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Bioethanol Production from Enzymatic Hydrolysis of Rio Sweet Sorghum Bagasse Grown in Kenya

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Abstract:
*Sweet sorghum (*Sorghum bicolor *(L) Moench) is a crop analogous to sugarcane with similar accumulation of sugars in its juicy stems and has a high yield of green biomass (20-30t/ha), and a huge amount of lignocellulosic residue are produced as byproduct of sweet sorghum. The present study was undertaken with the objective of determining the potential of sweet sorghum bagasse (SSB) to produce bioethanol hence find a sustainable source of biofuel and spur economies of rural areas and also mitigate climate change. Sixteen sweet sorghum varieties namely: Madhura, Theis, Rema, Ramanda, Rio, CMSXS633, CMSXS644, SPV1411, IESV91018LT, IESV92008DL, IESV92038/2SH, IESV93042SH, were planted at the Jomo Kenyatta University of Agriculture and Technology research farm. Rio sweet sorghum with the highest sucrose purity was harvested and juice extracted. SSB was dried, comminuted and pretreated with phosphoric acid and alkaline hydrogen peroxide. The pretreatment with alkaline hydrogen peroxide and phosphoric acid led to 63.40% (wt/wt) and 49.12% (wt/wt) yield of glucose per gram substrate. Hydrolysis was by cellulase produced by Trichoderm reesei and the % theoretical enzymatic sugar yield after 72h was 50%, 78% and 88% for untreated, phosphoric acid pretreated and sodium hydroxide pretreated bagasse, respectively. Fermentation was by baker's yeast and the results were 15.33%, 40.45% and 59.44% of the theoretical yield for untreated, sodium hydroxide pretreated and phosphoric acid pretreated bagasse, respectively. The rate of ethanol production was respectively, 0.001g/l.h, 0.016g/l.h and 0.019g/l.h for the untreated, phosphoric acid pretreated and NaOH pretreated bagasse. Therefore, bioethanol can be produced from SSB but further research should be done to increase the yield before piloting and later commercialization of the process.*

Keywords: *Rio sweet sorghum bagasse, pretreatment, hydrolysis, fermentation, bioethanol*

1. Introduction

Sweet sorghum [sorghum bicolor (L.) Moench] is a C_4 crop growing in geographical areas with a temperate climate and requires limited fertilizer rates and water. It also has a shorter growing season than sugarcane, higher sugar yield per hectare than sugar beet, and high resistant to drought and salinity (Barbanti et al., 2006; Dolciotti et al., 1998; Zhao et al., 2009). The juice extracted from the fresh stems of this plant contains approximately 16-18% fermentable sugars predominately sucrose, glucose and fructose. Furthermore, sweet sorghum has a high yield of green biomass (20-30t/ha), and a huge amount of lignocellulosic residue is produced as byproduct of sweet sorghum (Maiorella, 1985; Sipos et al., 2009; Teetor et al., in press; Wu et al., 2010). This lignocellulosic residue, so-called bagasse, has only non-food applications e.g., cattle feed roughage and soil fertilizer after composting (Sipos et al.,

2009). Sweet sorghum bagasse, like other lignocellulosic materials, contains considerable amount of carbohydrate polymers (cellulose and hemicellulose) and lignin. The carbohydrates can be hydrolysed to fermentable sugars by acids or enzymes and then fermented to ethanol. Ethanol production is suggested as a promising future utilization of sweet sorghum bagasse due to high biomass yield per hectare and lower production costs than many other plants (Li et al., 2010). However, since the cellulose is chemically stable and well protected by lignin in the natural structure, it requires a pretreatment prior to the hydrolysis (Cheng et al., 2008; Macesic & Darko, 2008; Maiorella, 1985; Taherzadeh & Karimi, 2008a, b).

