

# Production of fermentable sugar from elephant grass (*Pennisetum purpureum*)

## Obtención de azúcares fermentables a partir de pasto elefante (*Pennisetum purpureum*)

Received: 10-30-2014 Accepted: 11-28-2014

Marcelo Alexander Guancha Chalupud<sup>1</sup>  
Brandon Guerrero Olave<sup>2</sup>  
Katheryn Mondragón Escobar<sup>3</sup>  
Juan David Caicedo Salazar<sup>4</sup>  
Leydi Johana Aponza Múnera<sup>5</sup>  
Lesly Johana Briceño Barona<sup>6</sup>

<sup>1</sup> Colombian engineer. Chemist at the National University of Colombia. Instructor of National Technical Assistance Center for Industry - Sena. marceloguancha@MisenA.edu.co.

<sup>2</sup> Chemical Technologist Colombian National Technical Assistance Center for Industry - Sena. branguerrero@MisenA.edu.co

<sup>3</sup> Chemical Technologist Colombian National Technical Assistance Center for Industry - Sena. k-theryn@misena.edu.co

<sup>4</sup> Chemical Technologist four Colombian National Technical Assistance Center for Industry - Sena. jdcaicedo9@misena.edu.co

<sup>5</sup> Chemical Technologist Colombian National Technical Assistance Center for Industry - Sena. ljaponzam@misena.edu.co

<sup>6</sup> Chemical Technologist Colombian National Technical Assistance Center for Industry - Sena. lbriceno@misena.edu.co

### Abstract

The present study shows the results of obtaining fermentable sugars using elephant grass (*Pennisetum purpureum*) feedstock. The concentration effect of acetic acid ( $\text{CH}_3\text{COOH}$ ) and sodium chlorite ( $\text{NaClO}_2$ ) was assessed on the removal of lignin hemicellulose complex. A comparison was made about method, using basic pretreatment with sodium hydroxide ( $\text{NaOH}$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). The Saccharification step is carried out by enzymatic hydrolysis method that is performed using cellulase and  $\beta$ -glucosidase. The results show in the case of pretreatment with  $\text{NaClO}_2$  and  $\text{CH}_3\text{COOH}$  a percentage of saccharification of 80.87% compared to  $\text{NaOH}$  pretreatment with a percentage 56.82% and hydrogen peroxide with a percentage of 44.75%.

**Keywords:** Lignin; pretreatment; enzymatic hydrolysis; elephant grass; ethanol.

### Introduction

Petroleum and its subproducts are considered nowadays as one of the biggest pollutants worldwide. The production and usage of fossil fuels generates large amounts of carbon dioxide; these are emitted into the atmosphere and are cause of climate change and global warming. Therefore, it is necessary to investigate alternatives routed to the production of biofuels as commitment to the enforcement of Law 693 of 2001, "in which the laws typify the rules and code to use alcohols as fuels, creation of stimuli for the production, distribution and usage." (Secretaría General de la Alcaldía Mayor de Bogotá D.C, 2001). At present, the technology to integrate a system for ethanol production, exists. However, one of the problems of first-generation ethanol production is focused on alimentary safety (FAO, 2012), which has ignited a great controversy regarding the risk generated by deciding between producing ethanol and producing food;

for which, actually, viable alternatives are being looked for to produce second-generation biofuels, since Colombia has a great potential, due to its large availability and variety of natural resources, like lignocellulosic materials. In general, lignocellulosic materials are resistant to ethanol conversion under the presence of lignine. Therefore, a pre-treatment is required to augment its biodigestibility and making the cellulose more accessible to the generation of simple sugars. Of the lignine-hemicellulose mixture, a complex structure is created around the cellulose, which needs to be removed or modified to be able to make an efficient cellulose hydrolysis. For this, alkaline or acid aqueous pre-treatments are applied. (Singh *et al*, 2014).

In this study, *Pennisetum purpureum* is used as feedstock, its main advantages are that compared to other lignocelluloses, consists of a species that easily adapts to diverse characterizations of the soil. Furthermore, its structure is covered mainly of a hemicellulose-lignine network, with high biomass content. The stalk dimensions can reach values from 1.5 to 2.0 cm in diameter and can reach 3 meters high (Basso, *et al*. 2014).

