# Thermal and Morphological Evaluation of Chemically Pretreated Sugarcane Bagasse

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Abstract—Enzymatic hydrolysis is one of the major steps involved in the conversion from sugarcane bagasse to yield ethanol. This process offers potential for yields and selectivity higher, lower energy costs and milder operating conditions than chemical processes. However, the presence of some factors such as lignin content, crystallinity degree of the cellulose, and particle sizes, limits the digestibility of the cellulose present in the lignocellulosic biomasses. Pretreatment aims to improve the access of the enzyme to the substrate. In this study sugarcane bagasse was submitted chemical pretreatment that consisted of two consecutive steps, the first with dilute sulfuric acid (1 % (v/v)  $H_2SO_4$ ), and the second with alkaline solutions with different concentrations of NaOH (1, 2, 3 and 4 % (w/v)). Thermal Analysis (TG/ DTG and DTA) was used to evaluate hemicellulose, cellulose and lignin contents in the samples. Scanning Electron Microscopy (SEM) was used to evaluate the morphological structures of the in natura and chemically treated samples. Results showed that pretreatments were effective in chemical degradation of lignocellulosic materials of the samples, and also was possible to observe the morphological changes occurring in the biomasses after pretreatments.

*Keywords*—Alkaline solutions, bioethanol production, dilute acid, enzymatic hydrolysis, lignocellulosic biomass.

## I. INTRODUCTION

AFTER coal, petroleum and natural gas, biomass is the fourth largest energy source, providing about 14% of the world's primary energy consumption [1]. Currently biomass is used in different applications, including generating electricity, fueling vehicles and providing process heat for industries [2].

In Brazil, sugarcane, a lignocellulosic material is used to produce ethanol. This process requires the conversion of cellulose into glucose monomers, which are converted into ethanol using microorganisms and one of the produced residue is the bagasse [3].

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Lignocellulosic materials predominantly contain a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives, and ashes [4, 5]. The lignin and hemicellulose have an important role in the rate and yield of the enzymatic hydrolysis. Results reported in the literature show that the digestibility of the cellulose increases with the removal of these constituents [4, 6].

The key inhibitory role of lignin was attributed to the nonspecific adsorption of enzyme on their surface and inaccessibility to the cellulose, due to sterical impediment. Studies show that lignin and its derivatives are toxic to microorganisms and enzymes. Substrates with low lignin content have higher enzymatic activity and require a lower load of enzyme in the process [7]. Thus, to produce ethanol from sugarcane bagasse, a transformation must be made: conversion of cellulose and hemicellulose into their constituent monomers (glucose and xylose) and subsequent conversion of these monomers by the action of microorganisms. The lignocellulosic biomass must be pretreated and for this, diverse pretreatment processes have been evaluated technically and economically, aiming at improving enzymatic hydrolysis; these include acid or alkaline treatments, steam-explosion, and organic solvents [7].

Chemical pretreatments tend to solubilize hemicellulose and lignin in order to expose the cellulose component to acid and/or enzymatic hydrolysis [8]. The pretreatment changes the lignocellulosic matrix of the biomass, increasing the porosity of the material, reducing the crystallinity and consequently increasing the digestibility of cellulose [4, 7]. Acid pretreatment, mainly using sulfuric acid, and hydrothermal methods, based on the autocatalytic action of acetic acid released by hydrolytic cleavage of acetyl groups, have shown to be effective in the improvement of the enzymatic hydrolysis of cellulose [6].

The main issue when pre-treating lignocellulosic materials with diluted acid is to solubilize the hemicellulosic sugars keeping, at the same time, as much cellulose as possible in the remaining pretreated solid that may be enzymatically hydrolyzed [9]. Alkaline treatments, usually employing NaOH (sodium hydroxide) and Ca(OH)<sub>2</sub> (calcium hydroxide), tend to cause an increase in both pore volume and surface area, highly ordered dismantling the structure of cellulose and leading for a decrease in the crystallinity degree of the cellulose polymerization. Also, these processes cause a breakdown of lignin-carbohydrate linkages, as well as perturbations in the

lignin structure [7].