In order to enhance the yield of ethanol production from lignocelluloses, extensive pretreatments by physical, chemical or biological means were suggested (Maiorella, 1985; Taherzadeh & Karimi, 2007). The pretreatment process should alter or remove lignin, increase the accessibility of enzymes to the cellulose, and decrease the cellulose crystallinity (Jeihanipour & Taherzadeh, 2009; Sun & Cheng, 2002). Phosphoric acid is a non-corrosive, nontoxic, safe, and inexpensive chemical compared to other mineral acids. Furthermore, concentrated phosphoric acid is an ideal solvent, which can dissolve cellulose in presence of water without inhibitory effect in the subsequent hydrolysis and fermentation processes. The remaining acid may also be used as nutrient for the microorganism. Compared to other pretreatment technologies, this process utilizes lower temperatures and pressures and can be carried out at ambient conditions (Margeot et al., 2009; Zhang et al., 2010). Similarly, pretreatment by sodium hydroxide is among the pretreatment methods that received high attention over the years. It is a low energy intensive and relatively inexpensive technique, which has high impact on many feedstocks such as agricultural residues. Sodium hydroxide can simultaneously disrupt the structural linkages, affect the lignin barriers, reduce the cellulose crystallinity, and increase the cellulose accessibility, which results in a sharp increase in saccharification yield (Mosier et al., 2005; Taherzadeh & Karimi, 2008b; Xu et al., 2010). In short, alkali pretreatments proceed at ambient temperatures and at low pressures, which is advantageous as it eliminates the cost of maintaining the high temperatures and pressures that are usually required in other pretreatments. Alkali pretreatments increase the accessibility of the surface exposed to enzymatic hydrolysis through the removal of acetyl and uronic acid substituents on hemicelluloses (Chang & Holtzapple, 2000). Unlike the use of steam and acid-pretreatments, alkali pretreatments solubilise lignin and a small percentage of the hemicelluloses (Chang, 2007; Kaar & Holtzapple, 2000).

Global biofuel production has been increasing rapidly over the last decade, but the expanding biofuel industry has recently raised pertinent concerns. In particular, the sustainability of many first-generation biofuels, fuels made from edible sugars and starch, has been increasingly questioned over concerns such as reported displacement of food crops, effects on the environment and climate change. Therefore, an alternative raw material had to be sought and the answer could lie in sweet sorghum which will not use the current arable land under agriculture in Kenya, thus not affecting food and nutritional stability. This study sought to evaluate the potential of Rio SSB to give bioethanol that could have the potential of being blended with petrol and hence be used in petrol engines. The results could assist in optimization of the process before piloting and later commercialization.

2. Materials and Methods

2.1. Planting Materials and Experimental Design

The selected 16 sweet sorghum varieties were evaluated during the short rainy season of September – December 2012 and the long rains of April- July 2013 at the JKUAT experimental farm. The type of soils at JKUAT area is rhodicferralsols with pH of 6.2 with an annual rainfall of 856mm and a mean temperature of 25-27°C. The experimental design consisted of a randomized complete block design (RCBD) with three replications and each variety was sown in a plot size of 4 rows, 5 m long and 3 m wide (15m²), the spacing was 75cm by 30 cm and cultural practices such as weeding and disease control were done to obtain optimum stalk and sugar yields

2.2. Juice Extraction from the Stalks

After 16 weeks, 4 stalks of each variety were cut manually, seeds, pinnacle and leaves removed. The juice from the stalks was then removed using a sugarcane presser. The Brix of resulting juice was measured by a digital refractometer (Model PAL-1, Atago Co. Ltd., Tokyo, Japan) and put in 100ml plastic bottles and stored in a deep freezer at -20^0C awaiting glucose, fructose and sucrose analyses. Rio, sweet sorghum variety with the highest sucrose purity was harvested and the juice extracted using the sugarcane presser and put in 3lt plastic containers and stored in a deep freezer at -20^0C and its bagasse stored in the greenhouse to dry.

2.3. Comminution of Rio Bagasse

After 2 months of drying and storage in the greenhouse, the bagasse was ground using a diesel driven disintegrator, chopper and grinder (DPM-4, Brazil) to less than 0.84mm.This was further ground by a laboratory bench top mill to a size less than 0.42mm. The ground bagasse was then sealed in a black plastic bag and stored awaiting further analyses.

