American Society of Agricultural and Biological Engineers

An ASABE Meeting Presentation

Paper Number: 076041

Potential of Temulose™ for Ethanol Production (Part 2)

Froilan L. Aquino, Grad. Research Assistant

Biological and Agricultural Engineering, TAMU, College Station, TX

Joan R. Hernandez, Grad. Research Assistant

Biological and Agricultural Engineering, TAMU, College Station, TX

Sergio C. Capareda, Assistant Professor

Biological and Agricultural Engineering, TAMU, College Station, TX

Written for presentation at the
2007 ASABE Annual International Meeting
Sponsored by ASABE
Minneapolis Convention Center
Minneapolis, Minnesota
17 - 20 June 2007

Abstract. *Temulose™, a hemicellulose extract from wood has a large potential for ethanol production due to its high available sugar content. We have investigated the effect of delignification on the substrate by using various amounts of $CaCO_3$ and NaOH treatment. The presence of high amounts of inhibitory compounds in the substrate (i.e. phenolic compounds and furfurals) could limit ethanol production from yeast fermentation. The different $CaCO_3$ and NaOH treatments resulted to various pH and Brix levels for the substrate. It is expected that the pretreatment process will effectively remove much of the lignin inhibitors. After four consecutive days of fermentation, it is projected that ethanol will be produced and the highest ethanol yield will be observed from the sample with the highest pH level (5 % $CaCO_3$ and 1 % NaOH). The production of ethanol is also expected to decelerate and eventually level off after the fourth day of fermentation.*

Keywords. Temulose™, hemicellulose extract, wood sugar, delignification, fermentation, ethanol

Introduction

Ethanol production from different sources has been continuously increasing throughout the world, particularly in the developed countries such as the United States and Europe (Palmarola-Adrados et al., 2005, Saha et al., 2005; Sun et al., 2005; Haan et al., 2007a). Ethanol is a clean, renewable, alternative fuel to some petroleum-based products. Fermentation is the biochemical process of converting simple sugars to ethanol. Lignocellulosic biomass, such as those coming from municipal solid wastes, forestry by-products and agricultural crop residues, is abundant and contains high amounts of sugars which made it a very promising source of fuel ethanol (Haan et al., 2007b; Palmarola-Adrados et al., 2005). The main issue with lignocellulosic biomass is that the sugars are not readily available, thus, the hydrolytic processes (i.e. chemical or enzymatic) should be employed first to free the reducing sugars contained in the lignin before they can be used for fermentation. Moreover, hydrolysis of biomass is usually an expensive process and most of the time produces low yield (Sun et al., 2005; Haan et al., 2007b; Champagne, 2007; Borjesson et al., 2006).

Temulose™, a hemicellulose wood extract, is a product of Temple-Inland (Diboll, Texas) from the wood processing plant. It is primarily used as a substitute to cane molasses, which is a supplement for cattle feed due to its high nutritional content. According to the 5-year average compositional analysis published by Temple-Inland in 2006, Temulose™ has high carbohydrate content (57.6 %) containing 2.4 % arabinose, 6.8 % xylose, 4.4 % galactose, 5.2 % glucose, 17.8 % mannose and 19.5 % other sugars. Temulose™ is also 100 % water soluble and contains no more than 39 % moisture. It is also found that hemicellulose extract, such as Temulose™, contains high amounts of phenolic compounds which are beneficial to ruminants because of its capability of protecting the rumen from microbial degradation (Grainger, 2006). This is also the same reason (high phenols) why Temulose™ cannot be used directly as a substrate for ethanol fermentation.

According to Berlin et al., (2006), during cellulose hydrolysis of softwoods, lignin or lignin-carbohydrate complex (LCC) that produces high amounts of phenolic compounds inhibits the reducing enzymes such as cellulase, xylanase and β-glucosidase, which means that the conversion of cellulose and hemicellulose components to fermentable sugars is also reduced. Lignin-derived inhibitors such as hydroxymethylfuraldehyde, hydroxybenzaldehyde, vanillin and syringaldehyde could be present in some cellulosic biomass and could affect ethanol production (Davis et al., 2005). In order to optimize ethanol production from lignocellulosic materials, a detoxification (e.g. alkaline pretreatment) process is required (Champagne, 2007; Davis et al., 2005). Dawson and Boopathy (2007) have used H_2O_2 for the alkaline pretreatment of post-harvest sugarcane residue. The pretreatment process increases the pH level of the substrate and effectively removes lignin. Yu and Zhang (2003) on the other hand have used ten detoxification methods including either single addition of solid $Ca(OH)_2$ or its combinations with absorbents as pretreatments for cellulose pyrolysate. The most completely fermented substrate by *Saccharomyces cerevisiae* is the one that was neutralized by adding solid $Ca(OH)_2$ and treated with four adsorbents: activated carbon, diatomite, bentonite, zeolite (10 % w/v) by stirring for 80 minutes and filtering repeatedly.

