CHEMICAL PROCESSES (ACIDIC AND ALKALINE) IN SACCHARIFICATION OF SORGHUM BIOMASS FOR BIOFUEL PRODUCTION

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Biofuel production from lignocellulosic materials requires degradation of cell walls to specific polymers and hydrolysis of carbohydrates to monomer sugars. Cellulose, hemicellulose and lignin constitute three main lignocellulose components and they are bonded together forming a complex matrix, highly recalcitrant to chemical and biological conversion. One of the main factors behind the difficulties in biomass saccharification is the lignin, which intertwines with hemicellulose and covers the microfibrils of crystalline cellulose. All this forces to subject the material to pretreatments (physical, chemical and biological), which affects significantly the course of further stages in biofuel production. The present study evaluated the effect of sulfuric acid and sodium hydroxide on the amount of released reducing sugars (Miller's method). The chemical composition was determined for the solid fraction formed after the treatments, while for the liquid phase (after acidic treatment only) toxins and pentoses were determined. Also, FTIR spectra analysis was performed for sorghum biomass before and after the chemical treatment.

Keywords: lignocellulosic biomass, chemical treatment, biofuels

INTRODUCTION

Biofuels offer a promising alternative for fossil fuel consumption due to their renewability, biodegradability and generation of exhaust gases of acceptable quality.[1] The term "biofuels" refers to liquid, gas and solid fuels, produced predominantly from biomass. Bioethanol produced from organic raw materials is a renewable and clean resource for energy production. It is used as fuel and also in chemical, cosmetic and pharmaceutical industries. The main materials for bioethanol (first generation biofuels) production are cereals and maize grain, potatoes, as well as sugar beet. The bioenergetic use of these plants is controversial, as it implies reduction of the arable area for production of food and feed. Currently, one of the alternative materials for bioethanol production (second generation biofuels) to be considered is sorghum. It is an annual plant reaching the height of 4 m, tolerant to drought, and providing high yields of dry mass (28 t/ha) at the so called milk-wax phase of the seed.[2] Sorghum biomass contains high amounts of monosaccharides, mainly of fructose (9.75%), which indicates its high usability for obtaining bioethanol.[3] Additionally, the energetic value of combustion for sorghum is 15 MJ/kg.

Lignocellulosic biomass is characterized by a complex chemical composition, as it contains in its structure a polymeric complex called lignocellulose, which is relatively recalcitrant to biodegradation. The lignocellulosic complex found in plant cell walls is composed of cellulose, hemicellulose and lignin. Cellulose, a glucose polymer, and hemicellulose, which mainly consist of galactose, mannose, xylose and arabinose molecules, are potential substrates for efficient use in fermentation processes. Lignin, consisting of phenolic alcohol derivatives, such as *p*-coumaryl, coniferyl and sinapyl alcohols, structurally crosslinked by ester and carbon bonds, is a major obstacle in bioethanol production from plant biomass. Its aromatic character and complex structure make lignin difficult to

degrade. Lignin and its derivatives have a negative effect on biomass hydrolysis, as they physically hinder the access of cellulases to the microfibrils of crystalline cellulose and they bind cellulases, which leads to their deactivation.[4-6]

Biofuel production from lignocellulosic material requires decomposition of the cell wall into individual polymers, and hydrolysis of the carbohydrates into monomeric sugars. This forces to subject the biomass to pretreatment, which affects significantly the course of the further stages of bioethanol production, i.e. enzymatic hydrolysis and fermentation process, and which determines the final efficiency of the process.[7-8] In order to disintegrate the biomass and remove lignin, several pretreatment methods have been tested – physical, chemical, and biological methods or combinations thereof.[9-10]

Physical pretreatment methods for lignocellulosic biomass, including grinding, milling and chopping, all aim at reducing the size of the substrate, facilitating the access of bioactive substances to the surface, the reduction of polymerization and crystallization degree of lignocellulose. The physical methods also comprise an extrusion method (an integrated method of heating, mixing and shearing), ultrasound pretreatment, steam-explosion and liquid hot water (LHW).[11-12]

Chemical processes include dilute acid treatment (with sulfuric acid, more rarely hydrochloric acid), alkali treatment (sodium hydroxide, calcium hydroxide and also ammonia), neutral treatment (ionic liquids), and the organosolv process with organic solvents, SO_2-steam explosion, ammonia fiber explosion (AFEX), ammonia recycle percolation (ARP) and ozonolysis.[13-15]

Depending on the method, different changes occur within the lignocellulosic complex. In summary, alkali pretreatment involves mainly delignification and partial degradation of the hemicellulose, while acid pretreatment process results in the dissolution of most of the hemicellulose and of a little lignin. A neutral solvent mainly acts by depolymerizing the lignin, while hot liquid or steam treatments involve degradation of the hemicellulose and of a small quantity of lignin.

