BIOETHANOL PRODUCTION FROM COTTON GIN TRASH: A NEW PROCESS ROUTE

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Abstract

Cotton gin trash (CGT) is a ubiquitous cotton production waste resource. One of the solutions for large scale utilization of these wastes produced abundantly in the USA is the production of biofuels such as ethanol. In this research, various combinations of pretreatments to produce bio-ethanol from a highly lignocellulosic material such as cotton gin trash were evaluated. The combination of three different pretreatments: ultrasonication, steam explosion and ligninolytic enzymes were evaluated in 7 pretreatment combinations to determine their response to improve the cellulose conversion and the ethanol yield. The saccharification process was made using a combination of two commercial enzymes Accellerase 1500 and Accellerase XC (Genencor International) and the fermentation of the sugars to ethanol using ethanol red (*Saccharomyces cerevisiae)*. All the pretreatments combinations upgraded the quantities of sugar and ethanol produced compared with the unpretreated CGT biomass. However, the best results were achieved by the sequential combination of ultrasonication, steam explosion and ligninolytic enzymes with an approximately difference of around 10% with respect to the other pretreatments. All pre-treatments significantly improved the percentages of sugars originally present in the CGT biomass. These initial results will pave the way for new possibilities to improve and make more feasible the bio-ethanol production from CGT and other agro-industrial biomass.

Introduction

Cotton is the principal source of natural fibers for the textile industries, and thus one of the most important agro-industrial biomass with a production of 24 million tons in the world in 2006-2007 and which continue to increase by 2% each year (Sharma-Shivappa and Chen 2008). In the USA the cotton production was close to 4.1million tons in 2007 and in Texas, cotton production is 25% of the US crop, which represents over 6 million acres of cotton plants and a production of 4 million of bales per year (USDA, 2008).

The high level of cotton production is directly related to the high production of wastes and residues. In the USA, close to 2.5 million tons is produced each year (White, Coates and Wolf, 1996). The residues from cotton crop cultivation are of two types: the cotton plant trash (CPT) and the cotton gin trash (CGT) (Rogers, Poore and Paschal 2002). CPT is the residue that stays in the field after the harvest of cotton while CGT is the residue that comes from the ginning process. Of these two types of wastes, the CGT is very import to the researchers and the cotton producers due to the high production and accumulation at the cotton gins and its difficulties for final disposal.

The CGT is composed of pieces of sticks, leaves, bolls and soil cleaned from lint during the ginning operation. About 218 kg of cotton fiber generates 68-91 kg of CGT (Sharma-Shivappa and Chen 2008). Worldwide, the production of this waste is approximately 3.23 million Mg (Jeoh and Agblevor, 2001a). In the USA, the Cotton Belt Region produces about 2.8 million tons of CGT each year (Macias-Corral et al., 2005) and 80% of these total production is generated in the states of Arizona, California, New Mexico, Oklahoma, and Texas (Badger, 1999). Texas is the leading state in cotton production with 1.2 million tons per year (Hernandez, Capareda and Aquino, 2007) and an average of 995 short tons of cotton gin trash in 30 principal counties (TAMU, 2003).

With this large quantity of wastes, the final disposal becomes a major problem to the cotton industry, mainly during winter, when insects use these residues as a surviving site (White, Coates and Wolf, 1996).The conventional disposal method of CGT was incineration that produces several health hazards (Hernandez, Capareda and Aquino, 2007)and environmental pollution (Shen and Agblevor 2008) and was regulated by the federal law, making this disposal method economically unachievable (Mayfield, 1991). To provide a solution to this disposal problem, CGT have been used in different ways: in land filling, feed roughage additive (Pordesimo, et al., 2005), composting (Díaz, et al., 2002), microbial gum production (Smith, Tollner and Eiteman 1999), growth of edible fungus (Zervakis, et al., 2001), activated carbon production (Hernandez, Capareda, and Aquino. 2007, Klasson, et al., 2009), animal bedding(Grimes, Carter and Godwin, 2006), cellulase production(Tan and Wahab, 1997),

construction materials (Guler and Ozen, 2004)and bio-fuel production (Aquino, Capareda and Parnell, Jr., 2007, Jeoh and Agblevor, 2001b, Agblevor, et al., 2006).