The aim of this study was to evaluate the performance of sugarcane bagasse submitted to two-step treatments for delignification; the first with dilute sulfuric acid and the second with different concentrations of NaOH. Thermogravimetric Analysis (TGA) was applied for a semi-quantitative analysis of hemicellulose, cellulose and lignin content in biomasses. In addition, the morphology of such biomasses pretreated was investigated using *Scanning Electron Microscopy* (SEM).

# II. EXPERIMENTAL PROCEDURE

#### A. Substrate

Sugarcane bagasse samples used in the experiments are from the harvest (2011/2012) and provided by Raizen Group (São Paulo State, Brazil). Prior to analysis and pretreatment the sugarcane bagasse was dried at 45 °C for 48 h and left at room temperature for 24 h and then stored in plastic containers at room temperature until to be used [7].

# B. Acid and Alkaline Pretreatments

The sugarcane bagasse was initially treated to solubilize the hemicellulose, and a ratio solid-liquid 1:16 (grams of dry sugarcane bagasse/mL of 1% H<sub>2</sub>SO<sub>4</sub> solution) was used. After this pre-treatment, the samples were treated with alkaline solutions of 1%, 2%, 3% and 4% w/v to promote the delignification. The suspension containing sugarcane bagasse was autoclaved for 40 min at 1 atm and 120 °C. After this process, the samples were abundantly washed with distilled water to eliminate acid or alkaline excesses until a neutral pH [8].

# C. Thermal Analysis (TA)

Determination of moisture, cellulose, hemicellulose, lignin and ashes content *in natura* and chemically pretreated sugarcane bagasse samples have been done by thermoanalytical techniques, in particular Thermogravimetric Analysis and Derivative Thermogravimetry (TGA/DTG), and Differential Thermal Analysis (DTA) [1,10]. The employment of the Thermogravimetric Analysis (TGA) allows a semi-quantitative determination of this content in the biomasses [11].

After chemical pretreatments, the samples have been washed in running water to remove impurities, ground and sieved to give different size fractions by using ASTM standard sieves. The average particle sizes selected were 0.35 mm. Non-isothermal thermogravimetric experiments (TG/ DTG and DTA) were carried out in analyzers *Shimadzu* brand, TGA-50H and DTA-51 models, respectively. Experimental conditions used in both TG and DTA experiments are described as follows: carrier gas was synthetic air with flow rate of 100 mL min<sup>-1</sup>, heating rate of 10 °C min<sup>-1</sup> from room temperature up to 800 °C, sample mass of  $(10.0 \pm 0.5)$  mg and crucible of alumina. Tests were carried out in duplicate; the

mean values and standard deviations were considered and are shown in Table I.

# D. Scanning Electron Microscopy (SEM)

Morphological and structural analysis of all samples, including *in natura* and chemically pretreated (acid and alkaline medium) have been obtained on a Scanning Electronic Microscope Ltd. equipment brand, LEO 440 model. Samples used in this analysis have been fixed in an aluminum support and then submitted to the metallization gold process.

#### III. RESULTS AND DISCUSSION

## A. Thermal Analysis

Figs. 1, 2 and 3 present TG, DTG and DTA curves, respectively, for sugarcane bagasse samples *in natura* and chemically pretreatments with dilute acid ( $1\% \text{ H}_2\text{SO}_4$ ) and alkaline solutions (1, 2, 3 and 4% NaOH).

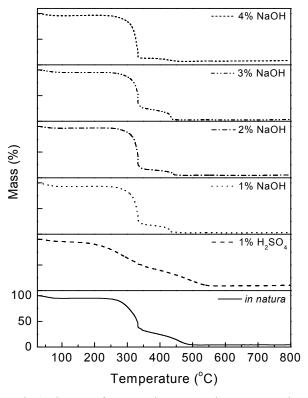


Fig. 1 TG curves of sugarcane bagasse samples *in natura* and chemically pretreated with dilute acid (1% H<sub>2</sub>SO<sub>4</sub>), and alkaline solutions (1, 2, 3 and 4% NaOH). Others graphics follow the scale of the first

TG curves (Fig. 1) show three thermal degradation steps. The first is related to moisture releasing ( $\approx 100$  °C), the second, with the greatest amount of weight loss, is characterized by the thermal decomposition of hemicellulose and cellulose, both in the same stage, around 200 to 300 °C (hemicellulose starts before than cellulose. Finally, the third step is related to the lignin content and the temperature range

is between 325-450 °C [12].

