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Sodium Hydroxide Pretreatment of Sunflower Stalks For Enzymatic Hydrolysis

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Abstract*—The effect of alkaline pretreatment parameters on the production of fermentable sugars from sunflower stalks was investigated. Dried and milled sunflower stalks were pretreated with NaOH (0.5, 2.0, 4.0% w/v) at different temperatures (60, 90 and 120˚C), and pretreatment times (15, 30, and 60 min). Enzymatic hydrolysis of the solid residue was carried out using a mixture of cellulase (60 FPU/g dry biomass) and β-Glucosidase (40 CBU/g dry biomass) at 50°C for 48 h. Glucose and xylose contents in the solutions were analyzed by HPLC. Raw sunflower stalks were found to contain 16% lignin, 32% cellulose, and 19% hemicellulose. After pretreatment, cellulose recovery ranged between 77% and 94%, and maximized with 2% NaOH, at 60˚C for 60 min. 52% of lignin was removed at 120˚C. While temperature increased from 60 to 120˚C, glucose recovery slightly increased up to 72% .

Keywords*—Lignocellulose, enzymatic hydrolysis, alkaline pretreatment, monomeric sugar

I. INTRODUCTION

Lignocellulosic biomass consist of mainly three components that are generally present as 40-50% cellulose, 20-30% hemicellulose, and 10-25% lignin. These plant materials can be used for production of fine chemicals, biofuels, enzymes and as a source of microbial fermentation [1].

The heterogeneous structure of lignocellulosic biomass requires some essential pre-processing before the enzymatic hydrolysis of the carbohydrates in this complex network. These pretreatments of lignocellulose alter its structure or remove some of the compounds in biomass. As a result of the pretreatment, the rate of enzymatic hydrolysis of cellulose and hemicellulose and the yield of fermentable sugars [2].

The main purpose of sodium hydroxide pretreatment on lignocellulosic biomass is removal of lignin. Sodium hydroxide pretreatment increases the porosity of biomass by breaking the ester bonds cross-linking lignin and hemicellulose [3].

Sunflower is the largest seed oil source in Turkey, with more than one million tons annual production [4]. After seed harvesting, sunflower stalks can be considered as readily available raw material for production of C-5, C-6 sugars.

The aim of this study was to investigate the effect of sodium hydroxide pretreatment on sunflower stalks for efficient enzymatic hydrolysis.

MATERIAL AND METHODS

A. Material

Sunflower stalks, used in this study were purchased from Trakya region, Turkey. The dried materials (9% moisture) were ground by grinder and screened with a sieve shaker to obtain particle sizes between 0.224-0.850 mm. The Aminex HPX 87H column, used in the HPLC studies, was purchased from Bio-Rad Laboratories (California, USA). All chemicals used were standard analytical grade. The standard reagents of xylose and glucose were purchased from Sigma-Aldrich.

B. Pretreatment

Alkali pretreatments were conducted in a high temperature alkali pretreatments were conducted in a high-pressure stirred 160 mL-stainless steel reactor.

Approximately 4 g of dry sunflower stalks were mixed with 80 mL NaOH (0.5, 2.0, 4.0% w/v) at different temperatures (60, 90 and 120 ^{o}C), and pretreatment times (15, 30, and 60 min). The solid to liquid ratio was kept at 1/20.

For high temperature alkaline pretreatment, the reactor was heated until desired temperature and pretreatment time was initiated. The solution was kept liquid under N_2 atmosphere. After the pre-treatment, the content of the reactor was cooled down to 80 °C. Then the wet material was filtrated for solid

recovery and washed with distilled water until the wash water's pH was neutral (pH= 7.0).

C. *Enzymatic Digestion of Sunflower Stalks*

Enzymatic hydrolysis was carried out in stoppered conical flasks (50 mL). The pH was adjusted to 4.8 with acetate buffer, and a mixture of cellulase (60 FPU/g dry biomass) and β-Glucosidase (40 CBU/g dry biomass) was added to the pretreated substrate in a total working volume of 10 ml. The hydrolysis reactions were carried out at 50 ^{o}C in an incubator for 48 h by shaking at 150 rpm. The reactions were stopped by boiling in a water bath for 15 minutes and hydrolysates were clarified by centrifuging at 5000 rpm for 5 min. Glucose and xylose contents of the supernatants were analyzed using HPLC.

