# Effect of different chemical pretreatment methods and enzymatic saccharification on chemical composition of sugarcane shoots and leaves

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## Abstract

Sugarcane shoots and leaves consist of 16.21% neutral detergent soluble (NDS), 38.43% hemicellulose, 34.06% cellulose, 5.51% lignin and 5.79% ash on dry solid (DS) basis and have the potential to serve as low cost feedstocks for ethanol production. To improve the enzymatic digestibility of these biomass and bioethanol production, four pretreatment methods had been investigated and compared, including: (1) alkali solution autoclaving pretreatment; (2) acid solution autoclaving pretreatment (3) two steps of alkali solution autoclaving followed by acid solution autoclaving pretreatment and (4) two steps of acid solution autoclaving followed by alkali solution autoclaving pretreatment. The results showed that cellulose conversion efficiencies throughout enzymatic saccharification of sugarcane shoots and leaves pretreated by alkali, (alkali+acid) and (acid+alkali) using autoclave at 121°C for 15 min were similar. But cellulose conversion efficiency of sugarcane shoots and leaves pretreated by acid using autoclave at 121°C for 15 min was significantly lower than other methods.

**Keywords:** Sugarcane shoots and leaves, lignocellulosic biomass, pretreatment and enzymatic saccharification, cellulosic ethanol production.

## Introduction

Bioethanol (ethyl alcohol, fuel ethanol) is the most-used liquid biofuel in the world. It is obtain from energy-rich crop such as sugar cane and corn. Ethanol can be directly employed as a sole fuel in vehicles or as gasohol oxygenate increasing its oxygen content and allowing a better hydrocarbon oxidation that reduces the amount of aromatic compounds and carbon monoxide released into atmosphere. For this reason, fuel grade ethanol is the market with the most rapid growth rate in America and Europe. Furthermore, the fuel ethanol can be obtains from lignocellulosic biomass as well, but its production is much more complex. Nowadays, great efforts are being made to diminish the production cost of lignocellulosic ethanol (Cardona et al. 2010).

Lignocellulose material is composed of heterogeneous complex of carbohydrate polymers. Cellulose and hemicellulose are densely packed by layers of lignin, which protect them against enzymatic hydrolysis. So it is necessary to have a pretreatment step to break lignin seal to expose cellulose and hemicellulose for enzymatic action. Pretreatment aims to decrease crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break lignin seal. Pretreatment makes cellulose more accessible to enzymes so that conversion of carbohydrate polymers into fermentable sugars can be achieved more rapidly and with more yields. Pretreatment includes physical, chemical and thermal methods and their combinations (Yang and Wyman 2008). Especially, chemical pretreatment is important to enzymatic digestibility by the most promising chemicals for pretreatment of lignocellulose is acid and alkali (Cardona et al. 2010).

Dilute acid pretreatment predominantly affect hemicellulose with little impact on lignin degradation. Acid pretreatment will solubilize the hemicellulose, and by this, making the cellulose better accessible to enzymes. Acid pretreatment is usually carried out using mineral acids like HCl and $H_2SO_4$. Following dilute acid treatment, the enzyme cellulase is needed for hydrolysis of the remaining carbohydrates in the treated biomass (Saha et al. 2005; Guo et al. 2008).

Alkali pretreatment involves the application of alkaline solutions like NaOH or KOH to remove lignin and a part of the hemicelluloses, and efficiently increase the accessibility of enzyme to the cellulose. The alkali pretreatment can result in a sharp increase in saccharification yields. Pretreatment can be performed at low temperatures but with a relatively long time and high concentration of the base. Compared with acid or oxidative reagents, alkali treatment appears to be the most effective method in breaking the ester bonds between lignin, hemicellulose and cellulose, and avoiding fragmentation of the hemicellulose polymers (Xu et al. 2010; Zhang et al. 2011)

The objective of this study was to compare the effects of different chemical pretreatment methods and enzymatic saccharification on sugarcane shoots and leaves composition between alkali pretreatment, acid pretreatment, alkali followed by acid pretreatment and acid followed by alkali pretreatment.

