a difference between the holocellulose and cellulose content

Additionally, the hemicelluloses content in the treated biomass was notably lower than that of the untreated sample, however, among other methods, ammonia-based pretreatment lose the least amount of xylan during procedure (Zhao et al. 2018). Loss of polysaccharides during alkali treatments are in large part due to peeling, hydrolytic reactions and dissolution. Hemicelluloses are generally much more vulnerable to chemical reactions in alkaline media than cellulose (Balan et al. 2012). In this research, as shown in the Table 1, hemicelluloses content was respectively 28.8% and 56.9% lower in case of biomass after the 50 °C and 90 °C procedures when compared to raw feedstock. Less intense xylan degradation may be beneficial considering that it can lead also to formation of enzymatic inhibitors including acetic acid and formic acid (Geng et al. 2018)

Moreover, extractives content declined after the alkali procedure, although decrease was lower in the biomass treated with more severe conditions, which may be result of formation of hemicelluloses and lignin degradation products. Overall, the pretreatment step opens and partly dissolves the fibre structure of corn stover, especially lignin and hemicelluloses, which accounts for considerable mass loss. Cellulose share in the SAA treated biomass grew extensively, however it was just apparent growth caused by hemicelluloses and lignin loss and deterioration.

As shown in Fig. 1. the soaking aqueous ammonia process highly increased the amount of simple sugars (glucose and xylose) obtained from enzymatic hydrolysis compared to sugars yield from untreated corn stover (10.5%). Cumulative sugar yield after saccharisation of treated biomass amounted for 59.5% and 71.6% respectively for procedures performed at 50 °C and 90 °C. With the removal of lignin and hemicelluloses the accessible surface area of cellulose in the substrates increases, which favourable influences the cellulose susceptibility to enzyme and improves the enzymatic conversion (Geng et al. 2018). Jia et al. (2013) reported 82% of the glucan conversion yield in the corn stover pretreated with the SAA method under the conditions of 75 °C and 21% of an aqueous ammonia solution.

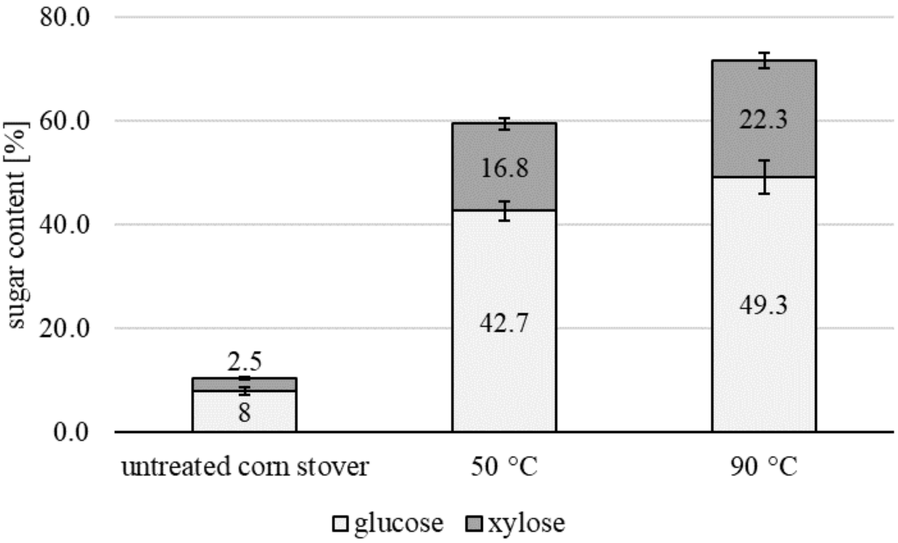

Fig. 1. The simple sugars content (glucose and xylose) after enzymatic hydrolysis of biomass obtained from corn stover both untreated and treated with SAA process.

Chemical composition of the SAA treated biomass indicated that hemicelluloses content was decreasing along with growing temperature of the process, nevertheless results of enzymatic hydrolysis did not confirm that. After alkali pretreatment performed at the 90 °C hemicelluloses content in corn stover amounted for only 11.8%, while xylose content after hydrolysis of this biomass equaled to 22.3%. That may be result of imperfections within the hemicelluloses content analysis method (calculated as a difference between the holocellulose and cellulose content), which do not take into account xylans embedded into cellulose structure, that are very hard to hydrolyze. Another reason for that may be loosening the structure of the biomass pretreated at 90 °C and despite lower hemicelluloses content its increased accessibility to the enzymes and hence improved susceptibility to hydrolysis.

CONCLUSIONS

Successful delignification of the corn stover treated with the SAA pretreatment was achieved at 15% ammonia solution and at 50 °C and 90 °C. The lignin content was respectively 38.7% and 68.9 % lower than in untreated feedstock. Furthermore, the ammonia-based pretreatment highly increased sugars yield after enzymatic hydrolysis with both glucose and xylose content improving along with the growth of the process temperature. In the biomass treated with the ammonia soaking method performed at the more severe conditions summary sugar content was as high as 71.6%. Assessment of aforementioned results indicate that corn stover is a very promising lignocellulosic substrate for bioconversion and that the soaking aqueous ammonia is a suitable initial treatment method aiming to facilitate subsequent hydrolysis and fermentation processes leading to bioethanol obtainment.

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Streszczenie: *Wpływ wstępnej obróbki słomy kukurydzianej w wodnym roztworze amoniaku (SAA) na jej skład chemiczny i hydrolizę enzymatyczną*. Celem badań było zbadanie wpływu zastosowania dwóch rożnych temperatur podczas obróbki wstępnej wodnym roztworem amoniaku słomy kukurydzianej na jej skład chemiczny oraz wydajność hydrolizy enzymatycznej. Próbki słomy natywnej, jak i słomy po 20 godzinach obróbki w 15% roztworze amoniaku i temperaturze 50 °C lub 90 °C zostały przebadane pod kątem zawartości celulozy, hemiceluloz, ligniny i substancji ekstrakcyjnych. Zarówno materiał natywny, jak i po obróbce został podany również hydrolizie enzymatycznej, a hydrolizaty zostały przebadane przy pomocy wysokosprawnej chromatografii cieczowej (HPLC). Uzyskane wyniki pozwoliły stwierdzić wysoce zwiększoną zawartość cukrów w hydrolizatach biomasy po obróbce SAA. Ponadto, osiągnięto 38.7% oraz 68.9% poziom delignifikacji odpowiednio po procedurze SAA przeprowadzonej w 50 °C i 90 °C.

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Corresponding author:

Florentyna Akus-Szylberg
Department of Wood Science and Wood Protection,
Institute of Wood Sciences and Furniture
Warsaw University of Life Sciences (SGGW)
159 Nowoursynowska St., 02-776 Warsaw, Poland
e-mail: *florentyna.akusszylberg@gmail.com*