Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 98 (2007) 1695–1699
BIORESOURCE TECHNOLOGY

Use of post-harvest sugarcane residue for ethanol production

Letha Dawson, Raj Boopathy *

Department of Biological Sciences, Nicholls State University Thibodaux, LA 70310, USA

Received 28 April 2006; received in revised form 10 July 2006; accepted 10 July 2006
Available online 28 August 2006

Abstract

Agricultural residues are produced in large quantities throughout the world. Approximately, 1 kg of residue is produced for each kilogram of grains harvested. This ratio of grain/residue translates into an excess of 40 billion ton of crop residue produced each year in the USA. These residues are renewable resources that could be used to produce ethanol and many other value added products. In this study, we demonstrate that the post-harvest sugar cane residue could be used to produce fuel grade ethanol. A chemical pre-treatment process using alkaline peroxide or acid hydrolysis was applied to remove lignin, which acts as physical barrier to cellulolytic enzymes. Yeast *Saccharomyces cerevisiae* ATCC strain 765 was used in the experiment. The pre-treatment process effectively removed lignin. Ethanol production in the culture sample was monitored using high performance liquid chromatography. The results indicate that ethanol can be made from the sugarcane residue. The fermentation system needs to be optimized further to scale up the process for large-scale production of ethanol from sugar cane residue.

Keywords: Ethanol; Cellulose; Lignin; Acid hydrolysis; Yeast

1. Introduction

Production of fuel alcohol from cellulosic feedstock is of growing interest worldwide. Cellulosic biomass is an abundant renewable resource on earth and includes various agricultural residues (Krishna and Chowdary, 2000). Some of these agricultural residues such as straw, cornhusk, and sugarcane residue represent an abundant, inexpensive, and readily available source of renewable lignocellulosic biomass (Gould, 1984). At the present time, this readily available biomass is considered as a waste and is disposed of through agricultural burning after harvest.

Sugar production is a major industry in south Louisiana, and for the past two hundred years sugarcane farming has been a vital component of Louisiana's economy and culture (LSU Ag Center, 2003). As of 2004, there were 461,738 acres of sugarcane grown by 718 producers within 24 Louisiana parishes. Approximately 424,799 acres were harvested for sugar, producing a total of 1,174,028 tons of sugar (LSU Ag Center, 2004). Every year after sugarcane is harvested, farmers typically reduce residue by open air burning. This is a cost-effective way to remove the fibrous content that would otherwise significantly reduce milling efficiency and decrease profits, as well as to clear residue from the field that hinders farming (Boopathy et al., 2002).

The open air burning practice not only affects the quality of air but also the quality of life to those who live in the area. Smoke from open air burning contains respirable particles that are less than 10 μ in size (Givens, 1996). National air quality standards have been set for particulate matter that is equal to or less than 10 μ. Studies have suggested that populations exposed to particles of that nature suffer from asthma and bronchitis in addition to pulmonary morbidity and mortality (Koren, 1995). One alternative to open air burning is the production of ethanol from sugarcane residue. Ethanol is a clean burning, renewable resource that can be produced from fermented cellulosic biomass (Yu and Zhang, 2004). In many parts of the world, demand for

* Corresponding author. Tel.: +1 985 448 4716; fax: +1 985 493 2496.
E-mail address: biol-rrb@nicholls.edu (R. Boopathy).

0960-8524/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2006.07.029

ethanol as an alternative fuel source has steadily increased (Sheoran et al., 1998) due to efforts in decreasing the overall amount of greenhouse gases emitted into the atmosphere (Martín et al., 2002), dwindling fossil fuel resources, (Yu and Zhang, 2004) and increased gasoline prices. Since 1970s, it has become clear that availability of domestic natural gas and petroleum cannot meet the growing demand for these energy sources. Therefore, there has been serious concern for developing renewable energy sources in an effort to ease the severity of the expected shortage. One possibility is the conversion of waste or grown organic matter into liquid and gaseous fuels (Graham et al., 1976). Currently, the United States produces approximately three billion gallons of ethanol from corn annually (Potera, 2004).