2.4. Proximate Analysis of Rio Bagasse

2.4.1. Moisture Determination

Moisture dishes which had been dried in a hot air oven for 1 hour and cooled in desiccators were weighed using 4 decimal analytical balance and approximately 2.00g of SSB sample put in them. Then the weight of dish plus sample was taken. This was placed in a hot air oven and the temperature set at 105^0C and dried for 2hrs.The samples were removed and put in a desiccator to cool to room temperature and then reweighed. They were then put back in the hot air oven for 30minutes and removed and put in a desiccator for 15minutes to cool to room temperature and then reweighed. This was stopped when a constant weight was observed.

2.4.2. Determination of Total Ash Content

Using a marker pen, 3 silica crucibles were marked and approximately 3g of RIO bagasse was weighed into the numbered crucibles in triplicate. The sample was then charred on a hot plate in the fume cupboard till no visible smoke was present. Once charred; the crucibles were placed on a heat resistant mat and transferred to a muffle furnace heated to 550^0C and heated in the furnace for 7hrs. Since the sample was not yet white or grayish white, it was moistened with distilled water and reheated on a hot plate in the fume cupboard, then transferred to the muffle furnace for further 9hrs, total time taken was16hrs. Samples were removed using tongs and cooled in a desiccator and weighed to determine the mass of ash.

2.4.3. Determination of Extractives by Soxhlet Extraction

Approximately 3g of Rio SSB was put in thimble in triplicate and numbered and thimble top closed with cotton wool. The thimbles were then inserted in the soxhlet apparatus and extracted with petroleum ether for 12hrs contained in pre-weighed round bottom flasks. After extraction, the solvent was distilled off from the round bottom flask using the rotary evaporator. The flasks and extractives (thimbles) were then dried on a hot plate in the fume cupboard and then weighed using the analytical balance and percentage extractives calculated

2.4.4. Determination of Lignin Concentration and Neutral Sugars

Extracted bagasse samples weighing 300mg in triplicate were mixed with 3ml of 72% H_2SO_4.The mixture was put in a water birth at 30^0C for 1hr for the hydrolysis reaction to take place. The acid was then diluted by adding 73ml of distilled water to a final concentration of 3%.The sample was then placed in an autoclave (Model Autoclave SS-325, Tomy Seiko Co., Ltd, Tokyo, Japan) at 125^0C for 1hr, cooled and filtered through filter paper number 1.The solids were dried to constant weight at 80^0C using a hot air oven and the resulting mass was taken as acid insoluble lignin. The filtrate was analyzed for neutral sugars namely glucan, galactan, mannan, and xylan using HPLC (Model LC-10AS, Shimadzu Corp., Kyoto, Japan) fitted with aminopropylsilyl column and a refractive index (RI) detector. The mobile phase was acetonitrile / water at ratio of 80:20 (v/v) at a flow rate of 1 ml/min, and the column and detector temperature was maintained at 35±1°C. The standard stock solution of glucan, galactan, mannan, and xylan were prepared with suitable concentrations.

2.5. Rio Bagasse Pretreatments

2.5.1. Phosphoric Acid- Acetone Pretreatment

Approximately 6g of bagasse were weighed using analytical balance and put in 50ml plastic centrifuge tubes and 60ml (85%) H_3PO_4 added. The mixture was then put in a water bath (MODEL: SHA-C, SN: 10706002) at 50^0C shaking at 90rpm for 30minutes.The treated slurry was washed with 20ml cold acetone and centrifuged (H-200 000, Kokusan Corporation, Japan) at 4000rpm for 20 minutes and temperature 27^0C. The bagasse was then washed three times with 40ml acetone, followed by three times with 40ml distilled water. The residual acetone from washing stages was removed from supernatant after simple evaporation in a fume hood. The treated bagasse was washed by hot distilled water several times till clear liquid was produced i.e pH 7(Jeihanipour & Taherzader, 2009; Zhang *et al.*, 2007). It was then dried in a hot air oven at 40 ± 1^0C for 2 days and kept in a refrigerator.