Biomass pretreatment is an extremely important step in the synthesis of biofuels from lignocellulosic biomass, and there is critical need to understand the fundamentals of various processes, which can help in making a suitable choice depending on the structure of the biomass substrate and the hydrolysis agent.

The present study aimed to compare the acidic and alkaline pretreatments of sorghum biomass during preparation of the material for bioethanol production.

EXPERIMENTAL

The material used in the study was plant biomass from Sucrosorghum 506 from the Experimental Farm of INF&MP in Sielec Stary (Poland) harvested in September 2013. The raw material was subjected to preliminary crushing and then dried at a temperature of 50 °C for 24 hours. Next, the material was disintegrated on a knife mill, with a sieve of 4 mm mesh size.

The effect of the chemical process with sulfuric acid was determined after 10 min treatment with 2% acid and the autoclaving process at a temperature of 121 °C for 60 minutes, whereas for the process with sodium hydroxide, after 300 min treatment with 1.5% alkali at a temperature of 90 °C.

The effect of sulfuric acid and sodium hydroxide on the content of released reducing sugars was evaluated by Miller's method with 3,5-dinitrosalicylic acid (DNS) in an enzymatic test.[16] The test was performed with the use of the enzymatic preparation Celluclast 1.5L (Novozymes) in a dose of 10 FPU/g. The raw material was incubated at a temperature of 55 °C in 0.05 M citrate buffer of pH 4.8 for 24 hours. Then, an absorbance measurement was taken against the reference sample at the wavelength of 530 nm.

The non-specificity of the acidic treatment led to the formation of complex sugars and compounds (organic acids, furan derivatives, phenolic compounds), which are inhibitory to the microorganisms used for ethanol production.

The chemical composition was determined for the solid fraction formed after the treatment, while for the liquid phase (after acidic treatment only), toxins and pentoses were determined with the liquid chromatography technique (HPLC) using an Agilent Technologies 1200 chromatograph with a DAD detector (toxins) and a RID detector (pentoses). The chemical components of sorghum biomass were determined, i.e. cellulose (TAPPI T17 m-55 method), hemicellulose – as the difference of holocellulose (TAPPI T9 m-54 method) and cellulose, and lignin (modified method TAPPI T13 m-54).

The analysis of FTIR spectra was carried out for sorghum biomass before and after the chemical treatment using an ISS 66v/S (Bruker) spectrophotometer at infrared wavelengths of 400-4000 cm^{-1}.

RESULTS AND DISCUSSION

The primary goal of chemical pretreatment is to improve the cellulose biodegradability by removing lignin and/or hemicellulose, and to a lesser extent, to reduce the polymerization degree (PD) and crystallinity of the cellulose component. An efficient pretreatment method is required for the enzymatic hydrolysis to give maximum sugar productivity. Therefore, the success of using renewable biomass for ethanol production depends on the physical and chemical properties of the biomass, the pretreatment methods, efficient microorganisms and on the optimization of the processing conditions.

Two types of pretreatment were compared: the acidic treatment – for 10 minutes with 2% sulfuric acid, followed by an autoclaving process at a temperature of 121 °C for 60 minutes, and the alkaline treatment – for 300 minutes with 1.5% sodium hydroxide at a temperature of 90 °C, were conducted for the sorghum biomass ground on a knife mill with a mesh size of 4 mm. In order to determine the efficiency of the chemical processing of the sorghum biomass, the content of reducing sugar was measured by Miller's method in the enzymatic test (Table 1).

The results allow concluding that the sodium hydroxide pretreatment is a more efficient method for sorghum biomass, compared to the pretreatment with sulfuric acid.

The action mode of dilute acid is to solubilize hemicellulose and leave lignin and cellulose intact, so that the enzymatic digestibility of cellulose is enhanced. Alkaline pretreatment involves basically a delignification process, in which a significant amount of hemicellulose is also solubilized. In comparison with other pretreatment technologies, alkali pretreatment usually needs lower temperatures and pressures. Pretreatment time, however, lasts for a few hours, which is much longer than the time required for other pretreatment processes.