While ethanol can be produced from fermented agricultural products, which are abundant renewable resources found world-wide (Krishna and Chowdary, 2000), there are major limitations to efficient ethanol production from agricultural residues. These limitations include the close physical and chemical associations between lignin and plant cell wall polysaccharides, together with cellulose crystallinity (Gould, 1984). Lignin forms a protective shield around cellulose and hemicellulose, protecting the polysaccharides from enzymatic degradation. To convert the biomass into ethanol, the cellulose must be readily available for cellulase enzymes (Krishna and Chowdary, 2000). Thus, by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation (Wyman, 1996). Therefore, a pretreatment must be applied to degrade the lignin in the sugarcane residue, decrease cellulose crystallinity, and increase the surface area for enzymatic activity (Krishna and Chowdary, 2000).

The current study was initiated to determine the optimal pretreatment conditions for high efficiency ethanol production from the post-harvest sugarcane residue, namely, leaf litter. The residue was subjected to alkaline hydrogen peroxide pretreatments and sulfuric acid pretreatments, followed by three weeks of fermentation using the ATCC yeast, *Saccharomyces cerevisiae* strain 765. The results indicated that ethanol can be made from the post-harvest sugarcane residue.

2. Methods

2.1. Microbial source

The yeast *Saccharamyces cerevisiae* strain 765 was obtained from the American Type Culture Collection (ATCC), Rockville, Maryland. This strain is known to produce ethanol from cellulosic material, specifically, from the bark of trees (Boopathy, 2005).

2.2. Alkaline pretreatment

The purpose of the alkaline pretreatment was delignification. The removal of lignin is necessary for cellulose to become readily available for the enzymes, which permit the yeast to convert the glucose into ethanol (Wyman, 1996). The amount of weight lost following chemical pretreatment of residue was due to lignin removal (Wyman, 1996). Greater weight loss equals more lignin loss. The percent weight lost was used to compare pretreatment effects on lignin removal. Delignification was tested by soaking each residue in various concentrations (0%, 1%, 2%, and 5%) of household hydrogen peroxide at various pHs (8, 11.5, and 13), for various time intervals (8, 24, and 48 h).

Post-harvest sugarcane residue was supplied by the United States Department of Agriculture (USDA) office in Houma, Louisiana. Three grams (g) of dry sugarcane leaf litter residue was weighed and placed in beakers. Subsequently, three, 1% H_2O_2 solutions were made. The pH of separate 1% H_2O_2 solutions was adjusted to 8, 11.5, or 13 by adding sodium hydroxide (NaOH) tablets. Enough of each treatment solution was added to the beakers to submerge the sugarcane residue, and allowed to soak for 8, 24, or 48 h. This experiment was repeated for H_2O_2 concentrations of 0%, 2%, and 5%. Deionized (DI) water was substituted for H_2O_2 for the 0% treatment level. In addition, a DI water control was conducted without adjusting the pH. Each H_2O_2, pH, and time treatment combination was repeated four times. After the allotted amount of time for soaking, the residue was removed from the solutions by filtering through a piece of cheesecloth. The residue was then triple rinsed for 30 min in DI water and oven dried at 100 °C for approximately 12 h. Finally, the residue was reweighed. The weight difference is equivalent to the amount of lignin removed.

Upon conclusion of the alkaline pretreatments, analysis of variance was used to determine the pretreatment conditions that removed the most lignin. The best pretreatment was then used for further fermentation experiments. Fermentation experiment was conducted using the yeast *S. cerevisiae* strain 765.

The pretreated leaf was used in the fermentation experiment. The pretreated residue was placed into anaerobic bottles containing 100 mL of sterile tap water and 5% (v/v) of the yeast *S. cerevisiae* strain 765. This experiment was run in duplicates along with duplicate controls without pH adjustment. Samples were taken on days 0, 6, 12, 18, and 21 with a 5 mL syringe. Samples were microcentrifuged at 10,000 rpm for 6 min and the supernatant was used to monitor ethanol production using HPLC analysis as described below in Section 2.4.