2.5.2. Alkaline Hydrogen Peroxide Pretreatment

Approximately 50ml of hydrogen peroxide was added to 700ml of distilled water and pH adjusted to 12.70 with sodium hydroxide. Four grams of bagasse was treated with 100ml of the pretreatment agent in 250ml flasks in a water bath at 2.5Hz for 4hrs. It was then washed with warm distilled water till pH was neutral and dried at 45^0C in a hot air oven.

2.6. Enzymatic Hydrolysis

Eight, 150ml conical flasks were labeled and approximately 1g of untreated, phosphoric acid pretreated and alkaline peroxide pretreated bagasse, in duplicate was put in the labeled conical flasks. The pH of citrate buffer was adjusted to 4.8 using 0.5M H_2SO_4 and 100ml of citrate buffer was added to each conical flask. These were then autoclaved at 121^0C for 1hr, and then allowed to cool in the autoclave to 50^0C. Approximately 3.3mg (20 Filter Paper Units) of cellulase produced by Trichoderma reesei was then added to each flask and incubated in a shaking water bath at 50^0C for 72hrs. Samples were drawn after 30h, 54h and 72h for sugar analysis and placed in a boiling water bath for 15minutes to deactivate the cellulase and then stored in a deep freezer at -20^0C.The resulting hydrolysate was then deactivated in a boiling water bath for 15minutes and then stored in a deep freezer at -20^0C awaiting fermentation.

The yield of enzymatic hydrolysis was calculated as a ratio of theoretical glucose production yield using the following equation 1.

$$\text{Yield of enzymatic hydrolysis } (\%) = \frac{\text{Produced glucose}(g/l)\times 100}{1.111\times \text{Substrate concentration}(g/l)\times F} \quad \ldots\ldots (1)$$

Where F in the denominator is the biomass glucan fraction, and is presented in Table 1 and 2, for untreated and different pretreated bagasse. The conversion factor of 1.111 was applied to consider the conversion of glucan to glucose.

2.7. Fermentation to Ethanol

The pH of the hydrolysates was adjusted to 6.0±0.1 using 0.5MNaOH or 0.5MH_2SO_4. Two more conical flasks for 1g glucose each to be used as the reference were added and autoclaved at 121^{0}C for 1hr and then cooled to 30^{0}C. Approximately 0.3g/100ml of yeast strain sacharomyce cerevisiae was added into each conical flask and supplemented with 0.005g/100ml of $(NH)_2HP0_4$ and 0.001g/100ml $MgSO_4.7H_20$ as nutrients. The samples were then placed in a water bath at 30^{0}C shaking at 150rpm and fermented for 48hrs.Approximately 5ml samples were collected from each flask and centrifuged at 13000rpm for 5minutes and the supernatants was then stored in a deep freezer at -20^{0}C for GC analysis. The fermentation broth was placed in a boiling water bath for 15minutes to deactivate the enzyme and then stored in a deep freezer at -20^{0}C. Equation (2) was used for calculation of ethanol production yield:

$$Yield\ of\ ethanol\ production\ (\%) = \frac{\text{Produced ethanol } (g/l) \times 100}{0.51 \times 1.111 \times \text{Substrate concentration } (g/l) \times F} \quad \ldots\ldots (2)$$

2.8. Gas Chromatography Analysis

The ethanol concentration was determined by a gas chromatography using a GC-9A Shimadzu, equipped with a packed column and a flame ionization detector. The temperature of the injector was 220^{0}C and that of the detector 240^{0}C. For the column a gradient of 50-150^{0}C was used and nitrogen was used as a carrier gas while hydrogen and air were used as the combustion gases. The ethanol (chromatographic grade) and butanol (0.5g/l) were used for the standard curve and internal standard substance separately. Samples of 1µl were directly injected into the column. All determinations were done by means of standard curves, and the final results were the average of two repetitions.