The lignocellulosic material from CGT can be used for bio-fuel production. Lignocellulose is a polysaccharide combination of cellulose, hemicellulose and lignin that belongs to the structure of the plant cell wall. Lignocellulose is more than 60% of the organic matter of the vegetal species in the earth. This biomass is considered as the most abundant source of organic material from the wastes of the agro-industrial activities. The use of CGT to produce renewable energy has been investigated specifically to be used in pyrolysis, biogas production and bioethanol production.

Bio-ethanol is one of the most important bio-fuel products that can be produced from lignocellulosic material, like CGT. The United States regulation requires producing 36 billion gallons of biofuels and from these 21 billion should be produced from lignocellulosic material or other new advanced fuels by 2020 (Sissine, 2007). This regulation opens opportunities to develop new processes and to improve other existing research processes to reach the goals of biofuel production. In general the production of bio-ethanol from lignocellulosic material is based on three principal steps: 1) pretreatment, 2) saccharification and 3) fermentation. In the first step, the aim is to reduce the quantity of lignin present in the biomass and make the cellulose and the hemicellulose readily available for the saccharification process. The second step is to obtain the mono-saccharides present in the cellulose (glucose) and the hemicellulose (xylose, arabinose, galactose and mannose). Finally, fermentation is the bio-ethanol production by microorganisms using the sugars produced during saccharification. The 3 step processes can be changed or improved for better production and yield of the bio-ethanol from lignocellulosic biomass.

The main objective of this paper was to determine the adequate pretreatments combinations among ultrasonication, high pressure steam explosion and ligninolytic enzymes that generate the best ethanol production from CGT.

Materials and Methods

Substrate

The samples of cotton gin trash were obtained from the Varisco Cotton Gin near College Station, in the Brazos Valley County, Texas. The CGT samples were ground in a Wiley mill to obtain an average particle size of approximately 1 mm in diameter. This particle size reduction is important for succeeding pre-treatment processes and would make the feedstock uniform in size. To perform the characterization of the CGT biomass the analytical protocols developed at the National Renewable Energy Laboratory (NREL) of the US Department of Energy (USDOE) were followed as follows: (a) Determination of Structural Carbohydrates and Lignin in Biomass (Sluiter, et al., 2008a), (b) Determination of Extractives in Biomass (Sluiter, et al., 2005), and (c) Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples (Sluiter, et al., 2008b). These procedures were made to determine the structural composition of the CGT biomass.

Pretreatments

In this research three different types of pretreatments (ultrasonication, high pressure steam explosion and ligninolytic enzymes) were evaluated and its combination to obtain the best ethanol production from CGT. The ultrasonication process was developed employing an ultrasonicator (Hielscher Ultrasonic Processors, Ringwood, NJ, USA) on a solution of at 10% of solids of CGT biomass. The steam explosion pretreatment was made in an autoclave using Erlenmeyer flasks with a solution of 10% CGT solids at 121°C, 15 psi for 1 hour. The ligninolytic enzymes pretreatment was made using the commercial laccase mediator systems PrimaGreen® EcoFade LT100 from GENENCOR, International. These enzymatic reactions were made in 250 ml Erlenmeyer flasks with an initial enzyme load of 3 grams, 50 ml of phosphate buffer 25mM at pH 6 with 10% solids of CGT and for 96 hours at a controlled temperature of 30°C and 150 rpm in an orbital incubator/shaker (Innova, New Brunswick Scientific, NJ). The 7 pretreatments evaluated were: Steam explosion (SE), ultrasonication (U), enzymes (E), ultrasonication+steam explosion (U+SE), ultrasonication+enzymes (U+E), steam explosion+enzymes (SE+E), ultrasonication+steam explosion+enzymes (U+SE+E). The experimental design was completely randomized with three replicates using like response variables such as cellulose conversion and ethanol yield.