D. Analytical Methods

The chemical composition of raw and pretreated sunflower stalks was determined according to NREL [5,6] methods. 0.3 g solid biomass was hydrolyzed by 3 mL of 72% (w/w) H_2SO_4 at 30^{o}C for 60 min; then, the reaction mixture was diluted to 4% (w/w) with distilled water and autoclaved at 121^{o}C for 60 minutes. Lignin content was determined by the remaining solid residue, cellulose and hemicellulose amounts were determined from the filtrate by using High Performance Liquid Chromatography (Agilent 1100). The HPLC system was mainly equipped with a Bio-Rad Aminex HPX-87P column (300 mm × 7.8 mm), and a refractive index detector. The analytical column was operated at 80°C with 0.2 µm filtered HPLC grade water as the mobile phase, which had a flow rate of 0.6 mL/min.

Total reducing sugars were determined by the dinitrosalicylic acid (DNS) method [7]. Enzyme activity of Celluclast 1.5L® was determined by NREL protocols and reported as Filter Paper Unit (FPU) [8]. One unit of FPU is defined as the amount of enzyme required to liberate 1µmol of glucose from Whatman no:1 filter paper per minute at 50 ^{o}C. One cellobiose unit (CBU) is the amount of enzyme that converts 1 µmol of cellobiose to 2 µmol of glucose per minute.

The following calculations were performed for the determination of percent cellulose recovery, lignin removal, hemicellulose removal, cellulose digestion, saccharification and glucose recovery:

$$Cellulose\ Recovery\ (\%) = \frac{\text{Amount of cellulose in pretreated solid}}{\text{Amount of cellulose in unpretreated solid}} x100$$

$$Hemicellulose\ Removal\ (\%) = \frac{\text{Amount of removed hemicellulose in pretreated solid}}{\text{Amount of hemicellulose in unpretreated solid}} x100$$

$$Lignin\ Removal\ (\%) = \frac{\text{Amount of removed lignin in pretreated solid}}{\text{Amount of lignin in unpretreated solid}} x100$$

$$Cellulose\ Digestion\ (\%) = \frac{\text{Amount of glucose producedx0.9}}{\text{Amount of cellulose in pretreated solid}} x100$$

$$Saccharification\ \ (\%) = \frac{\text{Amount of total reducing sugar producedx0.9}}{\text{Amount of cellulose and hemicellulose in pretreated solid}} x100$$

$$Glucose\ Recovery\ \ (\%) = \frac{\text{Amount of glucose producedx0.9}}{\text{Amount of cellulose in unpretreated solid}} x100$$

II. Results and Discussion

The composition of raw sunflower stalks was analyzed and found to be 32.45% cellulose, 19.12% hemicellulose and 15.52% lignin.

A. Effect of alkali concentration

The cellulose recovery, lignin and hemicellulose removal from the biomass was based on the solid fraction obtained after pretreatments of sunflower stalks at 60 ˚C, 30 min and 1/20 (wt/v) solid-liquid ratio. Effect of alkali concentration on the content of sunflower stalks is given in Fig. 1. Hemicellulose and lignin removal from sunflower stalks increased by increasing alkali concentration from 0.5 to 4%. Maximum cellulose recovery was obtained with 2wt %NaOH solution (91.41%)

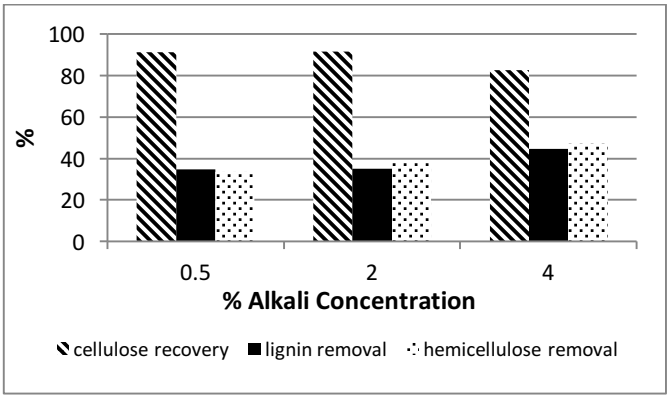

Fig. 1. Effect of alkali concentration on the content of sunflower stalks solid residue after pretreatment at 60 ˚C, 30 min and 1/20 (w/v) solid-liquid ratio