2.9. Data Analysis

The data analysis was done using Microsoft Excel.

3. Results and Discussion

3.1. Composition of Rio Sweet Sorghum Bagasse

The sweet sorghum bagasse used in this study contained glucan (38.26%), xylan (17.22%), Acid insoluble lignin (21.07%) and Ash (3.94%) (Table 1). Galactan, mannan and arabinan were not detected. This does not mean that they were absent because other researchers like Shen (2011) was able to detect them though their values were less than 2%. The glucan content was higher than that of 35.1% got by Shen (2011) but similar to that got by Wu (2011) of 38.7%. Approximately 60% of the total dry matter was polysaccharide, in which the largest fraction was glucan occupying 38.26% in total carbohydrate content. Thus, such high polysaccharide content could be potentially made SSB available for hydrolysis and subsequent ethanol fermentation. Moreover, the ash content accounted for 3.94% of the total dry matter of SSB, and it was lower than that obtained by Ballesteros (2004) for SSB of 4.8% but higher than that of sugarcane bagasse of 1.6% obtained by Rabello (2011). Lower ash content in lignocellulosic material was proved to be potentially beneficial for enzymatic hydrolysis (Yu & Chen, 2010). The extractives consisted of 18.34% of the total dry matter.

Carbohydrates (%wt/wt)				**Extractives (%wt/wt)**	**Acid Insoluble lignin(%wt/wt)**	**Ash (%wt/wt)**
Glucan	Xylan	Galactan	Mannan			
38.26±0.35	17.22±0.28	n.d.[a]	n.d.	16.34±0.25	21.07±0.43	3.94±0.22

Table 1: The composition of Rio sweet sorghum bagasse (weight percent of dry matter)
Values are presented as Mean ± SD, n=3 [a] *"n.d." means "not detected".*

3.2. Pretreatment

The compositions of the bagasse after the pretreatments were analyzed and the results are presented in Table 2. Glucan was the dominant component in the range of 49.12% to 63.4% and xylan was in the second in the range of "not detected" to 14.7%. There was a remarkable increase in glucan fraction after the pretreatments. The glucan fraction increased by 65.7% and 28.4% after the pretreatment by sodium hydroxide and phosphoric acid, respectively. On the contrary to glucan, xylan decreased by 14.6% when sodium hydroxide was used and it was not detected when phosphoric acid was used. According to Goshadrou (2011), 58.66% glucan was observed when sodium hydroxide solution only was used as the pretreatment agent (41.9% increase in glucan fraction) and 52.25% glucan (26.42% increase in glucan fraction) when phosphoric acid was used as the pretreatment agent. On the other hand xylan dropped by 29.2% and 33.85% when sodium hydroxide and phosphoric acid were used, respectively. The interest in the use of phosphoric acid is that after neutralization of hydrolysates with NaOH, the salt formed is sodium phosphate (Gámez *et al*., 2006). This salt can remain in the hydrolysates because it is used as nutrient by micro-organisms (Cardona, 2010). Therefore, a filtration operation of it is not needed with the subsequent advantages: improvement of process profitability (avoiding salts removal and decreasing the amount of nutrients needed for fermentation) and positive impact to the environment (the salt formed is not a waste).

Pretreatment Method		Glucan (%)	Xylan (%)	Mannan (%)	Galactan (%)
NaOH		63.40±0.76	14.70±0.60	n.d.	n.d.
H_3PO_4		49.12±2.31	n.d.[a]	n.d.	n.d.