Saccharification Process

The pretreated biomass samples were enzymatically hydrolyzed using the combination of 2 types of commercial cellulases from GENENCOR: Accellerase 1500 and Accellerase XY. The experiment had an initial enzyme loading

of 0.15 ml/g of Accellerase 1500 + 0.03 ml/g of Accellerase XY. The process was run in 250 mL erlenmeyer flasks with 50 ml of a solution of 50 mM sodium acetate buffer at pH 4.8 for 96 hours at 50^0C and 125 rpm in an incubator/shaker (Innova, New Brunswick Scientific, NJ). This process will be used to evaluate the glucose conversion. When the process was completed, the samples were centrifuged at 13,000 RPM for 20 minutes in a 2 ml eppendorf tube, and the supernatants were filtered through 0.5 µm hydrophilic PTFE syringe filters (Millipore, Billerica, MA). These samples were then analyzed for glucose, mannose, xylose, arabinose, galactose and cellobiose concentration using High Performance Liquid Chromatography (HPLC) (Waters 2690, Separations Module, Waters Corporation, Milford, MA) equipped with auto sampler, Shodex SP 810 packed column and a Refractive Index (RI) detector. Each sample was run for 25 minutes at a flow rate of 0.7 ml/min, 60°C using HPLC water as mobile phase. Cellulose and hemicellulose conversion will be determined from the percentage of lignocellulose converted into sugars. Efficiency is calculated by comparing sugars yield (g) before and after enzymatic hydrolysis using the equation: $\%\ glucose\ conversion = [(c \times V) / m] \times 100\%$, where, *c* is the concentration (g/L) of sugars in the sample hydrolyzed, as determined by HPLC, *V* is the total volume (L) hydrolyzed, *m* is the initial weight (g) of glucose or xylose determined through the protocols of the National Renewable Energy Laboratory (NREL) of USDOE. The statistical analysis was made using three repetitions for a one way Anova and the Duncan´s multiple range test in the statistical software SAS system 9.0.

Fermentation Process

The enzymatically treated biomass was used to develop the fermentation process. Ethanol Red (*Saccharomyces cerevisiae)* provided by Fermentis (Lesaffre Yeast Corp., Milwaukee, WI) and was used to perform the fermentation process. The activation of the strain was made using 0.5g of dry yeast in 10 mL of the inoculum broth. The composition of the inoculum broth was 0.2 g glucose, 0.05 g peptone, 0.03 g yeast extracts, 0.01 g KH_2PO_4, and 0.005 g $MgSO_4.7H_2O$. The inoculums were shaken at 200 rpm in an incubator shaker at 38 °C for 25-30 min. The fermentation process was made in 125ml Erlenmeyer flasks with 50 ml of the slurry supplemented with 0.3 g of yeast extract. The pH was adjusted to 4.2 to 4.3 with 2N hydrochloric acid. The slurry was then incubated with 1 ml of freshly activated dry yeast (Ethanol Red) and run for a period of 72 hours at 32 °C and 100 rpm. After the fermentation process the treatment samples were centrifuged at 12,000 RPM for 15 min and the supernatant was filtered through 0.5 µm hydrophilic PTFE syringe filters (Millipore, Billerica, MA). The filtered samples were injected in an HPLC (Waters 2690, Separations Module, Waters Corporation, Milford, MA) using a Shodex SP 810 packed column and a Refractive Index (RI) detector to detect the ethanol. Column temperature was maintained at 60 ^{0}C. Each sample was run for 25 minutes at a flow rate of 0.7 ml/min, using HPLC water as the mobile phase. The ethanol yield was calculated from the ratio between the average produced ethanol and the theoretical ethanol production of 51.1 g of ethanol generated per 100 g of glucose (Wu et al., 2006) in the biochemical conversion of the sugar. The response variable was the ethanol yield and was analyzed using the software SAS system 9.0 employing one way ANOVA and the Duncan´s multiple range test.