After the pretreatments, enzymatic hydrolysis was applied on recovered solids. It was observed that saccharifications increased by increasing alkali concentrations and the highest yield of cellulose digestion (98,34%) and glucose recovery (70.20%) were obtained for the pretreatment with 2 wt % NaOH solution (Fig. 2.)

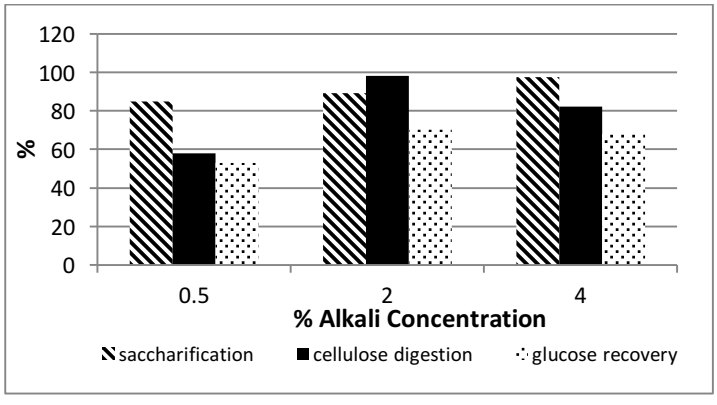

Fig. 2. Effect of alkali concentration on the saccharification, cellulose digestion and glucose recovery

B. Effect of temperature

The effect of temperature on solid residue compostion was investigated upon 30 min-pretreatment in the presence of 2 wt% NaOH (Fig. 3). While hemicellulose and lignin removal from sunflower stalks increased by rising the temperature from 60 to 120°C, cellulose recovery decreased. The highest percentage of cellulose recovery of 91.41% was achieved at 60˚C.

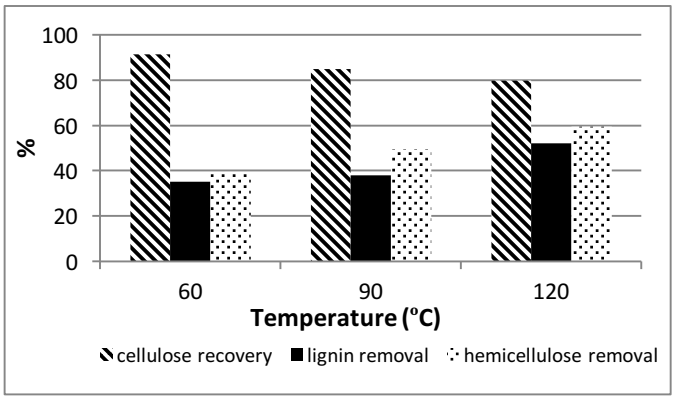

Fig. 3. Effect of temperature on the content of sunflower stalks

Upon enzymatic hydrolysis of the pretreated solids, saccharification (or production of total reducing sugars) increased slightly by raising the temperature; however, no significant change were observed in glucose recovery (Fig. 4). Saccharification yield was 89,3% at 60˚C and 97,27% at 120˚C. Glucose recovery yields were 70,20%, 70,64%, and 72,05%, for 60, 90 and 120°C, respectively.