2.3. Acid pretreatment

The purpose of acid hydrolysis was to remove lignin from the post-harvest sugarcane residue, which hinders enzymatic hydrolysis of cellulose for ethanol fermentation. Dilute sulfuric acid (H_2SO_4) concentrations (0.0, 0.2, 0.4, and 0.8 M) were used in this pretreatment. For the acid hydrolysis pretreatment, approximately 3 g of dry sugarcane leaf litter residue was placed into anaerobic bottles

containing 100 mL of DI water and 0.2 M H_2SO_4 and allowed to soak for 24 h. The bottles were subsequently autoclaved and allowed to cool before 5% (v/v) of the yeast *S. cerevisiae* strain 765 was added. Samples were taken on days 0, 6, 12, 18, and 21 using a 5 mL syringe, microcentrifuged at 10,000 rpm for 6 min and transferred to HPLC vials. Ethanol production was monitored using HPLC analysis as described below in Section 2.4. This experiment was repeated using 0.0, 0.4, and 0.8 M H_2SO_4, and each treatment had three replicates.

2.4. Analytical techniques

Ethanol production was analyzed by high performance liquid chromatography (HPLC) on a Varian Pro Star Autosampler Model 410 liquid chromatograph equipped with two solvent pumps, a model 210 programmable multi-wavelength detector set at 210 nm, a data module, and a model 320 system controller. The mobile phase was 0.0025 N H_2SO_4. Aliquots of 10 μL were injected into an organic acid column (Varian organic acid column, Cat #SN 035061) at 22 °C. The flow rate of the mobile phase was 0.6 mL/min, and the analysis was done under isocratic mode. Quantification of ethanol was done by using standard ethanol.

2.5. Statistical analysis

Data were subjected to analysis of variance (ANOVA) followed by a Tukey *post hoc* range test ($p \leqslant 0.05$; Neter et al., 1990).

3. Results and discussion

3.1. Alkaline pretreatment

The alkaline pretreatment of 2% H_2O_2 (pH 13) soaked for 8 h removed the most lignin in sugarcane leaf litter compared to other treatment combinations (Figs. 1 and 2). Therefore, this treatment was chosen for fermentation of the leaf litter. Treatment combinations consisting of pH 8 or soaking for 48 h were not significant ($p = 0.9$) (data not shown).

Lignocellulosic biomass cannot be saccharified by enzymes to high yields without a pretreatment, mainly because the lignin in plant cell walls forms a barrier against

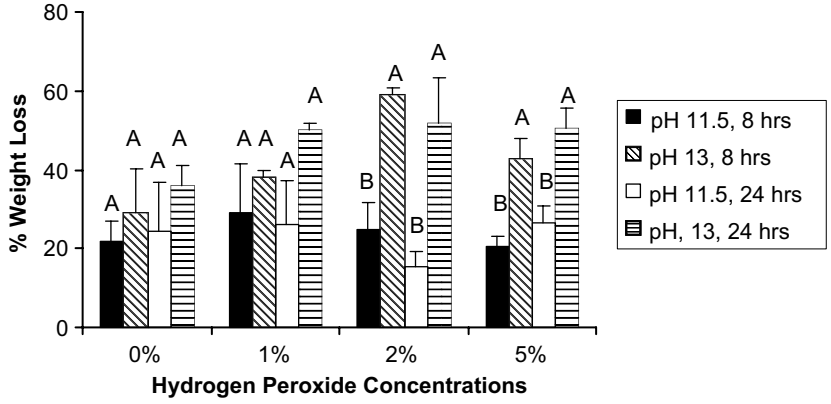

Fig. 1. Mean (±standard deviation) percent weight loss from sugarcane leaf litter after soaking in different H_2O_2 concentrations for 8 or 24 h at a pH of 11.5 or 13. Means denoted by the same letter are not significantly different from each other within treatments.