Results and Discussion

Cotton Gin Trash Composition.

The results of the structural compositional analysis of the cotton gin trash are shown in Table 1. The mass balance value (79.41) is lower compared with those obtained in the work of Agblevor, et al., 2006, where different sources of cotton gin trash were evaluated. In the cited study, the values ranged between 79 and 90. This difference is perhaps due to the diversity between the feedstocks, the ginning method and the sampling method (Agblevor, et al., 2006). The ethanol extractives of this research (10.56) and the acid insoluble lignin (23.17) corresponds to values found it in other CGT samples. Although it is important to note that not all the acid insoluble material part corresponds to lignin due to the different compounds like cottonseed, small leaf, and the hulls present in the CGT and can produce other insoluble acid compounds (Agblevor, et al., 2006).

Table 1. Structural composition of cotton gin trash.

Compound	Amount
Ethanol extractives	10.56±0.37
Acid insoluble material	23.17±0.60
Arabinose	1.47±0.21
Xylose	5.71±1.16
Mannose	1.15±0.27
Galactose	1.77±0.35
Glucose	24.88±0.51
Ash	10.69±1.82
Mass balance	79.41

The total carbohydrates content (35%, i.e. sum of all sugars) in the CGT samples was similar to the lowest values found literature (Agblevor, et al., 2006), where the total carbohydrate ranges were from 35% to 49%. In addition, the principal carbohydrates, xylose and glucose correspond with the highest values found in the same literature cited. However, the glucose content of the sample in this study is low compared with those reported in literatures. The reason for the lower value is related with more hulls, small leaf, high ash, and seed contents which are present in the CGT (Agblevor, et al., 2006).

Pretreatments Evaluation

The evaluation of the pretreatment processes was made using the cellulose conversions (Figure 1) and the ethanol yield (Figure 2). In the first case the variable was evaluated to know how the different pretreatments could influence the final glucose concentration from the original cellulose present in the CGT. In this response variable, differences in mean cellulose conversions for the treatments were statistically significant ($p < 0.05$), and the Duncan test was used to evaluate the differences presented among pretreatments. In this case the highest cellulose conversion was achieved with the use of the U+SE+E combination (Table 2) with a 23.4% of cellulose conversion. Any other pretreatment was statistically different to this treatment. The other pretreatments related with the steam explosion presented the next highest cellulose conversion showing the importance of this physical pretreatment to improve the cellulose conversion. The ultrasonication (alone) pretreatment presented an intermediate action with an average cellulose conversion of 11.8%. On the other hand, the enzymatic pretreatment alone showed no significant difference in cellulose conversion with the untreated CGT biomass.

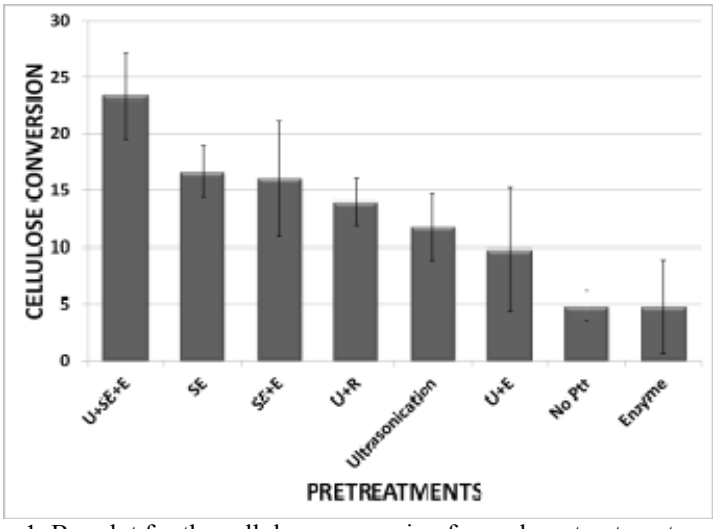

Figure 1. Bar plot for the cellulose conversion for each pretreatment process.