This research proposes the method of Sodium Chlorite delignification in acid media (CH<sub>3</sub>COOH), since it presents advantages in comparison to other inorganic acids, due to the easy recovery of it. Its production is low cost and can be obtained from through biological processes. Sodium Chlorite, in its place, it can degrade lignine into soluble products; it is important to mention that this reactive is very severe, degrading the cellulose chains to a certain point. A pre-treatment effectivity comparison was realized using Hydrogen Peroxide and Sodium Hydroxide, in temperature and concentration regulated conditions.

## Materials and Methods

### Materials

Elephant grass (*Pennisetum purpureum*) was obtained from the city of Guacari, Valle del Cauca, in the hacienda “El Edén”, next to Guabas river. After the process of collection, the material was transported to the laboratory and then properly prepared and stored.

### Feedstock preparation

Initially, the elephant grass was washed with water at room temperature filtered and dried at 60°C until its weight

became constant to remove impurities. In a following step, the particle size reduction was achieved with a bladed grinder, until a size of 53 µm was measured.

### Pretreatment with Acetic Acid and Sodium Chlorite

For the pretreatment trials, with CH<sub>3</sub>COOH AND NaClO<sub>2</sub>, a 2-3 factorial design was used with 2 more instances. The factors accounted for, are: Acetic Acid Concentration, (CH<sub>3</sub>COOH) 3M and 6M, Sodium Chlorite concentration, 2.0% and temperature. The grass was treated with a 1:1 CH<sub>3</sub>COOH and NaClO<sub>2</sub>; the solid to liquid ratio of 1:15. The pretreatment for 4 hours, in autoclave, according to the method proposed by Siqueira (Siqueira *et al*, 2012). The samples treated were filtered and cleansed with distilled water until neutral pH, were dried at a temperature of 50°C until constant weight; the resulting material was stored in sealed plastic bags in a dehumidifier. In table 1, the essay results can be appreciated.

**Table 1.** Pretreatment tests with CH<sub>3</sub>COOH/NaClO<sub>2</sub>

Sample	Concentration		Temperature [°C]
	NaClO <sub>2</sub> [%]	CH <sub>3</sub> COOH [mol/L]	
1	2.0	3.0	100
2	2.0	6.0	100
3	0.5	3.0	100
4	0.5	6.0	100
5	2.0	3.0	120
6	0.5	6.0	120
7	2.0	6.0	120
8	0.5	3.0	120

### Oxidizing and alkaline pretreatment

The alkaline pretreatment was realized according to the method proposed by Cardona (Cardona *et al*, 2014). The elephant grass was treated with a Sodium Hydroxide solution for 4 hours, in an autoclave, keeping a solid liquid ratio of 1:15. Each essay was duplicated; the different pretreatments are shown in table 2. After the alkaline pretreatment, the samples are filtered, washed with distilled water until neutral pH. The samples were dried until constant weight and stored in sealed plastic bags in a dehumidifier.

The same procedure was executed with Hydrogen Peroxide, whose concentrations were 2.0% and 4.0% at 40°C

for 4 hours in an autoclave. The solution pH was fixed to an approximate value of 11 with Sodium Hydroxide, 10.0M.

**Table 2.** Pretreatment tests with Sodium Hydroxide

Sample	NaOH [%]	Temperature [°C]
1	1.0	100
2	2.0	100
3	1.0	100
4	2.0	120

## Chemical composition of the samples

Analysis regarding humidity content, percentage of extractive substances and the lignine content, of the nontreated, as well as the treated samples, were realized in compliance to NREL/TP-510-42618. To determine the extractive percentage, a Soxhlet apparatus with 600 mL of Acetone, was used. 2 grams samples were weighed on 150 mm No. 41 filter paper and was inserted in the extractor. The extraction refluxes were conducted for 6 hours. (Schoening, A and Johansson, G, 2006); Sluiter et al., 2008).