Table 2: The carbohydrate content of pretreated Rio sweet sorghum bagasse in different conditions
[a] "n.d." means "not detected". Values are presented as Mean ± SD, n = 2

3.3. Enzymatic Hydrolysis (Saccharification)
Different preparations of Rio sweet sorghum bagasse were subjected to 72h enzymatic hydrolysis by addition of cellulase. The most important hydrolysis results are presented as percentages of theoretical sugar yield in Table 3. The yield of hydrolysis of native bagasse was effectively improved after sodium hydroxide and concentrated phosphoric acid pretreatments. Hydrolysis of the untreated bagasse resulted in 15% and 50% conversion after 30h and 72h, respectively. The hydrolysis yield increased from 50% to 78% after pretreatment by concentrated phosphoric acid. The best results of enzymatic hydrolysis were obtained in the hydrolysis of pretreated bagasse by NaOH solution where more than 88% of the theoretical glucose yield was obtained within 72h.
Table 3. Yield of enzymatic hydrolysis of untreated and different pretreated sweet sorghum bagasse (as percentage of theoretical yield).

Pretreatment Method	Yield of Enzymatic Hydrolysis(% Theoretical Sugar Yield)		
Hydrolysis time	30h	54h	72h
Untreated	15%	29%	50%
NaOH pretreated	32%	84%	88%
Phosphoric acid pretreated	22%	76%	78%

Table 3

3.4. Fermentation
Figures 1 and 2 show the results of fermentation. Pure glucose was selected as a reference in fermentation and a mass ethanol yield and productivity of 0.51g/g and 0.106g/l.h, respectively were observed. The results showed significant improvements in ethanol production from 15.33% of the theoretical yield for untreated bagasse to 40.45%-59.44% for the different pretreated materials depending on the pretreatment method. According to figure 1, phosphoric acid pretreatment improved the ethanol production yield to 59.44% (44.11% increment), which was the better result for ethanol production between the two applied pretreatment techniques. The results showed that pretreatment with sodium hydroxide increased ethanol yield by 25.12%.
The maximum volumetric ethanol productivity for untreated and all pretreated materials is presented in figure 2. The ethanol productivity for untreated bagasse hydrolyzate was 0.001g/l.h, while it was improved after the pretreatments. The materials pretreated with sodium hydroxide showed the highest productivity (0.019g/l.h) whereas the phosphoric acid pretreated bagasse had the productivity of 0.016g/l.h. Ethanol yield for the hydrolysates was lower than that obtained when glucose was used as substrate. According to Cheng (2010), when the fermentation process of any lignocellulosic material is completed, ethanol concentration in the fermentation broth is usually 10-15% (w/w) or 13-19% (v/v). Therefore the results observed of 0.009-0.646% (v/v) of ethanol in the fermentation broth are far much lower than those observed by Cheng (2010). This difference could be due to the amount and type of enzyme used and the conditions of fermentation and even the presence of fermentation inhibitors. For instance, the baker's yeast used in this research can only ferment hexoses and not pentoses thus affecting the ethanol yield observed.

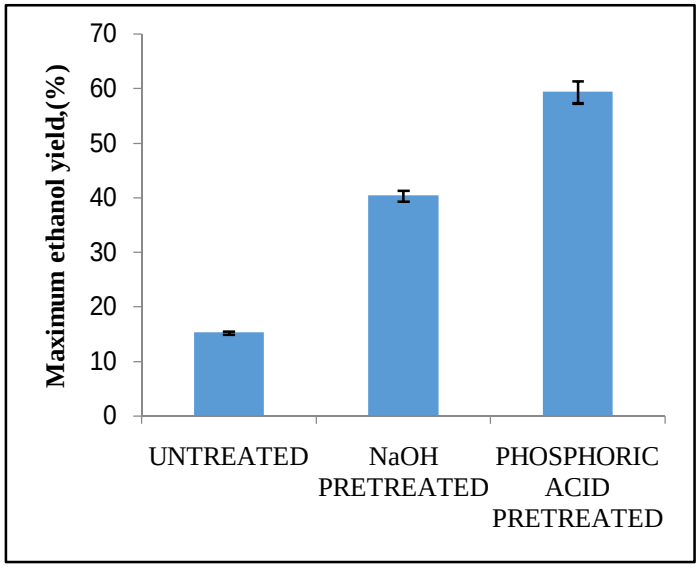

Figure1: Maximum ethanol yield (% theoretical yield)