The maximum cellulose conversion presented similar values at the ones found in literature using microbial pretreatment (Shi, et al., 2009) and sulfuric acid pretreatment for cotton stalks (Silverstein, et al., 2007). However, these values are quite low compared with those reported in literature, where the conversion is between 40% and the 80% (Jeoh and Agblevor 2001a, Agblevor, Batz and Trumbo, 2003). This difference is primarily due to the higher

temperatures and pressures used on those studies compared with this research. The goal of this study is to operate at a much lower temperature and pressure ranges due to developing cost effective piloting processes that are being planned in the future.

However, the combination of pretreatments used in this study agree quite well with the results achieved using sorghum lignocellulosic biomass to produce bioethanol (Capareda, 2011) and those reported by Goshadrou *et al.,* (2011). The use of ultrasonication improved the cellulose conversion when this process is used in conjunction with other pretreatments as reported as well by Goshadrou, et al., 2011. Additionally, this study also found the need of pretreatments before the enzymatic process to improve the cellulose conversion as reported by Shi, et al., 2009. It is important to note that the combination of the two physical pretreatments with enzymatic process allows a considerable time reduction compared with the microbial enzymatic pretreatment alone and the significant toxicity reduction compared with the high temperatures steam explosion due to the unnecessary production of inhibitory co-substrates (Shen and Agblevor, 2011) or other processes that uses too much lime (Agblevor, Batz and Trumbo, 2003).

Table 2. Duncan's multiple range test for the ethanol yield and cellulose conversion

Pretreatment	Cellulose Conversion Average	Duncan´s Statistic	Pretreatment	Ethanol Yield Average	Duncan´s Statistic
U+SE+E	23.4	A	U+SE+E	31.6	A
SE	16.6	B	SE	22.2	B
SE+E	16.0	BC	SE+E	21.6	B
U+SE	14.0	C	U+SE	18.5	C
Ultrasonication	11.8	C	Ultrasonication	15.7	C
U+E	9.80	C	U+E	13.3	C
No Pretreatment	4.82	D	Enzyme	6.54	D
Enzyme	4.81	D	No Pretreatment	6.45	D

Similarly, the cellulose conversion into ethanol yielded significant statistical differences ($p<0.05$) among the evaluated pretreatments. In this step, the pretreatment that presented the highest ethanol yield was the U+SE+E combination with an ethanol yield of 31.6% and it resulted in a difference of close to 10% with the other combinations (Table 2). The importance of the steam explosion is also observed in the ethanol yield where the 4 SE related pretreatments presented the highest ethanol production. On the other hand, the ultrasonication and the ligninolytic enzymes presented low conversion percentages when worked alone, but when they are mixed with the steam explosion, better results are generated. In this case the combination of ultrasonication and ligninolytic enzymes with the steam explosion can result to a reduction of toxic compounds produced compared with other steam explosion-related studies from other literatures (Agblevor, Batz & Trumbo 2003). Other research showed an increase in the delignification (Meza et al. 2006, Grönqvist et al. 2005) and change the cellulose structure (Hu, Foston and Ragauskas, 2011) using these combined processes.