To determine the amount of Lignine, a 0.3 gr sample was taken anhydrous, free of extractables, and was inserted in a 100 mL precipitation flask. 3 mL of 72% pure sulfuric acid were added and left to react for 1 hour. Subsequently, distilled water was added, until the concentration of the acid was reduced to 4%. The sample was inserted in an autoclave for 1 hour, at 120°C. Then, the sample was separated through a #3 Gooch filter. To determine the insoluble lignine, the sample was neutralized with distilled water and was dried at 100°C, until constant weight was achieved. To determine soluble lignine in the filtered water, absorbancy readings were taken at 205 nm, using as a reference, sulfuric acid at 4% and a 1100 mL-AU/g molar extinction coefficient (Schoening, A., y Johansson, G., 2006; Siqueira et al., 2012; Sluiter et al., 2008).

To achieve anhydrous, extract-free cellulose determination, a mixture of ethanol and nitric acid, in a 4:1 ratio, was added to 1.0 gr of sample, taken to reflux in water bath for 30 minutes and then, filtered. The residue was washed with hot distilled water for an hour, then with a saturated solution of Sodium Acetate, followed by hot distilled water. It was dried at 105°C, cooled in a dessicator and weighed (Hernado et al., 2009).

## Thermal Properties

These samples were analyzed thermogravimetrically: untreated Elephant grass, pretreated with  $\text{CH}_3\text{COOH}/\text{NaClO}_2$ ,  $\text{NaOH}$  and  $\text{H}_2\text{O}_2$ . Besides, to acquire a comparison criteria, a thermogravimetric analysis of a commercially-available cellulose sample. The thermograms were obtained in a DSC/TGA 2STAR SYSTEM, Mettler-Toledo, with a temperature range from 30°C to 600°C, with a heating rate of 10°C per min<sup>-1</sup>, nitrogen supply (20 mL/min<sup>-1</sup>) and aluminum oxide crucibles with 8 mg of sample, approximately. (Solorza et al., 2013; Zhang et al., 2014).

## Enzymatic Hydrolysis

For the enzymatic hydrolysis,  $\beta$ -glucosidase was used. Sigma Aldrich 49290 with enzymatic activity 6UI/gr (international units) and *Trichoderma reesei* ATCC 26921 with an enzymatic activity of 700 EGU/gr (endoglucanase units). The hydrolysis took place in a thermostatic bath at 50°C in 20 mL test tubes. 0.5 gr samples of pretreated with  $\text{NaOH}$ ,  $\text{CH}_3\text{COOH}/\text{NaClO}_2$ ,  $\text{H}_2\text{O}_2$  and commercially-available cellulose are infused with 1000uL of cellulase enzyme and 0.5 (500uL) of  $\beta$ -glucosidase. The volume was raised to 10 mL with citrate tampon (50mM) and a 4.8 pH. Each essay was replicated 3 times. To determine the total reductor sugars, 500uL quotes were taken periodically at 1,2,3,6,12,24,48 and 72 hours, respectively (Cuadros y Celis, 2007). The concentration of total reductor sugars was determined by the DNS (3,5-Dinitrosalicylic acid) with glucose, as pattern. The concentration of reductor sugars was determined by absorbancy using a 540 nm wavelength, in accordance to the method proposed in 2010 by Rabelo (Rabelo, 2010).

## Discussion and Results

### Composition of the samples

In table 3 the composition of cellulose and lignine extracted from the treated and untreated samples are shown. The pretreatments conducted at the highest concentration and temperature. The fraction that corresponds to "others" includes compounds such as uronic acid groups, acetyl and traces along with minerals, waxes, fats, resins and gums (Cardona et al., 2014; Cuadros y Celis, 2007).

According to the results of table 3, it is observed that there is an increase in the cellulose content of the pretreated samples, due to the partial hydrolysis of lignine and

hemicellulose, (Rabelo, 2010; Cuadros y Celis, 2007). The NaOH pretreated samples presented a cellulose content of 62.20% in comparison to the H<sub>2</sub>O<sub>2</sub> treated samples (58.42%). Such raise in the cellulose content during the alkaline

pretreatments, resulted more efficient due to the high elimination of lignine, since it actuates on the ester bonds and N-glicosidic chains of lignine.(Cuadros y Celis. 2007; Rabelo, 2010).