Figure 1 presents the chemical composition (cellulose, hemicellulose, lignin) of the sorghum biomass before and after chemical pretreatment (in the solid fraction) with sulfuric acid and sodium hydroxide.

The acidic treatment offers good performance in terms of recovering hemicellulose sugars (especially pentoses in the liquid phase), but it also has some drawbacks. The hemicellulose sugars might be further degraded to furfural and hydroxymethylfurfural (HMF), strong inhibitors to microbial fermentation. In the case of alkaline treatment, due to its mild conditions, the degradation of sugars to furfural, HMF and organic acids is limited.

Figure 2 presents the content of toxins (furfural, HMF, acetic acid) and pentoses (xylose, arabinose) in the liquid phase after acidic treatment of sorghum biomass.

The FTIR technique can be applied to examine the structural changes in the biomass during pretreatments. The spectra of untreated sorghum biomass are presented in Figure 3, along with those of biomass pretreated with sulfuric acid and sodium hydroxide.

Comparing the FTIR spectra of untreated and pretreated (acidic and alkaline) fibers, as regards the transmittance at 1730 cm^{-1} (C=O of carbonyl group), 1510 cm^{-1} (C-C of aromatic ring) and 1270 cm^{-1} (C-O of guaiacyl ring), it may be concluded that the spectra indicate the delignification of sorghum biomass, especially after the treatment by sodium hydroxide. Also, it may be observed an increase in the band intensity at 898 cm^{-1} and a decrease at 1427 cm^{-1}, which indicates lower crystallinity and an increase of the amorphous form of cellulose as a result of chemical pretreatments, in particular, of the alkaline treatment.

An ideal pretreatment technique should be able to maximize the recovery of available carbohydrates, such as cellulose and hemicellulose, while minimizing the degradation of sugars and the generation of possible inhibitors.

Table 1
Reducing sugar content in sorghum biomass after chemical pretreatment

Chemical pretreatment	Content of reducing sugar, mg/g
Acidic	212
Alkaline	577

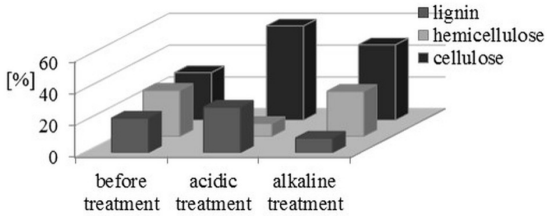

Figure 1: Chemical composition of sorghum biomass before and after chemical pretreatment

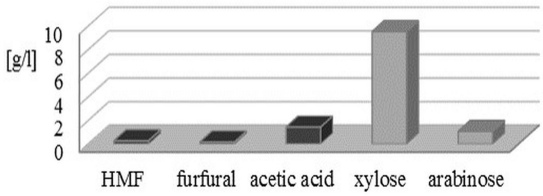

Figure 2: Content of toxins and pentoses in the liquid phase after acidic pretreatment of sorghum biomass

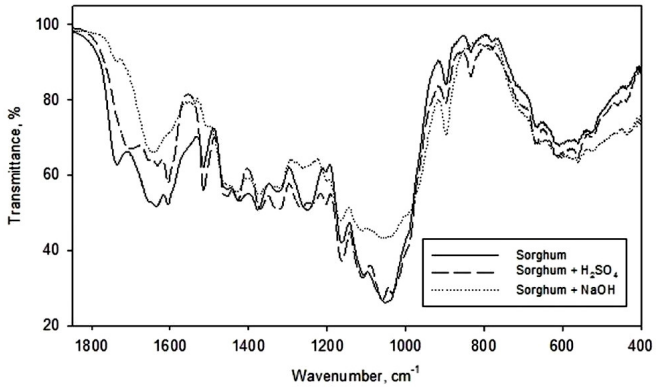

Figure 3: FTIR spectra of sorghum biomass before and after chemical treatments

CONCLUSION

It is suggested that the sorghum biomass is a valuable feedstock for ethanol production due to its easy cultivation, favorable properties and high amounts of monosaccharides.

Enzymatic hydrolysis of sorghum biomass could be significantly improved after pretreatment by sodium hydroxide and sulfuric acid. The main effect of the alkaline pretreatment is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides. The acidic pretreatment involves the depolymerization of hemicellulose sugars, but cellulose and lignin are only slightly affected. However, it seems that the sodium hydroxide is a more efficient pretreatment method before production of fermentable sugars from sorghum biomass for further processing.

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