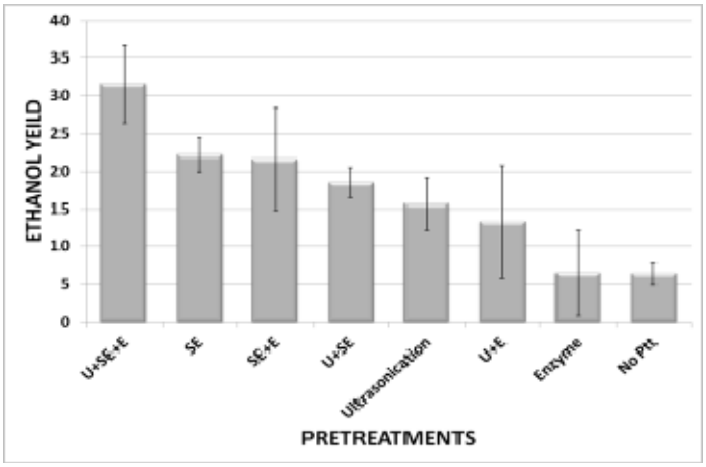

Figure 2. Bar plot for the ethanol yield for each pretreatment

The ethanol yield obtained by the U+SE+E (31.6% vs. 27%) is comparable with one of the CGT sources (Wakefield) evaluated by Agblevor, et al., (2003). However, these ethanol conversion values are still quite low compared with those reported in literature using much higher temperature and pressure for steam explosion processes (Jeoh and Agblevor, 2001a). As noted earlier, these conditions would give rise to more substances that would inhibit final ethanol conversion processes if not properly treated. Additionally it is important to note that other research studies having the highest reported production of ethanol were obtained using transgenic microorganisms while in this study, we have used conventional fermentation yeast (*Saccharomyces cerevisiae)* for the final ethanol conversion process and commercial enzymes for cellulose to sugar conversion.

Summary

In this study, the best combinations of pretreatments to generate the highest cellulose and ethanol yields were the use of ultrasonication, steam explosion and ligninolytic enzymes sequentially. It was also shown that the use of mild steam explosion showed relatively higher conversion efficiencies when combined with other pretreatments. More importantly, the use of steam explosion in combination with the enzymatic process will need to be further analyzed to find the optimal conditions for the CGT pretreatment without having to use higher temperatures and pressures. This study has shown that the use of steam explosion followed by enzymatic process produced the next highest relative amounts of sugars released from the CGT and perhaps the enzymatic pretreatment helped to reduce the toxicity of the slurry and upgraded the total delignification of the CGT biomass. Additionally, other enzymes may have to be used to further convert other sugar types such as xylose present in the hydrolyzates to obtain high ethanol concentrations from the CGT biomass. Conventional yeast (*saccharomyces cerevisiae*) cannot convert these types of sugars into ethanol effectively. The initial results obtained in this research using the combination of ultrasonication, steam explosion and ligninolytic enzymes showed an interesting mix of pretreatments for future cost-effective process to produce bioethanol from CGT.

Acknowledgements

The Colombian government and the Fulbright USA are being acknowledged for the financial support via the granting of FULBRIGHT-COLCIENCIAS for the PhD studies of Jersson Escobar in the USA. The BioEnergy Testing and Analysis Laboratory of Dr. Capareda at the Biological and Agricultural Engineering Department and the Texas Agri-Life Research of Texas A&M University provided most of the funds and equipment for the conduct of this research. The Genencor International is acknowledged for the enzymes used in the study.

References

Agblevor, F.A., Cundiff, J.S., Mingle, C. and Li, W. 2006, "Storage and characterization of cotton gin waste for ethanol production", *Resources, Conservation and Recycling,* vol. 46, no. 2, pp. 198-216.

Agblevor, F., Batz, S. and Trumbo, J. 2003, "Composition and ethanol production potential of cotton gin residues", *Applied Biochemistry and Biotechnology,* vol. 105, no. 1, pp. 219-230.

Aquino, F. L., S. C. Capareda, and C. B. Parnell, Jr. 2007, *Elucidating the Solid, Liquid and Gaseous Products form Pyrolysis of Cotton Gin Trash. Transactions of the ASABE, Vol. 53, No.3, pp. 651-658, ASABE, Sr. Joseph, MI.*

Badger, P.C. 1999, Solid fuels. In: CIGR Handbook of Agricultural Engineering, Volume 3: Energy and Biomass Engineering. Ed. O. Kitani, pp. 248-288.

Capareda, S. C. 2011. Production of ethanol from sorghum hybrids developed at Texas A&M University. Paper presented at the 31st Philippine-American Association of Science and Engineering (PAASE) Annual Conference held on June 15-18, 2011 at the University of the Philippines, Diliman, Quezon City, Philippines, Sponsored by the PAASE.