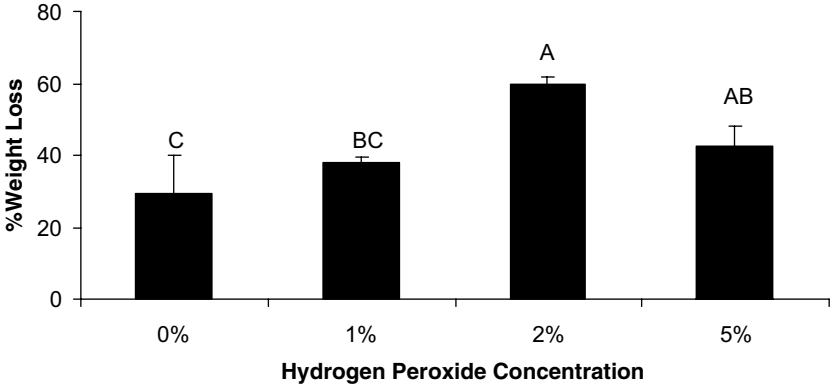

Fig. 2. Mean (±standard deviation) percent weight loss from sugarcane leaf litter after soaking in different H_2O_2 concentrations for 8 h at a pH of 13. Means denoted with same letter are not significantly different from each other.

enzymatic attack (Sewalt et al., 1997). An ideal pretreatment would reduce the lignin content and crystallinity of the cellulose and increase the surface area (Krishna and Chowdary, 2000). Lignin is degraded in nature by various organisms, but the mechanism of natural degradation is largely unknown. It is thought that oxidants such as H_2O_2 may play an important role. Under certain conditions, H_2O_2 is known to react with lignin and has been widely used to bleach high-lignin pulps (Gould and Freer, 1984). Gould (1984) recently reported that under suitable conditions, H_2O_2 will delignify wheat straw and other crop residues to a point where the cellulose can be enzymatically converted to glucose with near quantitative yield. According to Gould and Freer (1984), H_2O_2 treated lignocellulosic materials can be rapidly fermented to ethanol with greater than 90% efficiency in the presence of cellulase. In the present study, sugarcane leaf litter residue pretreated with 2% H_2O_2 at a pH of 13 soaked for 8 h (Figs. 1 and 2) removed 58.97% of the total weight of the sample; therefore, removing more lignin than any other pretreated sample.

These results are similar to those concluded from other research. Maximum delignification of wheat straw occurred at a pH of 11.5 or higher and the increase in saccharification efficiency was nearly complete after eight hours at room temperature (Gould, 1984). Krishna and Chowdary (2000) concluded that alkaline peroxide pretreatments were effective in providing fractionation of the hemicellulose and lignin components and resulted in efficient hydrolysis in linn leaves.

In another study by Gould and Freer (1984), wheat straw treated for several hours at room temperature with 1% H_2O_2 at a pH of 11.5 released slightly more than one-half of its lignin as water-soluble degradation products. They found that increased concentrations of H_2O_2, more alkaline pH, or repeated H_2O_2 treatments did not alter the total amount of lignin solubilized. However, based upon the present research, increased pH levels did remove more lignin than lower pHs. Furthermore, Gould and Freer (1984) concluded that in the absence of H_2O_2 only a very small fraction of the lignin present in the straw was released. Boopathy (2005) also obtained similar results in research conducted on sugarcane residue. Pretreatments soaked in 1% H_2O_2 at a pH of 11.5 for 8 h at room temperature removed 40% of lignin.

Sugarcane leaf litter pretreated in 2% H_2O_2 (pH 13) for 8 h was subjected to fermentation. Sugarcane leaf litter was fermented for 15 days and sampled every five days. Optimal fermentation conditions for pretreated sugarcane leaf litter residue at 2% H_2O_2, pH 13, 8 h was determined to occur on day 10, producing a mean of 130.5 mg/L ethanol (Fig. 3).

Results from this research were slightly higher than those reported by Boopathy (2005), where ethanol production was 118 mg/L. However, Gould and Freer (1984) reported that alkaline pretreated corn cobs, corn husks, and corn stalks produced ethanol with an overall 90% efficiency, while kenaf and oak shavings produced enhanced ethanol yields, although significantly below the theoretical maximum. It must be noted that Gould and Freer (1984) added cellulase enzyme prior to fermentation.