**Table 3.** Composition of the samples

Sample	Cellulose [%]	Lignin [%]	Extractives [%]	Others (%)
Without Pretreatment	35.29	31.11	16.32	17.28
CH <sub>3</sub> COOH 6M/NaClO <sub>2</sub> 2%	58.42	11.68	10.23	19.68
H <sub>2</sub> O <sub>2</sub> (4%,40°C)	59.62	11.43	10.94	18.02
NaOH (2%,120 °C)	62.20	8.44	3.91	25.45

### Results of the pretreatment with CH<sub>3</sub>COOH/NaClO<sub>2</sub> alkaline and oxidant.

The data resulting from the removal of lignine in the essays conducted to the elephant grass samples with CH<sub>3</sub>COOH/NaClO<sub>2</sub> is presented on table 4. On table 5, the results of the lignine removal with NaOH and H<sub>2</sub>O<sub>2</sub>.

The lignine removal from elephant grass with the use of CH<sub>3</sub>COOH/NaClO<sub>2</sub> shows that the removal percentage is higher (62.46% and 72.73%) at 6.0 M CH<sub>3</sub>COOH concentrations and 120°C of temperature. Similar results were generated when treated with NaOH (72.86%) and H<sub>2</sub>O<sub>2</sub> (63.26%). All of them are coherent with the data obtained by Cardona (Cardona et al. 2014), whose percentages of lignine removal are in a range of 76.2% to 88.4%.

**Table 4.** Results of lignine removal pretreatment with CH<sub>3</sub>COOH/NaClO<sub>2</sub>

Sample	NaClO <sub>2</sub>	CH <sub>3</sub> COOH [mol/L]	Temp [°C]	Lignine[%]	Lignine removed[%]
1	0.5	6.0	100	17.1	45.00
2	2.0	6.0	100	12.24	60.67
3	0.5	3.0	100	12.84	58.72
4	2.0	3.0	100	16.57	46.73
5	0.5	6.0	120	8.48	72.73
6	2.0	6.0	120	11.68	62.46
7	0.5	3.0	120	10.96	64.77
8	2.0	3.0	120	13.67	56.07

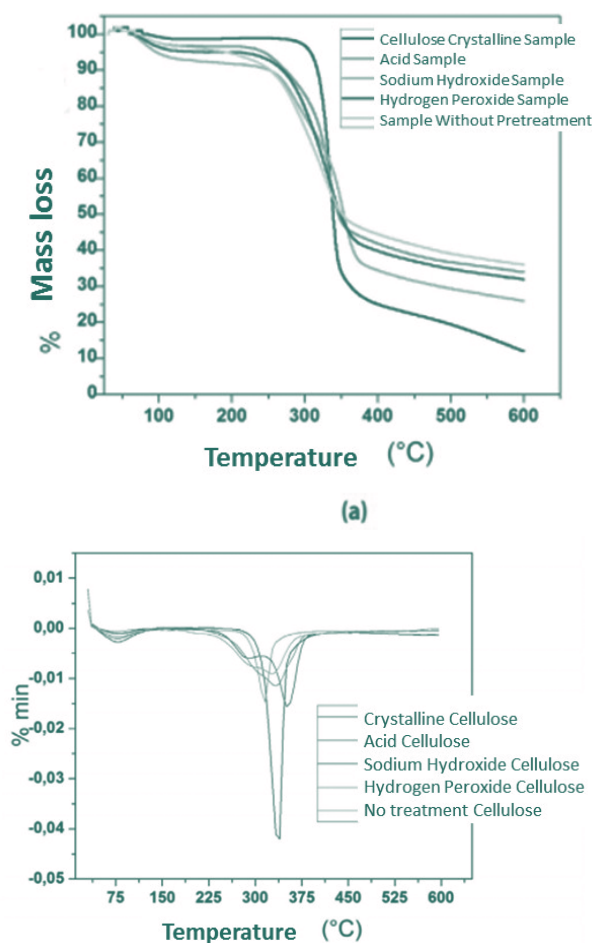
**Table 5.** Results of lignine removal alkaline pretreatment