Díaz, M.J., Madejón, E., López, F., López, R. and Cabrera, F. 2002, "Composting of vinasse and cotton gin waste by using two different systems", *Resources, Conservation and Recycling,* vol. 34, no. 4, pp. 235-248.

Goshadrou, A., Karimi, K. and Taherzadeh, M.J. 2011, "Bioethanol production from sweet sorghum bagasse by Mucor hiemalis", *Industrial Crops and Products,* vol. 34, no. 1, pp. 1219-1225.

Grimes, J., Carter, T. and Godwin, J. 2006. "Use of a litter material made from cotton waste, gypsum, and old newsprint for rearing broiler chickens", *Poultry science,* Vol. 85, No. 3, pp. 563-568.

Grönqvist, S., Viikari, L., Niku-Paavola, M.-., Orlandi, M., Canevali, C. and Buchert, J. 2005, "Oxidation of milled wood lignin with laccase, tyrosinase and horseradish peroxidase", *Applied Microbiology and Biotechnology,* vol. 67, no. 4, pp. 489-494.

Guler, C. and Ozen, R. 2004, "Some properties of particleboards made from cotton stalks (*Gossypium hirsitum* L.)", *European Journal of Wood and Wood Products,* vol. 62, no. 1, pp. 40-43.

Hernandez, J. R., S. C. Capareda and F. L. Aquino. 2007, *Activated Carbon Production from Pyrolysis and Steam Activation of Cotton Gin Trash. In: Proceedings of the 2007 Beltwide Cotton Conferences held in New Orleans, LA from January 9-12, 2007 and organized by the National Cotton Council of the USA, Memphis, TN.*

Hu, Z., Foston, M.B. and Ragauskas, A.J. 2011, "Biomass Characterization of Morphological Portions of Alamo Switchgrass", *Journal of Agricultural and Food Chemistry,* vol. 59, no. 14, pp. 7765-7772.

Jeoh, T. and Agblevor, F.A. 2001a, "Characterization and fermentation of steam exploded cotton gin waste", *Biomass and Bioenergy,* vol. 21, no. 2, pp. 109-120.

Jeoh, T. and Agblevor, F.A. 2001b, "Characterization and fermentation of steam exploded cotton gin waste", *Biomass and Bioenergy,* vol. 21, no. 2, pp. 109-120.

Klasson, K.T., Wartelle, L.H., Lima, I.M., Marshall, W.E. and Akin, D.E. 2009, "Activated carbons from flax shive and cotton gin waste as environmental adsorbents for the chlorinated hydrocarbon trichloroethylene", *Bioresource technology,* vol. 100, no. 21, pp. 5045-5050.

Macias-Corral, M. A., Z. A. Samani, A. T. Hanson, R. DelaVega and P. A. Funk. 2005. "Producing Energy and Soil Amendment from Dairy Manure and Cotton Gin Waste. Transactions of the ASABE, Vo. 48, No. 4, pp. - 1521-1526, ASABE, St. Joseph, MI.

Mayfield, W. 1991. Gin trash utilization: What are the options? In: Proceedings of the 1991 Beltwide Cotton Production Research Conferences, pp. 496-497 held in San Antonio, Texas from January 6-10, 1991 and Sponsored by the National Cotton Council of America, Memphis TN.

Meza, J.C., Sigoillot, J., Lomascolo, A., Navarro, D. and Auria, R. 2006, "New Process for Fungal Delignification of Sugar-Cane Bagasse and Simultaneous Production of Laccase in a Vapor Phase Bioreactor", *Journal of Agricultural and Food Chemistry,* vol. 54, no. 11, pp. 3852-3858.

Pordesimo, L.O., Ray, S.J., Buschermohle, M.J., Waller, J.C. & Wilkerson, J.B. 2005, "Processing cotton gin trash to enhance in vitro dry matter digestibility in reduced time", *Bioresource technology,* vol. 96, no. 1, pp. 47-53.

Rogers, G.M., Poore, M.H. and Paschal, J.C. 2002, "Feeding cotton products to cattle", *Veterinary Clinics of North America: Food Animal Practice,* vol. 18, no. 2, pp. 267-294.