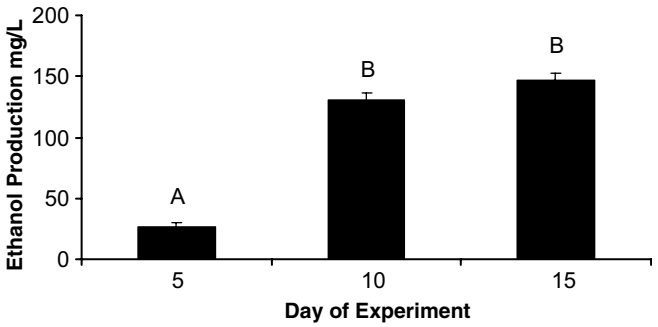

Fig. 3. Mean (±standard deviation) ethanol production (mg/L) from fermentation of alkaline pretreated leaf litter residue, 2% H_2O_2 (pH 13) 8 h for a 5, 10, and 15 day fermentation period. Means represented with the same letter are not significantly different from each other.

3.2. Acid hydrolysis

The sugarcane leaf litter acid treatment of 0.8 M H_2SO_4, fermenting for 12 days produced more ethanol than any other treatment combination up to day 12. Fermentation for more than 12 days did not increase ethanol production (Fig. 4).

For acid hydrolysis, the optimal concentration of H_2SO_4 was 0.8 M H_2SO_4. Results for sugarcane leaf litter show that fermenting for 12 days was the most efficient acid hydrolysis treatment for ethanol production, producing 335.67 mg/L ethanol (Fig. 4). In acid hydrolyzed experiments of waste cotton conducted by Yu and Zhang (2004), 0.2 mol/L H_2SO_4 was the optimal acid treatment, producing 14.2 g/L of ethanol in 24 h.

After comparing alkaline H_2O_2 and H_2SO_4 acid treatments, it was shown that acid hydrolysis produced the most ethanol from the residue. More ethanol was produced from sugarcane leaf litter when treated with 0.8 M H_2SO_4 for 12 days compared to alkaline pretreated residue at 2% H_2O_2 (pH 13) 8 h fermented for 10 days (Fig. 5). This preliminary

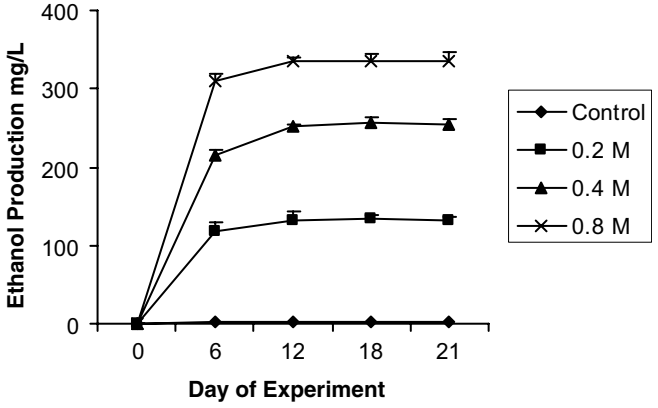

Fig. 4. Mean ethanol production (mg/L; ±standard deviation) from sugarcane leaf litter subjected to different concentrations of acid hydrolysis over time.

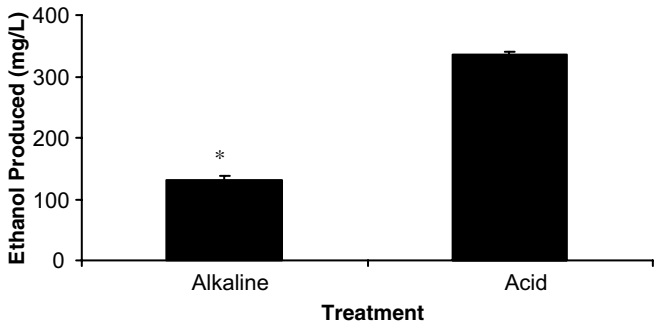

Fig. 5. Mean (±standard deviation) ethanol production (mg/L) from acid (0.8 M H_2SO_4) treated leaf litter residue for 12 days and alkaline (2% H_2O_2 (pH 13) 8 h) pretreated residue fermented for 10 days. Asterisk denotes a significant difference at $p = 0.05$.

study showed that ethanol production from post-harvest sugarcane residue is possible. Lignin prevents the degradation of cellulose mainly by acting as a physical barrier between the cellulolytic enzyme and its substrate. Consequently, the rate and extent of enzymatic cellulose degradation in lignocellulosic materials is inversely related to the lignin content (Gould, 1984) with maximum cellulose degradation occurring only after 50% or more of the lignin has been removed. In this study, we achieved a significant removal lignin from the sugarcane residue, which resulted in higher production of ethanol. Further research is needed to optimize the conditions for maximum production of ethanol from the sugarcane residue.