Treat.	Concentration [%]	Temp [°C]	Lignine[%]	Lignine removed[%]
NaOH	1	100	10.99	64.66
	2	100	10.57	66.03
	1	120	10.04	67.74
	2	120	8.44	72.86
H <sub>2</sub> O <sub>2</sub>	2	40	14.23	54.27
	4	40	11.43	63.26

## Thermal properties

In figure 1 (a), thermal decomposition curves for commercially available cellulose and pretreated samples with acetic acid/sodium chloride at 3.0 M and 6.0 M, and the untreated sample are shown. In the figure 1b, a differential thermal analysis (b) obtained from acid pretreatment (  $\text{CH}_3\text{COOH}$  6.0 M, Sodium Chlorite 0.5%, temperature 120°C), hydrogen peroxide (4%  $\text{H}_2\text{O}_2$  at 40°C) and a sample of commercially available cellulose.

You can observe in figure 1b that the mass loss for each one of the pretreated samples, starts at 75°C, which indicates water loss, except for the commercially available cellulose. The highest temperature peak for commercially available cellulose is 340°C, which is the degradation temperature for cellulose. The samples obtained from this research have a lower degradation temperature due to the presence of hemicellulose traces that were not removed during the pretreatment (Zhang *et al*, 2014).



**Figure 1.** (a) Thermo gravimetrical analysis cellulose elephant grass treatment with acid (b) Thermo differential analysis.

The ART results in function of enzymatic hydrolysis time for the pretreated samples, untreated samples and commercially available cellulose are exhibited in figure 2.

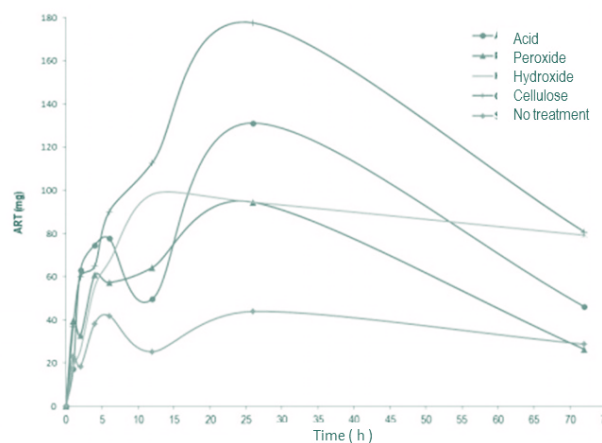
The highest conversion of cellulose to glucose took place in the NaOH treated samples; the saccharification process presented a higher yield in the samples treated with  $\text{CH}_3\text{COOH}/\text{NaClO}_2$  (80.87%) in comparison to the NaOH treatment (56.82).

**Table 6.** Percentage of saccharification of the pretreated samples

Sample	ART [%]
$\text{CH}_3\text{COOH}/\text{NaClO}_2$	80.87
NaOH	56.82
$\text{H}_2\text{O}_2$	57.06
Without pretreatment	44.75
Crystalline Cellulose	93.94

The cellulose has some ramifications denominated hemicelluloses, which gives the material an amorphous structure (Rabelo *et al*, 2011). Hemicellulose has a hydrophilic character, soluble in alkaline media and easily hydrolyzable in acid media. The lignine, that is the external layer is a polymer that contains aliphatic and aromatic compounds. It is completely amorphous and hydrophobic, soluble in alkaline media (Wörmeyer *et al.*, 2011), (Zhang *et al.*, 2014).

Therefore, if hemicellulose is easily hydrolyzable in acid media, this pretreatment can contribute to an increase in the total reducing sugars.



**Figure 2.** Variation of the ART with the enzymatic Hydrolysis time.

## Conclusions

In accordance to the  $\text{CH}_3\text{COOH}/\text{NaClO}_2$  and  $\text{NaOH}$  pretreatments, the percentages of higher lignine removal were presented in high concentration acids, hydroxide and  $120^\circ\text{C}$  of temperature.

The results evidence that even though the acid treatments allowed a good delignification with a 72.73% of removed lignine at  $120^\circ\text{C}$ , the higher percentages of removed lignine were obtained from the treatments conducted with an alkaline agent and hydrogen peroxide, denoting higher cellulose content to be hydrolyzed in the samples pretreated with sodium hydroxide (62.20) in comparison to the samples pretreated with hydrogen peroxide (59.62%) and acetic acid/sodium chloride (58.42%).