Sharma-Shivappa, R.R. and Chen, Y. 2008, "Conversion of Cotton Wastes to Bioenergy and Value-Added Products", Transactions of the ASABE, Vol. 51, No. 6, pp. 2239-2246, ASABE, St. Joseph, MI.

Shen, J. and Agblevor, F. 2011, "Ethanol production of semi-simultaneous saccharification and fermentation from mixture of cotton gin waste and recycled paper sludge", *Bioprocess and Biosystems Engineering,* Vol. 34, No. 1, pp. 33-43.

Shen, J. and Agblevor, F.A. 2008, "Optimization of enzyme loading and hydrolytic time in the hydrolysis of mixtures of cotton gin waste and recycled paper sludge for the maximum profit rate", *Biochemical engineering journal,* Vol. 41, No. 3, pp. 241-250.

Shi, J., Sharma-Shivappa, R.R., Chinn, M. and Howell, N. 2009, "Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production", *Biomass and Bioenergy,* vol. 33, no. 1, pp. 88-96.

Sissine, F. 2007. Energy Independence Act of 2007: A summary of major provisions. Congressional Research Service Report for Congress, Order Code RL34294, Washington, D.C.

Silverstein, R.A., Chen, Y., Sharma-Shivappa, R.R., Boyette, M.D. and Osborne, J. 2007, "A comparison of chemical pretreatment methods for improving saccharification of cotton stalks", *Bioresource technology,* vol. 98, no. 16, pp. 3000-3011.

Sluiter, A., Ruiz, R. Scarlata, C. Sluiter, J.and Templeton D. 2005. "Determination of Extractives in Biomass". National Renewable Energy Laboratory technical report. Available at: http://www.nrel.gov/biomass/pdfs/42619.pdf. Accessed October 2011.

Sluiter, A. Hames, B. Ruiz, R. Scarlata, C. Sluiter, J. Templeton, D. and Crocker. 2008a. "Determination of Structural Carbohydrates and Lignin in Biomass Laboratory Analytical Procedure (LAP)". National Renewable Energy Laboratory technical report. Available at: http://www.nrel.gov/biomass/pdfs/42618.pdf. Accessed October 2011.

Sluiter, A., Hames, B., Hyman, D., Payne C., Ruiz, R., Scarlata, C. Sluiter, J. Templeton, D. and Wolfe J. 2008b. "Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples Laboratory Analytical Procedure (LAP)". National Renewable Energy Laboratory technical report. Available at: http://www.nrel.gov/biomass/pdfs/42621.pdf. Accessed October 2011.

Smith, J.W., Tollner, E.W. and Eiteman, M.A. 1999, "Microbial gum formation from the decomposition of cotton gin trash", *Bioresource technology, V*ol. 69, No. 3, pp. 215-222.

TAMU, 2003. *Texas Is Cotton Country* . Available: http://cotton.tamu.edu/cottoncountry.htm [2011, 11/18/2011].

Tan, Y.H. and Wahab, M.N. 1997, "Extracellular enzyme production during anamorphic growth in the edible mushroom, Pleurotus sajor-caju", *World Journal of Microbiology and Biotechnology,* vol. 13, no. 6, pp. 613-617.

USDA, 2008. National Agricultural Statistics Service Data and Analysis for 2007. Accessed at http://www.nass.usda.gov/Data_and_Statistics/index.asp.

White, D.H., Coates, W.E. and Wolf, D. 1996, "Conversion of cotton plant and cotton gin residues to fuels by the extruder 'feeder liquefaction process", *Bioresource technology,* vol. 56, no. 1, pp. 117-123.

Zervakis, G., Philippoussis, A., Ioannidou, S. and Diamantopoulou, P. 2001, "Mycelium growth kinetics and optimal temperature conditions for the cultivation of edible mushroom species on lignocellulosic substrates", *Folia microbiologica,* vol. 46, no. 3, pp. 231-234.