In a previous study presented in the literature [13], it was shown that TG curves for sugarcane bagasse and other biomass samples *in natura* presented similar behavior with the TG curves demonstrated here. Also it was observed that in thermal decomposition process of biomasses, the dominant step of mass loss is related to the hemicellulose and cellulose degradation step [13].

Samples chemically treated with all concentrations of NaOH exhibited the same behavior of thermal decomposition as *in natura* samples, *i. e.*, in those samples basically three main steps of mass loss are presented. However, a significant difference can be observed for the sample pretreated with sulphuric acid, which can be evidenced by the profile of the TG curve between 200-550 °C. This behavior is attributed to the solubilization of cellulose without the effective removal of hemicellulose from lignocellulosic matrix [14].

In the acid pretreated samples there is evident changing in the TG profile when compared with other samples. These alterations possibly are due to destruction of molecular structures and linkages of cell walls. From this statement, it can be predicted that after the acid treatment the lignocellulosic structure is more readily to the anaerobic digestibility and to solubilize the hemicellulose, which makes the cellulose more accessible to the further enzymatic treatment [14].

During the dilute acid pretreatment, once that the hemicellulose is partially converted into soluble sugars, it is observed that a chain lignocellulosic complex is broken in very parts and thus the compact structure of the sugarcane bagasse is lost [15]. According to [15] the profile of the TG/ DTG curves for dilute acid pretreated revealed the destruction of the lignocellulosic structure.

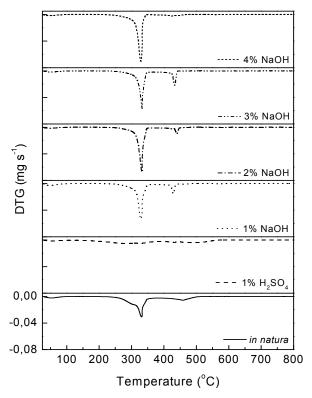


Fig. 2 DTG curves of sugarcane bagasse samples *in natura* and chemically pretreated with dilute acid (1% H<sub>2</sub>SO<sub>4</sub>), and alkaline solutions (1, 2, 3 and 4% NaOH). Others graphics follow the scale of the first

This interpretation is based on other studies [14, 16-17]. Where the authors [16] reported that a chain lignocellulosic complex is broken in order to reduce the crystallinity degree of the cellulose and increase the fraction of amorphous cellulose, the most suitable form for enzymatic attack. According to [14] and [17] it was observed that the hemicellulose removal process by dilute acid treatment has a positive effect, increasing the degradability of the cellulose by enzymes.

By means of the DTG curves (Fig. 2) it is possible to observe the presence of a shoulder (double-peak) around in the *in natura* samples, which characterize the thermal degradation of the hemicelulose prior to cellulose [12, 15]. For the *in natura* sample this shoulder is more pronounced than in the alkaline treated samples, evidencing the break of the lignocellulosic structure by means of the chemical treatment, as described before. Three peaks of the *in natura* sample can be observed: 310, 330 and 460 °C and they are identified as hemicelulose, cellulose and lignin, respectively.

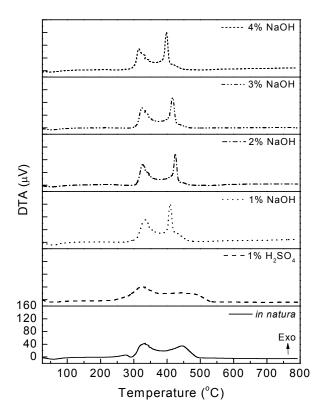


Fig. 3 DTA curves of sugarcane bagasse samples *in natura* and chemically pretreated with dilute acid (1% H<sub>2</sub>SO<sub>4</sub>), and alkaline solutions (1, 2, 3 and 4% NaOH). Others graphics follow the scale of the first

However, for dilute acid treatment samples these peaks had not been identified, possibly due the desestructuring of lignocellulosic constituents by the acid. The peaks for these samples can be observed at 285, 330 and 500 °C, for hemicelulose, cellulose and lignin, respectively and also they more wide than for the other samples. Also was noted a high thermal decomposition of hemicellulose and cellulose, that can is related with the increase of the concentrations of alkaline solutions (around 330 °C) and low thermal decomposition of lignin (around 430 °C) with small displacement of the peaks in these region.