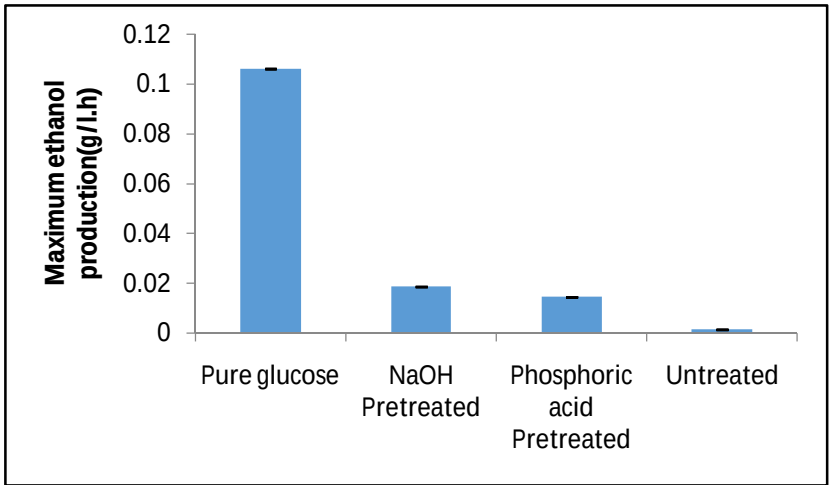

Figure 2: Rate of ethanol production (g/l.h)

4. Conclusion

From the study, Rio SSB can be used to produce bioethanol and the best pretreatment method was phosphoric acid which gave an approximate % theoretical ethanol yield of 60 but NaOH pretreatment gave the highest rate of ethanol production of 0.019g/l.h. Further research should be done to increase the bioethanol yield before piloting and later commercialized.

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5. References

i. Ballesteros, M., Oliva, J.M. and Negro, M.J. (2004). Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with kluyveromyces marxianus CECT 10875. Process Biochemistry, 39, 1843-1848.

ii. Barbanti, L., Grandi, S. and Vecchi, A. (2006). Sweet and fibre sorghum [Sorghum bicolor(L.)Moench], energy crops in the frame of environmental protection from excessive nitrogen loads. Eur.J. Agron., 25(1), 30-39.

iii. Cardona, C.A., Quintero, J.C. and Paz, I.C. (2010). Production of bioethanol from sugarcane bagasse: Status and perspectives. Bioresource Technology, 101, 4754-4766.

iv. Chang, M.C.Y. (2007). Harnessing energy from plant biomass. Curr. Opin. Chem. Biol., 11, 677-684.

v. Chang, V.S. and Holtzapple, M.T. (2000). Fundamental factors affecting biomass enzymatic reactivity. Appl. Biochem. Biotechnol., 84, 5-37.

vi. Cheng, K.K., Zhang, J.A. and Ping, W.X. (2008). Sugarcane bagasse mild alkaline/oxidative pretreatment for ethanol production by alkaline recycle process. Appl. Biochem. Biotechnol., 151(1), 43-50.

vii. Cheng, J.J. (2010). Advanced Biofuel Technologies: Status and Barriers. Development Research Group. The World Bank, September.

viii. Fei, S., Jack N. Saddler and Ronghou, L. (2011). Evaluation of steam pretreatment on sweet sorghum bagasse for enzymatic hydrolysis and bioethanol production. Carbohydrate Polymers, 86, 1542-1548.

ix. Gámez, S., González-Cabriales, J.J. and Ramirez, J.A. (2006). Study of the hydrolysis of sugar cane bagasse using phosphoric acid. J. Food Eng., 74, 78-88.

x. Goshadrou A., Karimi K. and Taherzadeh, M.J. (2011). Bioethanol production from sweet sorghum bagasse by Mucor hiemalis. Industrial Crops and Products, 34, 1219-1225.

xi. Jeihanipour, A. and Taherzadeh, M.J. (2009). Ethanol production from cotton-based waste textiles. Bioresour. Technol., 100(2), 1007-1010.

