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Monosaccharides from industrial hemp (*Cannabis sativa* L.) woody core pretreatment with ammonium hydroxide soaking treatment followed by enzymatic saccharification

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ABSTRACT

Ammonia soaking treatment was introduced for hemp woody core pretreatment to increase enzymatic saccharification of polysaccharides. Portions of the xylan, cellulose, and lignin were removed by aqueous ammonia soaking, which improved the enzymatic saccharification of cellulose and xylan. Following ammonia soaking, 37% (50℃-6 day treatment) to 61% (90℃-16 h treatment) of the cellulose was converted to glucose and 33% (50℃-6 day treatment) to 48% (90℃-16 h treatment) of the xylan to xylose. Cellulose responded better to enzymatic saccharification than did xylan after the ammonia soaking treatment. Aqueous ammonia soaking pretreatment was more effective than electron beam irradiation for increasing enzymatic saccharification of xylan and cellulose in hemp woody core.

Keywords: *ammonium hydroxide soaking, cellulose, xylan, lignin, enzymatic saccharification, industrial hemp* (*Cannabis sativa*L.)

1. Introduction

Global warming through carbon dioxide accumulation and depletion of fossil fuels has led to increasing interest in new and renewable energy sources. Plant biomass is renewably produced by sunlight and carbon dioxide and returns to energy and carbon dioxide. Plants fix solar radiation into the biomass body by photosynthesis via chlorophyll and many other biochemical reactions.

This plant biomass consists primarily of cellulose, hemicelluloses and lignin. Hemicelluloses in annual plants and hardwood are primarily composed of xylan with minor components such as glucomannan. Hemicelluloses in softwood are primarily of glucomannan with arabinoxylan as minor component.

Cellulose is a linearly linked polymer made up of glucose with β-1,4-glucosidic linkages and xylan is made up of xylose with β-1,4-xylosidic linkages. Saccharification of cellulose and xylan in biomass of

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annual plants by acids or enzymes produces glucose and xylose for use of bioenergy and biorefinery raw materials through appropriate fermentation technologies (1).

Pretreatment techniques have been developed to improve the conversion of polysaccharides in plant biomass to monosaccharides (2-5). Electron beam irradiation of hemp woody core altered the microstructure and improved saccharification of xylan and cellulose (6). Aqueous ammonia soaking and ammonia fiber explosion techniques have also been developed for biomass pretreatment (4, 5). Using corn stover as a raw material, ammonia soaking or ammonia fiber explosion techniques converted to the portion of the xylan and lignin in the biomass to a soluble form which improved enzymatic saccharification of corn stover. Although leaking of ammonia could present an environmental hazard, easy evaporation to ammonia gas provides a simple recycling process. In this study, aqueous ammonia soaking treatments were applied to increase saccharification of polysaccharides in hemp woody core and resulting chemical compositional changes were investigated. The effects of the pretreatment with aqueous ammonia soaking were also compared to electron beam irradiation.

2. Materials and Methods

2.1 Hemp woody core

Industrial hemp was kindly supplied by Dangjin Agricultural Technology Center (Dangjin, Chungnam, Korea) as agricultural residuals. The hemp, which was cultivated for hemp seed production, was harvested in November, 2006. Supplied hemp was debarked by hand to separate the bast fiber from the woody core, and these separated components were milled to less than 40 mesh using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) for chemical analysis.

2.2 Ammonium hydroxide soaking treatment

Hemp woody core meal (2.5 g oven-dried weight), was placed in a mini-reactor with 45.0 mL, 15.0 % aqueous ammonia solution, the reactor was sealed , and the target temperature and time were set. Treated samples were cooled in cold water and then filtered to collect the undissolved fraction. Non-dried samples were used for subsequent enzymatic saccharification and air-dried samples were used for chemical compositional analysis.

2.3 Enzymatic saccharification

Enzymatic hydrolysis was carried out to investigate the effects of ammonia soaking on carbohydrate composition and hydrolysis rate. The ammonia-soaked hemp powder solids were hydrolyzed using a commercial cellulase mixture, Celluclast 1.5L (20 FPU/g biomass) supplemented with the β-glucosidase preparation Novozyme 342 (25.0% of the volumetric Celluclast addition), both from Novozyme (Bagsvaerd, Denmark). All hydrolysis reactions were conducted at 50°C for 48 h in a shaking incubator (120 rpm).