Acknowledgements

Funding for this work was provided by Louisiana Board of Regents through the Clean Power Energy Research Consortium (CPERC) and the American Sugarcane League. The authors like to thank Dr. Quenton Fontenot for the statistical analysis.

References

Boopathy, R., 2005. Use of microbes in managing post-harvest crop residues: sugarcane residue as a case study. In: Ray, R.C. (Ed.), Microbial Biotechnology in Agriculture and Aquaculture. Science Publishers, Plymouth, UK, pp. 433–446.

Boopathy, R., Asrabadi, B.R., Ferguson, T.G., 2002. Sugar cane (*Saccharum officinarum* L.) burning and asthma in southeast Louisiana, USA. Bulletin of Environmental Contamination and Toxicology 68, 173–179.

Givens, J.D., 1996. Air quality annual report. Louisiana Department of Environmental Quality. Baton Rouge, LA.

Gould, J.M., 1984. Alkaline peroxide delignification of agricultural residues to enhance enzymatic saccharification. Biotechnology and Bioengineering 26, 46–52.

Gould, J.M., Freer, S.N., 1984. High-efficiency ethanol production from lignocellulosic residues pretreated with alkaline H_2O_2. Biotechnology and Bioengineering 26, 628–631.

Graham, R.W., Reynolds, T.W., Hsu, Y., 1976. Preliminary assessment of systems for deriving liquid and gaseous fuels from waste or grown organics. US Department of Commerce, National Technical Information Service, 1–40.

Krishna, S.H., Chowdary, G.V., 2000. Optimization of simultaneous saccharification and fermentation for the production of ethanol from lignocellulosic biomass. Journal of Agricultural and Food Chemistry 48, 1971–1976.

Koren, H.S., 1995. Associations between criteria air pollutants and asthma. Environmental Health Perspectives 103, 235–242.

LSU Ag Center. 2003. Annual Report. Baton Rouge, LA.

LSU Ag Center. 2004. Sugarcane summary for crop year 2004.

Martín, C., Galbe, M., Wahlbom, C.F., Hahn-Hägerdal, B., Jönsson, L.J., 2002. Ethanol production from enzymatic hydrolysates of sugarcane bagasse using recombinant xylose-utilizing accharomyces cerevisiae. Enzyme and Microbial Technology 31, 274–282.

Neter, J., Wasserman, W., Kutner, M.H., 1990. Applied linear statistical models: Regression, analysis of variance, and experimental designs, third ed. IRWIN, Burr Ridge, Illinois.

Potera, C., 2004. Efficient recombinant yeast yields ethanol from straw residues. ASM News 70, 442–443.

Sewalt, V.J.H., Glasser, W.G., Beauchemin, K.A., 1997. Lignin impact on fiber degradation: reversal of inhibition on enzymatic hydrolysis by chemical modification of lignin and by additives. Journal of Agricultural and Food Chemistry 45, 1823–1828.

Sheoran, A., Yadav, B.S., Nigam, P., Singh, D., 1998. Continuous ethanol production from sugarcane molasses using a column reactor of immobilized Saccharomyces cerevisiae HAU-1. Journal of Basic Microbiology 38, 123–128.

Wyman, C.E., 1996. Chapter 1. Ethanol production from lignocellulosic biomass: overview. In: Wyman, C.E. (Ed.), Handbook on bioethanol: production and utilization. Taylor & Francis, Washington, DC, pp. 11–12.

Yu, Z., Zhang, H., 2004. Ethanol fermentation of acid-hydrolyzed cellulosic pryolysate with *Saccharomyces cerevisiae*. Bioresource Technology 93, 199–204.