## Methodology

Substrate

Sugarcane (*Saccharum officinarum* Linn) shoots and leaves (procured from sugarcane growers in Kamphaeng Phet province, Thailand) were sundried and milled in a hammer mill, then pass through a 2.0 mm screen. The milled sugarcane shoots and leaves were stored at room temperature and used as substrate in the experiments.

Alkali pretreatment

Milled sugarcane shoots and leaves 2.4 kg dry weight were slurried in 16 L of 2% w/v NaOH in 20 L stainless steel reactor. They were then pretreated in an autoclave at 121 °C, 15 lb/in$^2$ for 15 min. After pretreatment and cooled down, the solid residue was separated from liquid by filter through muslin cloth. The solid residue was then washed with water several times. After adjusted to pH 5.0, the solid was again separated from liquid by filtering through muslin cloth and used as substrate for enzymatic saccharification and analysis chemical composition of sugarcane shoots and leaves after saccharification.

Acid pretreatment

Milled sugarcane shoots and leaves 2.1 kg dry weight were slurried in 14 L of 2% w/v $H_2SO_4$ in 20 L stainless steel reactor. They were then pretreated in an autoclave at 121 °C, 15 lb/in$^2$ for 15 min. After pretreatment and cooled down, the solid residue was separated from liquid by filter through muslin cloth. The solid residue was then washed with water several times. After adjusted to pH 5.0, the solid was again separated from liquid by filtering through muslin cloth and used as substrate for enzymatic saccharification and analysis chemical composition of sugarcane shoots and leaves after saccharification.

(Alkali + Acid) pretreatment

Wet pretreated sugarcane shoots and leaves by alkali 0.8 kg dry weight (average 59.93 % moisture, adjust pH to 2) were slurried in 5.33 L of 2% w/v $H_2SO_4$ in 10 L stainless steel reactor. They were then pretreated in an autoclave at 121 °C, 15 lb/in$^2$ for 15 min. After pretreatment and cooled down, the solid residue was separated from liquid by filter through muslin cloth. The solid residue was then washed with water several times. After adjusted to pH 5.0, the solid was again separated from liquid by filtering through muslin cloth and used as substrate for enzymatic saccharification and analysis chemical composition of sugarcane shoots and leaves after saccharification.

(Acid + Alkali) pretreatment

Wet pretreated sugarcane shoots and leaves by acid 0.9 kg dry weight (average 49.22 % moisture, adjust pH to 10) were slurried in 6 L of 2% w/v NaOH in 10 L stainless steel reactor. They were then pretreated in an autoclave at 121 °C, 15 $lb/in^2$ for 15 min. After pretreatment and cooled down, the solid residue was separated from liquid by filter through muslin cloth. The solid residue was then washed with water several times. After adjusted to pH 5.0, the solid was again separated from liquid by filtering through muslin cloth and used as substrate for enzymatic saccharification and analysis chemical composition of sugarcane shoots and leaves after saccharification.

Enzymatic saccharification

Enzymatic saccharification was done by using wet pretreated solid residue (525 g DS), washed with water and adjusted to pH 5.0. Cellic® CTec2 (5.325 FPU/g DS) was then added to the pretreated solid residue fraction and saccharification in fermenter reactor at 50 °C, 200 rpm for 48 h. the solid was again separated from liquid by filtering through muslin cloth and solid use to analysis chemical composition of sugarcane shoots and leaves after saccharification.

Analytical methods

The contents of neutral detergent soluble (NDS), cellulose, hemicellulose, acid detergent lignin (ADL) and acid insoluble ash (AIA) were determined according to the method of Van Soest et al. (1991). All experiments were conducted in triplicate.

**Results**

Composition of raw material

Sugarcane shoots and leaves used in this work contained 16.21% NDS 35.2% cellulose, 23.4% hemicellulose, 12.6% lignin (ADL) and 6.59% ash (AIA) on dry solid (DS) basis.