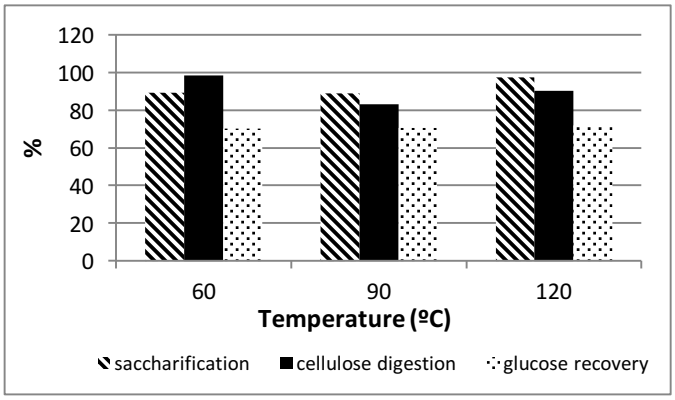

Fig. 4. Effect of temperature on the saccharification, cellulose digestion and glucose recovery

C. Effect of time

Effect of pretreatment duration (15, 30 and 60 min) was investigated at 60˚C and 2 wt % NaOH concentration. Increasing pretreatment time from 15 to 60 min improved the percent cellulose recovery from 84.74% to 94,44% (Fig. 5). Lignin removal was also enhanced from 25.77% to 30.75%. Hemicellulose removal increased by increasing pretreatment time.

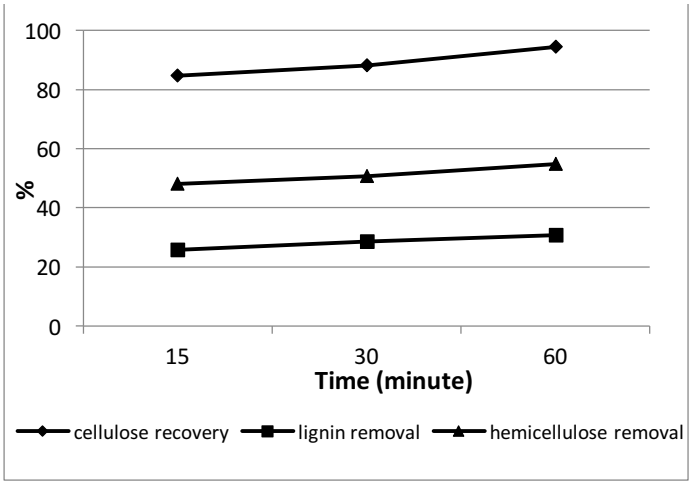

Fig. 5. Effect of time on the content of sunflower stalks

Enlongation of pretreatment duration had a positive effect on saccharification and provided a slight increase on glucose recovery (Fig. 6). Glucose recovery yields after 15-30-60 min pretreatments were 69.16%, 70,20% and 72,60%, respectively. The highest cellulose digestion value was 98,34% for 30 min-pretreatment.

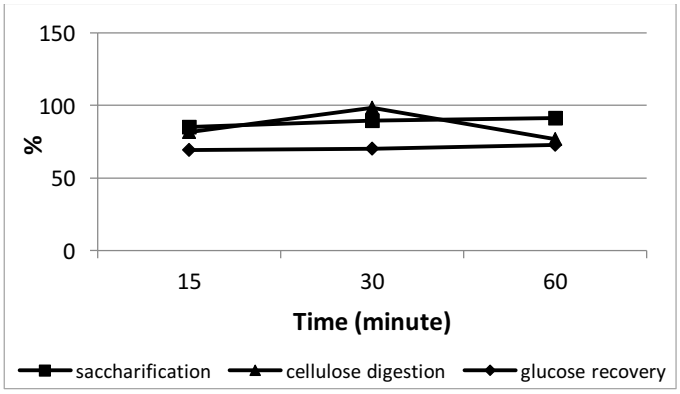

Fig. 6. Effect of time on the saccharification, cellulose digestion and glucose recovery

CONCLUSION

Alkaline pretreatment was conducted on sunflower stalks to investigate its effect on digestibility of the lignocellulosic biomass. Pretreatment parameters such as % NaOH, process duration and temperature influenced both the composition of the lignocellulosic biomass as well as the production of monomeric sugars upon enzymatic hydrolysis. Increasing alkali concentration and temperature during pretreatment resulted in efficient removal of lignin and hemicellulose; however, slight decrease in cellulose recovery was observed. Extending pretreatment time enhanced the removal of lignin and the recovery of cellulose; maximum cellulose digestion was observed after 30min- pretreatment. Hence, optimum pretreatment condition was selected as 2% NaOH, 60°C and 30min - processing time.

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