2.4 Chemical analysis of untreated and ammonium hydroxide-soaked hemp woody core

Organic solvent-soluble extractives and hot water-soluble extractives contents were analyzed using the US National Renewable Energy Laboratory (NREL) procedure, except acetone was used instead of ethanol-benzene solvents (7). Acetone- and hot water-extracted hemp woody core samples were used for Klason lignin determination and carbohydrate compositional analysis.

Klason lignin content was analyzed following the NREL procedure; carbohydrate composition was analyzed by acid hydrolysis followed by ^{1}H-NMR spectroscopy(8).

The saccharified samples (200 μL) were diluted with 900 μL of acetonitrile and water (1:1 v/v), followed by centrifuging to precipitate any solid impurities in the diluted solutions. The concentrations

of xylose and glucose in the diluted solutions were measured using an evaporative light scattering detector (ELSD Model ELSD2000, tube temperature 80°C, gas flow 2.0 L/min, gain 4 Alltech Assts., Deerfield, IL, USA) and an isocratic HPLC (Waters 1525 Binary HPLC Pump, Waters 717 Plus Autosampler Waters Corp, Milford, MA, USA) with an apHera™ NH_2 column (4.6 mm i.d. × 250 mm Supelco, Bellefonte, PA, USA). An acetonitrile/water mixture (80:20 v/v) was used as the mobile phase with a 1.2 mL/min flow rate. The retention times for xylose and glucose were 7.9 and 12.0 min, respectively.

3. Results and Discussion

3.1 Chemical compositional changes due to ammonia soaking pretreatment

Longer-duration or higher-temperature ammonium hydroxide soaking pretreatment resulted in greater removal of ammonium hydroxide-soluble components (5). In this study, 50ºC for 6 day and 90ºC for 16 h were selected to compare the effects of temperature and time in the ammonium hydroxide soaking pretreatment on cellulose, xylan, and lignin in hemp woody core biomass.

Unlike yellow poplar or corn stover, hot water-soluble extractives in hemp woody core were relatively resistant to the ammonium hydroxide soaking treatment and most of the hot-water-soluble extractives remained as residuals (Table 1) (9, 10). More lignin was removed in the lower temperature–longer duration soaking than in the higher temperature–shorter duration soaking (Table 1). For carbohydrates, the higher temperature soaking treatment removed more xylan; however, the lower temperature soaking treatment removed more cellulose (Table 2). Overall, carbohydrate component removal was higher in the lower temperature–longer duration treatment.

Table 1. Chemical compositional changes due to ammonium hydroxide soaking pretreatment of hemp woody core

		Chemical Composition (%)			
	Residual (%)	Acetone solubles	Hot-water solubles	Lignin	Carbohydrates
90 ℃-16 h	80.4	0.2	9.4	15.0	55.8
50 ℃-6 day	77.2	0.2	10.0	13.4	53.6
Untreated	100.0	0.8	10.4	17.2	71.6

Table 2. Carbohydrate compositional changes by ammonium hydroxide soaking treatment to hemp woody core

	NMR peak areas		Relative composition of carbohydrates (%)		Composition of Carbohydrates (%)	
	Glucose	Xylose	Glucose	Xylose	Cellulose	Xylan
90 ℃-16 h	0.3749	1.6926	69.3	30.7	37.7	16.7
50 ℃-6 day	0.4774	1.7159	64.2	35.8	34.2	19.0
Untreated	0.4800	1.7036	64.0	36.0	39.7	22.4

3.2 Enhancement of enzymatic saccharification by ammonium hydroxide soaking pretreatment

Xylan- or lignin-removing lignocellulosic biomass pretreatments improve enzymatic saccharification (11-13). Ammonium hydroxide soaking of hemp woody core removed part of the lignin and xylan (Tables 1 and 2), which improved enzymatic saccharification of cellulose (Fig. 1) and xylan (Fig. 2).

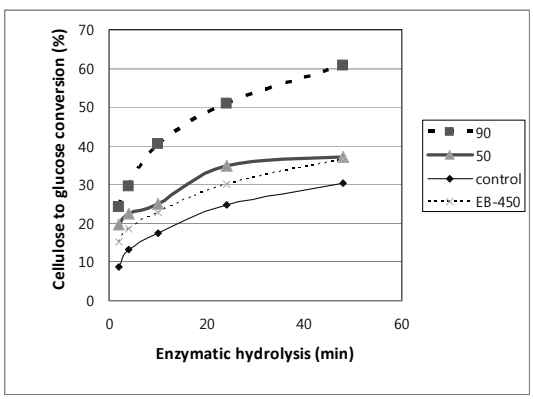

Fig. 1. Enzymatic saccharification of ammonium hydroxide-soaked hemp woody core with various treatment temperatures and times.