Alkali pretreatment and enzymatic saccharification

The average chemical composition of pretreated sugar cane shoots and leaves by alkali was 6.95 g NDS, 8.67 g hemicellulose, 1.51 g lignin, 32.23 g cellulose and 1.17 g ash and the average weight was 50.53 g DS. After enzymatic saccharification of alkali pretreated sugar cane shoots and leaves, the average weight of solid residue was found to be 19.05 g. The average chemical composition of solid residue was 7.25 g NDS, 3.36 g hemicellulose, 1.37 g lignin, 6.75 g cellulose and 0.32 g ash (figure 1).

Acid pretreatment

The average chemical composition of pretreated sugar cane shoots and leaves by acid was 9.71 g NDS, 4.58 g hemicellulose, 5.56 g lignin, 34.97 g cellulose and 1.52 g ash and the average weight was 56.33 g DS. After enzymatic saccharification of acid pretreated sugar cane shoots and leaves, the average weight of solid residue was found to be 39.25 g. The average chemical composition of solid residue was 9.39 g NDS, 1.62 g hemicellulose, 5.90 g lignin, 20.39 g cellulose and 1.41 g ash (figure 2).

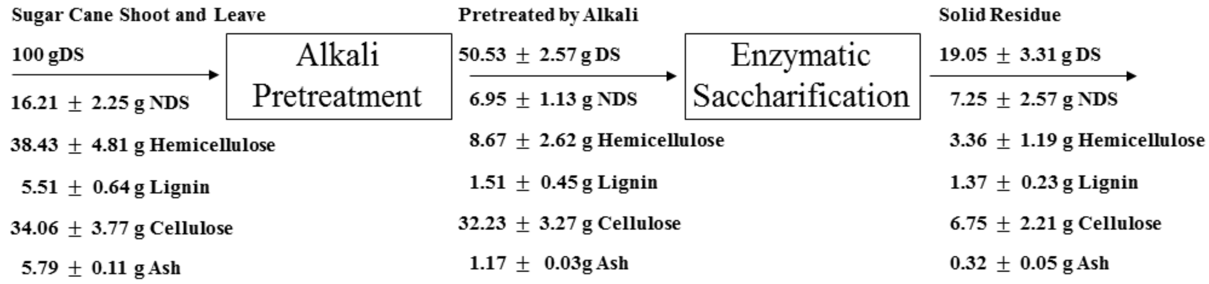


**Figure 1.** Material balance of sugarcane shoots and leaves after alkali pretreatment and enzymatic saccharification basis initial raw material is 100 g DS.

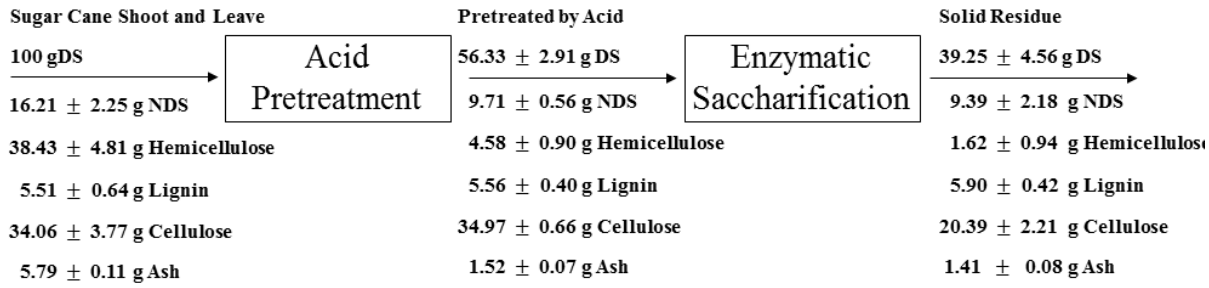


**Figure 2.** Material balance of sugarcane shoots and leaves after acid pretreatment and enzymatic saccharification basis initial raw material is 100 g DS.