DTA curves (Fig. 3) present enthalpic processes that occur during the thermal degradation where both exothermic and endothermic peaks represent the steps of degradation related to organic components, volatile materials and changing in the macromolecular structure [18, 19]. In all samples only the first step is an endothermic event which is related to moisture content and the two others, are exothermic events. But an interesting point that was noted is the intensity of the peaks. While in the DTG curves the stage related to hemicellulose and cellulose decomposition presents a higher and narrow peak ( $\approx 330~{}^{\circ}\text{C}$ ) in the DTA curves the peaks are lower and wider. And a contrary behavior is observed for the lignin degradation from 400 to 430  ${}^{\circ}\text{C}$ , *i. e.*, lower DTG peaks and higher and pronounced DTA peaks.

In addition, the intensity of the peaks, mainly related to the

lignin decomposition (350-550 °C), differs for each treatment, which suggest that there is differences on remaining lignin amount in the lignocellulosic structure when different treatments are applied.

TABLE I PERCENT THERMAL DEGRADATION OF SUGARCANE BAGASSE SAMPLES BEFORE AND AFTER CHEMICAL PRETREATMENTS  $H_2SO_4$  - Sulfuric Acid; NaOH - Sodium Hydroxide (chemical

PRETREATMENTS)				
Samples	Moisture (%)	Hemicellulose/ Cellulose (%)	Lignin (%)	Ash (%)
in natura	5.2±0.2	64.5±2.3	22.8±0.3	4.8±0.3
$1\% H_2SO_4$	5.4±1.2	55.0±1.5	$32.9\pm0.7$	$7.6 \pm 1.0$
1% NaOH	5.7±0.0	66.5±0.8	23.5±0.3	$4.4\pm0.4$
2% NaOH	$3.3\pm0.2$	$75.2 \pm 0.7$	$17.1\pm1.0$	$4.2\pm0.0$
3% NaOH	$4.7\pm1.2$	67.5±1.1	$21.2\pm0.0$	$7.0\pm0.0$
4% NaOH	$4.0\pm0.3$	82.0±2.3	$6.3\pm1.6$	$7.0\pm0.3$

The data presented in the Table I have been obtained from TG curves. Except for moisture and ash contents, the breakdown process occur under inert atmosphere and therefore it is a reflection of the combustion of the released volatiles during the partially decomposed organic materials.

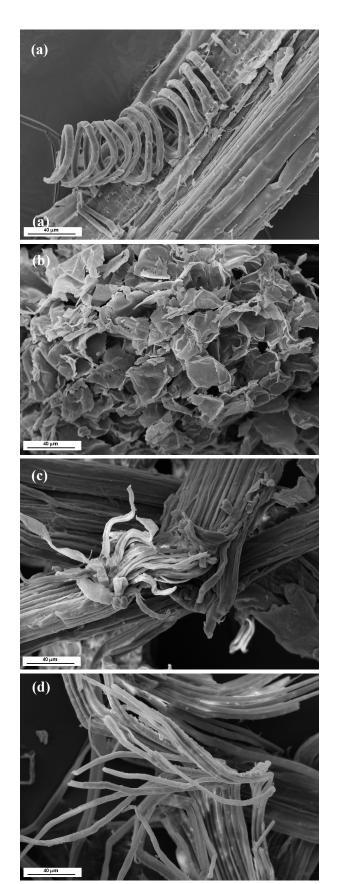
Moisture content varies a little with the treatment, only for samples treated with 2% NaOH presented (3.2%), for the others varies from 4.7 up to 5.7%. Related to the hemicellulose and cellulose contents, we have been considered in this study the degradation in the same step, because it is difficult to distinguish distinct steps. But for lignin, in all cases it is possible distinguish it separately from other steps.

From TG curves were calculated hemicellulose and cellulose together, but from DTG curves and through a qualitative analysis, can to be observed that the shoulder at 310°C is evident only for *in natura* sample, and not for all other samples. From this observation it can be understand that the hemicellulose was removed and then releasing the cellulose from the lignocellulosic structure.

The final residues (ashes content) are related to the inorganic materials and varied from 4.2 up to 7.2% depending on the treatment. Other factors also were observed by other researchers, justifying that can to lead the large ashes content in the *in natura* samples, as difference in cultivation method, species variation, soil composition, fertilizers used, and climate in where they have been grown [13, 20-21].