The acetic acid/sodium chloride pretreatment presents similar results regarding lignine removal in comparison to the sodium hydroxide pretreatment (72% of removed lignine). For the acetic acid case, the saccharification grade is 80.8% and for the sodium hydroxide is 56.0%, which indicates that the pretreatment with acid and sodium chlorite besides the removal of lignine, there is an increase of the amorphous regions in the cellulose of the elephant grass which leads to an increase in saccharification.

## Acknowledgements

The authors of this article would like to thank the members of the Product and Material Development Research Group (GIDEMP) of the ASTIN Center – SENA, the Technological Development, Innovation and Research Group, SENNOVA for the financial support through the SENA Micro, Nano and Biotechnology contest in 2013-2014, and the ASTIN Center subdirector, Engineer Aura Elvira Narváez.

## References

Basso, V., Machado, J., Da Silva, F., Da Costa, J., Fontana, R., Dillon, Al., and Camassola, M. (2014). Different elephant grass (*Pennisetum purpureum*) accessions as substrates for enzyme production for the hydrolysis of lignocellulosic materials. *Biomass and bioenergy*, 71: 155 – 161.

Cardona, E., Rios, J. Peña, J., and Rios, L. (2014). Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass. *Fuel*, 118: 41–47.

Cuadros J.F y Celis O.J. (2007). *Efectos del pretratamiento con ácido diluido e hidrólisis enzimática del bagazo de caña para la producción de glucosa*. Universidad Industrial de Santander, Bucaramanga, Colombia.

Hernado, L., Rojas, D., y Giraldo, G. (2009). *Cuantificación de celulosa proveniente de la industria del Mueble. Ingeniera de Recursos Naturales y del Medio Ambiente ocho*. Universidad del Quindío.

Organización de las Naciones Unidas para la Alimentación y la Agricultura. (2014). *Los biocombustibles y su efecto sobre la seguridad alimentaria*. Recuperado de: <http://www.fao.org/>.

Rabelo, S. (2010). *Avaliação e otimização de pré-tratamentos enzimática do bagaco e Hidrólise de cana de açúcar para a produção de etanol de segunda geração*. Campinas . Tese de Doutorado. Universidade Estadual de Campinas, Campinas, Brasil.

Secretaría General de la Alcaldía Mayor de Bogotá D.C. (2001). *Ley 693 de 2001*. Diario Oficial 44564.

Singh, R., Shukla, A., Tiwari, S., and Srivastava, Monika (2014). *A review on delignification of lignocellulosic biomass for enhancement of ethanol production potential*. *Renewable and Sustainable Energy Reviews* 32: 713–728

Siqueira, G, Várnai, A. Ferraz, A., and Milagres, A. (2013) Enhancement of cellulose hydrolysis in sugarcane bagasse by the selective removal of lignin with sodium chlorite. *Applied Energy*, 102: 399–402.

Sluiter A, Ruiz R, Scarlata C, Sluiter J, Templeton D. (2008). *Determination of determination of extractives in biomass, laboratory analytical procedure (LAP) NREL/TP-510-42618 Revised July 2011*: National Renewable Energy Laboratory.

- Schoening, A. and Johansson, G. (2006). Acid-insoluble lignin in wood and pulp (Reaffirmation of T 222 om-02).
- Rabelo, S., Amezquita, N., Andrade A., Filho R., Costa A. (2011). Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide. *Biomass and Bioenergy*, 35: 2600-2607
- Wörmeyer, K., Ingram, T., Saake, B. Brunner, G. Smirnova, I. (2011). Comparison of different pretreatment methods for lignocellulosic materials. Part II: Influence of pretreatment on the properties of rye straw lignin. *Bioresource Technology*, 102: 4157–4164.
- Zhang, J., Feng, L., Wang, Zhang, D., Ruihong G., and Cheng, G., (2014). Thermogravimetric analysis of lignocellulosic biomass with ionic liquid pretreatment. *Bioresource Technology* 153:379–382