(Alkali + Acid) pretreatment

The average chemical composition of pretreated sugar cane shoots and leaves by alkali followed acid was 6.99 g NDS, 3.21 g hemicellulose, 1.52 g lignin, 32.59 g cellulose and 0.22 g ash and the average weight was 44.54 g DS. After enzymatic saccharification of alkali+acid pretreated sugar cane shoots and leaves, the average weight of solid residue was found to be 19.71 g. The average chemical composition of solid residue was 7.12 g NDS, 3.19 g hemicellulose, 1.42 g lignin, 7.74 g cellulose and 0.22 g ash (figure 3).

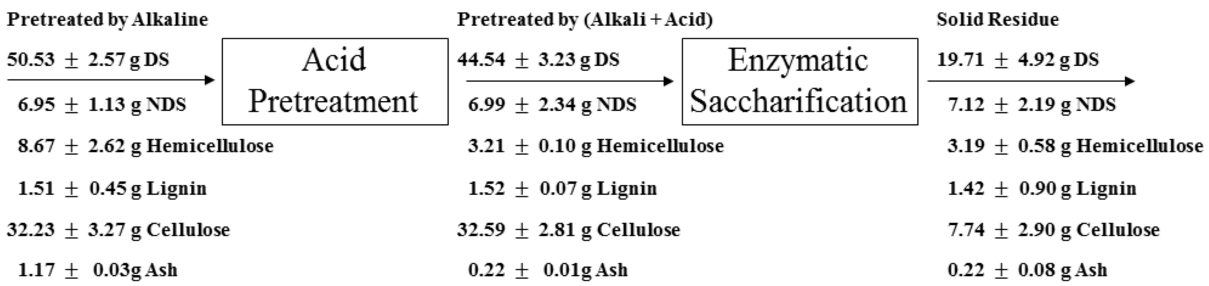


**Figure 3.** Material balance of sugarcane shoots and leaves after alkali followed by acid pretreatment and enzymatic saccharification basis initial raw material is 100 g DS.

(Acid + Alkali) pretreatment

The average chemical composition of pretreated sugar cane shoots and leaves by acid followed alkali was 6.49 g NDS, 2.34 g hemicellulose, 1.69 g lignin, 23.49 g cellulose and 0.13 g ash and the average weight was 34.13 g DS. After enzymatic saccharification of acid+alkali pretreated sugar cane shoots and leaves, the average weight of solid residue was found to be 14.68 g. The average chemical composition of solid residue was 6.48 g NDS, 2.45 g hemicellulose, 0.98 g lignin, 4.71 g cellulose and 0.06 g ash (figure 4).

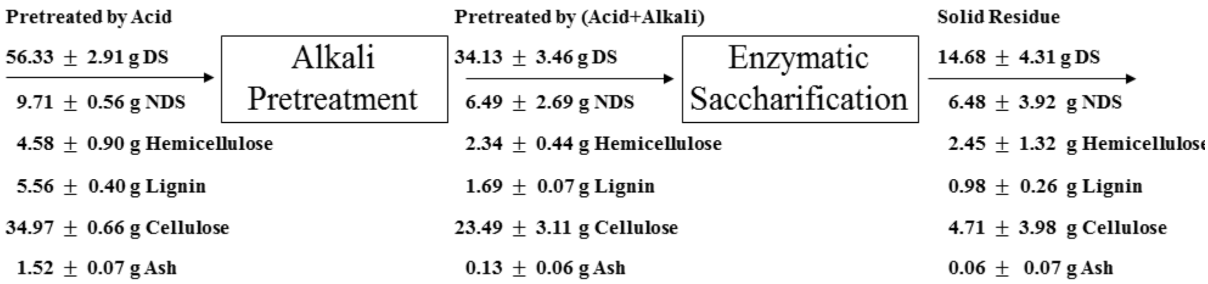


**Figure 4.** Material balance of sugarcane shoots and leaves after acid followed by alkali pretreatment and enzymatic saccharification basis initial raw material is 100 g DS.