# B. Scanning Electron Microscopy

Fig. 4 (a)-(f) show SEM images (morphological and structural features) of sugarcane bagasse samples *in natura* and treated with dilute acid and alkaline solutions.



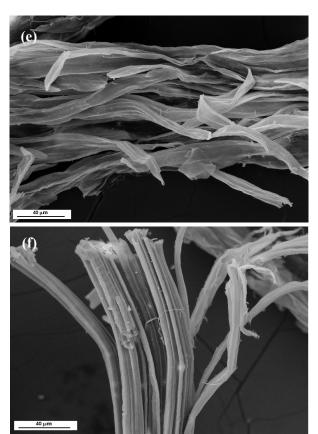


Fig. 4 SEM images of sugarcane bagasse: (a) *in natura*; (b) 1% H<sub>2</sub>SO<sub>4</sub>; (c) 1% NaOH; (d) 2% NaOH; (e) 3% NaOH, and (f) 4% NaOH pretreatments

Fig. 4 (a) presents the in natura sample and it is possible to observe the presence of elongated parallel fibers, massive beams and piths (more fragile and fragmented structure containing pits), which is in according to [8] and [22]. In addition, the samples are partially covered by a material on the surface, where a structure in swirl shape appears, possibly formed due cut blades effects during the milling process in the sugarcane mill. Fig. 4 (b) (1% H<sub>2</sub>SO<sub>4</sub>) shows that there is not a surface with elongated fibers evidencing the effect of the dilute acid on the sugarcane bagasse, In Fig. 4b it is also possible to observe the increasing of the amount piths, differently than the results observed by [8], in which study the authors observed the presence of small holes on surfaces and removing residues. On the other hand, the results of this study are consistent with [16], where the authors observed the destruction of SBP (Sugar Beet Pulp) fibers by dilute acid pretreatment, by removing the inner parts of the cells and consequently increasing the structural changes of the lignocellulosic matrix. The authors also verified that the dilute acid pretreatment increased the enzyme-accessible to the cellulose, leading to an enhanced degradability of pretreated SBP. Fig. 4 (c)-(f) show the alkaline solutions treated samples and in all of them the shape of the fiber is predominant but with some differences. Fig. 4c shows that there is peeling of fiber bundles of internal

structures. Thin fibers bundles stay freer and are best evidenced in this sample (Fig. 4 (d)). In the Fig. 4 (e) there is a high fibers detached amount, indicating that occurred size reduction and mechanical stress, which contribute to enhance of the chemical degradation. It was observed in the Fig. 4f that there was separation of elongated fibers bundles in tube shape, presence of cut in the ends, and residual material along the fibers. In their study [6] observed that regions more or less affected by the chemical pretreatments can to be found in any part of these samples, also was noted that the fibers are less affected by the treatments than piths.

# IV. CONCLUSIONS

In this study, sugarcane bagasse underwent treatments by dilute acid and alkaline solutions with the purpose to understand the effect of these treatments on the lignocellulosic structure of the biomass to be used for further enzymatic treatment. Thereby, TGA/ DTG and DTA analysis and morphological structure of the in natura samples were used.

By means from TG/ DTG and DTA curves it was possible to observe that the dilute acid and alkaline solutions caused modifications by breaking the linkages and lignocellulosic structure of the sugarcane bagasse samples. With alkaline treatments, hemicellulose and lignin content decreased due to their solubilization, while for samples treated with dilute sulfuric acid the amount of these compounds remained in the structure. The alkaline pretreatment demonstrates another important aspect that is the change of the cellulose structure to a form that is denser and thermodynamically more stable than the *in natura* cellulose.

Also was possible to observe the results through SEM images, where the samples pretreated with dilute acid underwent corrosive effect in their surfaces increasing the piths amount by destruction of the cell structures. Samples with alkaline solutions treated presented separation, peeling, swelling fibers and removing material of the surfaces.

The best pretreatment conditions were with samples treated 1% H<sub>2</sub>SO<sub>4</sub> and 4% NaOH, therefore showed significant reduction of lignin in the sugarcane bagasse. This compound, together with the surface area and crystallinity degree, are identified as the most important factors on the susceptibility of cellulose to enzymatic hydrolysis.

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