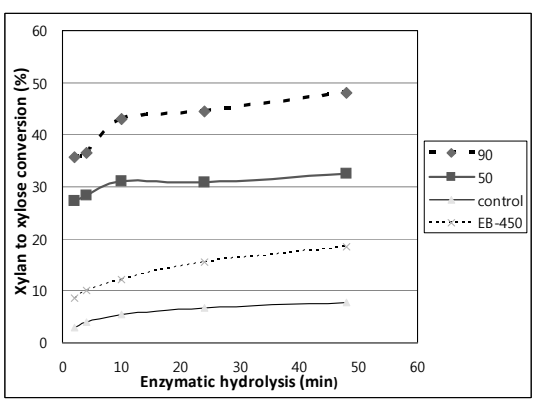

Fig. 2. Enzymatic saccharification of xylan to xylose in ammonium hydroxide-soaked hemp woody core with various treatment temperatures and times.

For untreated hemp woody core, 25% of the cellulose has been reported to be converted to glucose (6, 14). Electron bean irradiation can improve cellulose saccharification to 34−37% (6). Ammonia-soaked hemp woody core had a better response to enzymatic saccharification, with 37% glucose conversion for the 50ºC−6-day treatment and 61% for the 90ºC−16-h treatment.

For ammonium hydroxide-soaked hemp woody core, 33% of the xylan was converted to xylose for the 50ºC−16-h treatment and 48% was converted for the 90ºC−6-day treatment. Cellulose responded better to enzymatic saccharification than did xylan in hemp woody core, whether pretreated with electron beam irradiation (6) or ammonium hydroxide soaking. During cellulose hydrolysis, enzymatic conversion of cellulose to glucose slowed from 10 to 48 h (Fig. 1); however, this trend was less prominent than for xylan to xylose conversion (Fig. 2). In cellulose to glucose hydrolysis for the 90ºC−16-h treatment, 40.6% of the cellulose had been converted by 10 h hydrolysis and 60.7% by 48 h hydrolysis; 33.1% of the cellulose to glucose conversion occurred during the extended hydrolysis time from 10 to 48 h incubation. For the 50ºC−6-day treatment, 32.9% of the cellulose to glucose conversion occurred during extended hydrolysis from 10 to 48 h incubation. For xylan hydrolysis, the results of the ammonium hydroxide soaking treatment were different from those of cellulose hydrolysis, as shown in Figs. 1 and 2. For the 90 ºC−16-h treatment, the gain in the xylose yield during the extended hydrolysis time from 10 to 48 h was 5.0%, and was 1.5% for the 50ºC−6-day treatment (Fig. 2). For the 50ºC−6-day treatment, only 1.5% of the xylan to xylose conversion occurred during extended hydrolysis from 10 to 48 h; 95.4% of the xylan to xylose conversion had occurred by 10 h. For the 90ºC−16-h treatment, 89.6% of the xylan to xylose conversion had occurred by 10 h, and 10.4% of the hydrolysis occurred during the extended hydrolysis from 10 to 48 h. As the ammonium hydroxide soaking temperature increased from 50 to 90ºC, both the xylan

and cellulose hydrolysis yields improved.

Compared to electron beam irradiation (6), the aqueous ammonia soaking pretreatment was more effective; the cellulose to glucose conversion was 60.7% for the 90ºC−16-h treatment and 36.0% for the 450-kGy electron beam treatment. Greater xylan to xylose conversion occurred for the 90ºC−16-h aqueous ammonia soaking treatment (48.0%) than for the 450-kGy electron beam treatment (19.0%).

4. Conclusions

Ammonium hydroxide soaking treatment dissolved and removed portions of the cellulose, xylan, and lignin in hemp woody core, improving enzymatic saccharification of cellulose and xylan. With ammonium hydroxide soaking, 37% (50ºC treatment) to 61% (90ºC treatment) of the cellulose was converted to glucose and 33% (50ºC treatment) to 48% (90ºC treatment) of the xylan to xylose. Cellulose responded better to enzymatic saccharification following the higher-temperature ammonia soaking treatment.

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