## Discussion

Lignocellulosic biomass requires pretreatment, mainly because the lignin in plant cell walls forms a barrier against enzyme attack. An ideal pretreatment is to reduce the lignin content and crystallinity of the cellulose and increases surface areas (Tahagi et al. 1977). In the present study, four pretreatment methods was alkali, acid and two stages of alkali followed by acid, acid followed by alkali pretreatment were compared. The cellulose conversion efficiencies obtained after enzymatic hydrolysis of substrate pretreated with alkali, acid, (alkali+acid) and (acid+alkali) were found to be 79.06, 41.69, 76.25, and 79.95%, respectively. Diluted alkali pretreatment of lignocellulosic materials has been found to cause swelling, leading to an increase in internal surface area of that material and disruption of the lignin structure (Cao et al. 2012). Cellulose fraction would be retained to obtain as much as glucose for ethanol production. Taherzdeh and Karimi (2008) reported that elevated temperature could enhance lignin removal from lignocellulosic materials. Silverstein et al. (2007) also reported that 2% NaOH at 121 °C was the best pretreatment condition. The lignin removal increased enzyme effectiveness by eliminating nonproductive adsorption sites and by increasing access to cellulose and hemicellulose (Kumar et al. 2009).

## Conclusion

The cellulose conversion efficiency obtained after enzymatic saccharification of substrate pretreated with alkali, (alkali+acid) and (acid+alkali) using autoclave at 121°C for 15 min were similar.

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## References

Cardona, C.A., Sanchez, O.J., Gutierrez, L.F. (2010) Process synthesis for fuel ethanol production, Taylor & Francis Group Manizales, Colombia pp. 61 - 62.

Cao, W., Sun, C., Liu, R., Yin, R., Wu, X. (2012) Comparison of the effects of five pretreatmnent methods on enhancing the enzymatic digestibility and ethanol production from sweet sorghum bagasse. Bioresource. Technology 111:215-221.

Guo, G.L., Chen, W.H., Men, W.H., Hwang, W.H. (2008) Characterization of dilute acid pretreatment of silvergrass for ethanol production. Bioresource Technology 99:6046-6053.

Kumar, P., Barrett, D.M. Delwiche, M.J., Stroeve, P., (2009) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production, Industrial Engineering Chemical Research 48:3713-3729.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, V.Y. (2005) Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol, Process Biochemical. 40:3693-3700.

Silverstein, R.A., Chen, A., Sharma-Shivappa, R.R., Boyette, M.D., Osborne, J. (2007) A comparison of chemical pretreatment methods for improving saccharification of cotton stalks, Bioresour. Technol. 98:3000-3011.

Taherzadeh, M.J., Karimi, K. (2008) Pretreatment of lignocellulosic waster to improve ethanol and biogas production: A review, Inter Journal of Molecular. Science 9:1621-1651.

Takagi, M., Abe, S., Suzuki, S., Emert, G.H., Yara, N. (1997) A method for production of alcohol directly from cellulose using cellulase and yeast, in: T.K. Ghose, I.I.T. Delhi (Eds.), Proceedings of Bioconversion of Cellulosic Substances into Energy, Chemicals and Microbial Protein, pp. 551-571.

Van Soest, P.J., Robertson, J.B., Lewis, B.A. (1991) Method of dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74:3583-3597.

Xu, J., Cheng, J.J., Sharma-Shivappa, R.R.., Burn J.C. (2010) Sodium hydroxide pretreatment of switchgrass for ethanol production. Energy Fuels 24:2113-2119.

Yang, B. and Wyman, C. E., (2008) Pretreatment: The key to unlocking low-cost cellulosic ethanol. Biofuels Bioproduction and Biorefining 2:26 – 40.

Zhang J., Ma, X., Yu, J., Zhang, X., Tan, T. (2011) The effects of four different pretreatments on enzymatic hydrolysis of sweet sorghum bagasse, Bioresour. Technol. 102:4585-4589.