The study aims to investigate the possibility of producing ethanol from Temulose™ by employing a novel pretreatment method for detoxification. Calcium carbonate ($CaCO_3$) in powder form and sodium hydroxide (NaOH) pellets were used as adsorbents and neutralizer for Temulose™. We used commercially available yeast (*S. cerevisiae*) for the fermentation process which lasted for five days. The sugar content as well as the ethanol yield was determined using an HPLC system equipped with refractive index detector.

Objectives

The study generally aims to asses the feasibility of producing ethanol from Temulose™, specifically it aims to:

1) determine the effect of calcium carbonate ($CaCO_3$) washing at varying amounts on the yield of ethanol;
2) determine the effect of pH on the trend of ethanol production; and
3) suggest an optimum condition for ethanol production from Temulose™ based on the parameters used in the study.

Methodology

Materials

The substrate, Temulose™ (hemicellulose extract), was provided by Temple-Inland, Diboll, Texas. The substrate came from extracts of hydrolyzed wood in the wood processing plant and has the following characteristics: black liquid, viscous, and sweet odor. A commercially available yeast, *S. cerevisiae*, that has a high alcohol tolerant (>12 %), was used for the fermentation process.

Calcium carbonate ($CaCO_3$) and sodium hydroxide (NaOH) were reagent grade and were obtained from local merchants. Sugar standards (sucrose, glucose, arabinose, mannose, cellubiose, xylose and galactose) and ethanol standard were chromatographically pure and were obtained directly from Sigma. The other chemicals used in the experiment were obtained locally and of the highest purity.

Preparation and pretreatment of Temulose™

Temulose™ was first vacuum filtered through a filter paper with 0.45 um diameter pores to remove the residues. One hundred (100) milliliters of substrate was transferred into each 250 ml Erlenmeyer flasks and was then treated with varying amounts of $CaCO_3$ (1 %, 2 %, 3 %, 4 % and 5 % w/v). Each flask was added with one (1) gram of NaOH pellet. The mixture was then heated until boiling while continuously stirred on a stirrer-hotplate (Fischer Scientific) for 40 minutes. The mixtures were vacuum filtered for several times after cooling to remove the residual $CaCO_3$ from the substrate. The filtered mixtures were then sterilized at 121 ^{0}C and 15 psi for 15 min. This step was done to eliminate other microorganisms that might be present in the substrate and could affect the fermentation process.

Inoculation and fermentation

Brewer's yeast (*S. cerevisiae*) was propagated in the prepared yeast malt (YM) broth medium containing 200 g starch substrate, 3 g peptone, 1 g KH_2PO_4, and 1 g NH_4Cl at pH 5.6. The medium was then inoculated with 48 h yeast culture (10 % v/v) and was incubated in a rotary shaker (150 rpm) at 35 ^{0}C for 72 h.

Ten (10) milliliters (10 % v/v) of the prepared inoculum was added to each of the prepared substrate having different alkaline treatments. A substrate without any treatment (control) was also inoculated for comparison. All fermentation samples were placed inside a shaker-incubator with settings at 32 ^{0}C and 150 rpm for five consecutive days. The experimental setup was done in duplicate to verify the results and the average values were reported in this paper.

Analytical procedures

An aliquot (ca. 10 ml) of sample was taken everyday from each treated sample for sugar and ethanol analysis. A high performance liquid chromatograph (HPLC) from LCD Analytical (Model Consta Metric 3200) equipped with an autosampler, a Shodex SP 18 packed column and a refractive index (RI) detector was used for identification and quantification of different sugars as well as the ethanol from the samples. The column temperature was set at 78 ^{0}C and the flowrate of eluent (HPLC grade water) was set at 0.8 ml min^{-1}. The temperature was running in an isocratic mode and the total run time for the analysis was set to 20 min per sample.