xii. Kaar, W.E and Holtzapple, M.T. (2000). Using lime pretreatment to facilitate the enzymatic hydrolysis of corn stover. Biomass Bioenerg., 18, 189-199.

xiii. Li, B.Z., Balan,V. and Yuan, Y.J. (2010). Process optimization to convert forage and sweet sorghum bagasse to ethanol based on ammonia fiber expansion (AFEX) pretreatment. Bioresour. Technol., 101(4), 1285-1292.

xiv. Long, W., Mitsuhiro, A. and Masakazu, I. (2011). Low temperature alkali pretreatment for improving enzymatic digestability of sweet sorghum bagasse for ethanol production. Bioresource Technology, 102, 4793-4799.

xv. Margeot, A., Hahn-Hagerdal, B. and Edlund, M. (2009). New improvements for lignocellulosic ethanol. Curr. Opin. Biotechnol., 20(3), 372-380.

xvi. Mosier, N., Wyman, C. and Dale, B. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol., 96(6), 673-686.

xvii. Macesic, D. and Darko, U. (2008). Biomass production and ethanol potential from sweet sorghum in Croatia. Cereal Res. Commun., 36(5-1) 527-530.

xviii. Maiorella, B.L.(1985). Ethanol. In:Moo-Young, M. (Ed.),Comprehensive Biotechnology., first ed. Pergamon Press Ltd., Oxford, pp. 862-909.

xix. Rabelo, S.C., Amezquita Fonseca, N.A. and Andrade, R.R. (2011). Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide. Biomass and Bioenergy, xxx, 1-8.

xx. Sipos, B., Reczey, J. and Somorai, Z. (2009). Sweet sorghum as feedstock for ethanol production: enzymatic hydrolysis of steam-pretreated bagasse. Appl. Biochem. Biotechnol., 153(1-2), 151-162.

xxi. Teetor, V.H., Duclos, D.V. and Wittenberg, E.T. Effects of planting date on sugar and ethanol yield of sweet sorghum grown in Arizona. Ind. Crops Prod., (Corrected proof), in press.

xxii. Wu, X. R., Staggenborg, S. and Propheter, J.L. (2010). Features of sweet sorghum juice and their performance in ethanol in ethanol fermentation. Ind. Crops Prod., 31(1), 167-170.

xxiii. Taherzadeh, M.J. and Karimi, K. (2008a). Bioethanol: market and production processes. In: Nag, A.(Ed.), Biofuels Refining and Performance. McGraw-Hill, New York, pp. 69-106.

xxiv. Taherzadeh, M.J. and Karimi, K. (2008b). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. Int. J. Mol. Sci., 9(9), 1621-1651.

xxv. Taherzadeh, M.J. and Karimi, K. (2007). Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. Bioresources, 2(4), 707-738.

xxvi. Sun, Y. and Cheng, J.Y. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour. Technol., 83(1), 1-11.

xxvii. Xu, J., Cheng, J.J. and Sharma-Shivappa, R.R. (2010). Sodium hydroxide pretreatment of switchgrass for ethanol production. Energy Fuels, 24(3), 2113-2119.

xxviii. Yu, B. and Chen, H. (2010). Effect of the ash on enzymatic hydrolysis of steam-exploded rice straw. Bioresource Technology, 101(23), 9114-9119.

xxix. Zhao, Y., Dolat, A. and Steinbergerc, Y. (2009). Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. Field Crops Res., 111(1-2), 55-64.

xxx. Zhang, J., Zhang, B. and Ouyang, P. (2010). Effect of phosphoric acid pretreatment on enzymatic hydrolysis of microcrystalline cellulose. Biotechnol. Adv., 28(5), 613-619.

xxxi. Zhang, Y.H.P., Ding, S.Y. and Mielenz, J.R. (2007). Fractionating recalcitrant lignocelluloses at modest reaction conditions. Biotechnol. Bioeng., 97(2), 214-223.