Preliminary Results: Pretreatment process

The detoxification of Temulose^{TM} was carried out using different amounts of $CaCO_3$ powder and NaOH pellets. The pretreatment process was started by filtering the substrate, adding the neutralizing chemicals, heating and continuous boiling to dissolve NaOH and to bind most of the lignin with $CaCO_3$, and repeated filtration to remove the residues. After the pretreatment process, the pH levels of each treated sample were increased at increasing values. The yeast was added into the substrate and was placed in a shaker-incubator to facilitate fermentation.

We expect the following results after the completion of the research:

1. Inhibitory chemicals will be removed by the new suggested pretreatment methods.
2. Ethanol will be produced from the substrates with different pretreatments.
3. The higher the amount of chemical treatment (detoxification) in the substrate, the higher the ethanol yield expected.
4. The higher the pH value of Temulose^{TM} the more suitable is the substrate for ethanol fermentation.
5. The production of ethanol will be completed after four consecutive days and will eventually level off.

Conclusion

The detoxification of Temulose^{TM} was performed by applying a novel method using $CaCO_3$ and NaOH. These chemicals are expected to successfully remove much of the lignin and neutralize the phenolic compounds in the substrate that could cause inhibitory effects to reducing enzymes and yeast. It is also expected that high ethanol yield will be observed on the substrate with the highest amount of $CaCO_3$ and NaOH treatment. Although producing ethanol was the ultimate goal of the study, eliminating the inhibitory compounds to make the substrate yeast-friendly was also achieved. Further study is needed to determine the effect of using other chemical adsorbents to be used for the detoxification of Temulose^{TM} and make it more suitable for ethanol production.

Acknowledgements

The authors would like to thank the following groups or individuals for providing significant contribution in the conduct of the study:

1) Temple-Inland, Diboll, Texas
2) Texas Agricultural Experiment Station (TAES)
3) Jacquelyn Michalka and BAEN student workers

References

Berlin, A., M. Balakshin, N. Gilkes, J. Kadla, V. Maximenko, S. Kubo, J. Saddler, 2006. Inhibition of cellulase, xylanase, β-glucosidase activities by softwood lignin preparations. Journal of Biotechnology. 125: 198-209.

Borjesson, J., R. Peterson, F. Tjerneld, 2007. Enhanzed enzymatic conversion of softwood lignocellulose by poly(ethylene glycol) addition. Enzyme and Microbial Technology. 40: 754-762.

Champagne, P., 2007. Feasibility of producing bio-ethanol from waste residues: A Canadian perspective feasibility of producing bio-ethanol from waste residues in Canada. Resources, Conservation and Recycling. 50: 211-230.

Davis, L., Y.J. Jeon, C. Svenson, P. Rogers, J. Pearce, P. Peiris, 2005. Evaluation of wheat stillage for ethanol production by recombinant *Zymomonas mobilis*. Biomass and Bioenergy. 29: 49-59.

Dawson, L., R. Boopathy, 2007. Use of post-harvest residue for ethanol production. Bioresource Technology. 98: 1695-1699.

Grainger, R., 2006. Temulose™ Guaranteed analysis. Temple-Inland, Diboll, Texas. www.templeinland.com. Accessed date: May 10, 2007.

Haan, R.D., S.H. Rose, L.R. Lynd, W.H. Van Zyl, 2007a. Hydrolysis and fermentation of amorphous cellulose by recombinant *Saccharomyces cerevisiae*. Metabolic Engineering. 9: 87-94.

Haan, R.D., J.E. Mcbride, D.C. La Grange, L.R. Lynd, W.H. Van Zyl, 2007b. Functional expression of cellubiohydrolases in *Saccharomyces cerevisiae* towards one-step conversion of cellulose to ethanol. Enzyme and Microbial Technology. 40: 1291-1299.

Palmarola-Adrados, B., P. Choteborska, M. Galbe, G. Zacchi, 2005. Ethanol production from non-starch carbohydrates of wheat bran. Bioresource Technology. 96: 843-850.

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

Sun, Y. and J. Cheng, 2005. Dilute acid pretreatment of rye straw and Bermuda grass for ethanol production. Bioresource Technology. 96: 1599-1606.

Yu, Z. and H. Zhang, 2003. Pretreatments of cellulose pyrolysate for ethanol production by *Saccharomyces cerevisiae, Pichia* sp., YP-1 and *Zymomonas mobilis.* Biomass and Bioenergy. 24: 257-262.