ABSTRACT

DIAZ, JOSCELIN TERESITA. Green Liquor Pretreatment for Hardwood chips to Ethanol conversion strategies. (Under the direction of Drs. Lucian Lucia and Hassan Jameel.)

Modified Green Liquor pretreatment is a novel high sulfide pretreatment method that enhances carbohydrate stability.

The influence of hardwood chips pretreatment with green liquor was investigated under alkaline conditions of 12% and 16% TTA, and sulfidities of 0%, 12.5%, 25%, and 37.5% to see its effect on the sugar recovery. Also, ethanol was added in the pretreatment conditions of 12%TTA and 25%S as an Organosolv pretreatment method.

Quantitative analyses of glucose, xylose, mannose, and carbohydrates yield is discussed. The influence of pretreatment conditions such as alkalinity and sulfidity charge were the main variables controlled to analyze their influence in the carbohydrate yield. The results suggest that higher sulfidity promotes an increase in delignification, and also that this delignification is more notable in the samples pretreated with ethanol. The pretreated pulps were then hydrolyzed with cellulase, xylanase, and β-glucosidase enzymes. After enzymatic hydrolysis with these commercial cellulases enzymes, higher glucose yields were obtained at higher sulfidity and higher ethanol concentration in the liquor. The results also indicated that higher enzyme activity exhibited higher carbohydrates and glucan yield. The carbohydrates yield was 70.6% for hardwood chips pretreated with 16%TTA, 37.5%S, and hydrolyzed with cellulases at 20 FPU/g pulp. However, the higher carbohydrates yield obtained was 75.7% for the pulps pretreated at 20% ethanol and 20 FPU/g pulp.

Different techniques were applied in the samples 16%TTA, 0%S and 37.5%S, and 12%TTA, 25%S, 20%ethanol to determine its pretreatment effect on lignin. ToF-SIMS technique was

used to detect the changes in cellulose/lignin composition and then compare the results with ones the obtained from the sugar analysis using Ion Exchange Chromatography. ToF-SIMS suggested that the removal of lignin is mainly localized on the sample surface.

The molecular weight distribution of lignin using Size Exclusion Chromatography technique was also studied. The results shown a decrease in the molecular weight when sulfidity was increased. It is hypothesized that this happened mainly due to cleavage of lignin in the β-O-4 linkage that decrease the chain length of the residual lignin.

Green liquor has the advantage of altering the lignin structure, and preserving the celluloses and hemicelluloses present in the biomass.

An economic analysis based in different assumptions was elaborated to compare all the study cases. In general the results shown that samples pretreated with higher sulfidity always shown better profit per liter compared with lower sulfidity. Also, in the case of the samples pretreated with ethanol, the profit was even better than without ethanol. But, variations in ethanol lost during the process shown that this method is not as attractive as the pretreatment at high sulfidity and high total titratable alkali.

Green Liquor Pretreatment for Hardwood chips to Ethanol conversion strategies

by
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DEDICATION

I would like to dedicate this thesis to my father Francisco Diaz, who taught me that dreams can become truth once I really believe in that; and to my mother Merida de Diaz, who taught me how to be patient to persevere.

Without their wisdom, guidance, supports, and knowledge I would never been who I am.

Thanks for all the love you give me each day of my life. That has given me the strength to continue my search for success and happiness. I will love you forever.

BIOGRAPHY

Joscelin Teresita Diaz was born in Barquisimeto, Venezuela. She got her bachelor degree in Chemical Engineering at the University of Los Andes in December 2006, after a brief experience as informatics engineering major.

Later than working six month as a trainee in Petroleos de Venezuela (PDVSA) in 2006, she entered the graduate Master program in spring 2008 in the Department of Wood and Paper Science at North Carolina State University under the supervision of Drs. Lucian Lucia and Hassan Jameel.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Introduction

Bioenergy has become a very hot topic nowadays because of the increasing prices of fossil fuels such as oil, gasoline, and natural gas, and the need to establish national security independence. World governments and associated local communities have therefore not surprisingly become aligned in their position to promote the use of alternative energy sources. Bioenergy, or energy from renewable materials like wood, grasses, and agricultural residues to produce liquid fuels, electricity, and heat, is rapidly becoming accepted and mandated. In general, the escalating push to produce bioenergy is mainly flourishing for two reasons: to reduce the dependence on imported oil and to diminish the release of greenhouse gases.

Bioenergy as far as most recognize it is in the form of biofuels such as ethanol and is derived mainly from starch-based food crops such as corn. Corn possesses readily saccharifiable sugars for ethanol production. Or the use of sugar cane, a non-starch food crop, but readily saccharifiable energy crop, is nevertheless a recognized alternative that is seeing great success in Brazil. The second generation of biofuels will come from non-food crops like wood residues, switchgrass, tree biomass, cellulose wastes, and crops residues. These non-traditional bioenergy sources represent new and very valuable energy sources for ethanol production that typically do not interfere with the food chain, land arability or productivity, and green house gas emissions.

Different countries are therefore investing heavily in the bioenergy field by focusing on producing cellulosic ethanol to avoid the drawbacks mentioned above that are associated with food crops.

However, there are some biggest challenges that are necessary to consider when exploring investment in the bioenergy field: least capital and operation costs, highest yields and commercialization potential, best pretreatment methods, and utilizing the most promising feedstock(s) [1].

Despite the fact that no simple, cost-effective technology yet exists to generate ethanol from wood, it is only recently that its use as a renewable material to obtain high ethanol yield has been extensively promoted because it is a very abundant, cheap, very dense, non-food, and low cost ethanol feedstock.

The very first recorded research on wood-to-ethanol started in 1819 when Braconnot investigated wood hydrolysis and discovered that cellulose could be dissolved in concentrated acid solutions to produce sugars (saccharification). Then, the sugars (glucose) obtained could be fermented to produce ethanol [2].

However, cellulose is not alone in the wood cell; hemicellulose and lignin are also present. Cellulose is the principal compound that mainly has a crystalline structure, which impedes its hydrolysis (breakdown) by enzymes. The more easily hydrolysable fraction of the cellulose is the amorphous region which represents approximately 15% of the total cellulose structure [3]. Enzymatic hydrolysis of cellulose is probably the most promising technology to hydrolyze it into fermentable reducing sugars.

Increasing accessibility in lignocellulosic sources through pretreatment has been identified as perhaps the most critical step limiting the efficiency of enzymatic hydrolysis [4, 5].

In general, feedstock pretreatment technologies are used to open up the cellulose matrix allowing more rapid diffusion of the enzyme into the matrix pores. During pretreatment, the lignin is loss and the hydrolysis procedure is faster because the barriers have been removed. Pretreatment is also very important because it represents approximately 40% of the total production cost to obtain high sugar yields.

The most important factors that should be considered in choosing a convenient pretreatment method are: preservation of the cellulose and hemicellulose fractions, low formation of byproducts such as furfural and acetic acid (due to degradation of pentoses and hexoses respectively, which inhibit microorganisms), high recoverability of carbohydrates, and low energy demand [6]. Different pretreatment techniques to obtain ethanol have been reported [6]. The most common ones studied are mechanical, chemical, and thermal pretreatments. The studies that make use of chemicals offer the highest yields and lowest costs, essential to economic success. Thus, uncovering the most efficient and most effective pretreatment method to break down the cellulose into sugars is paramount to achieving a viable wood-to-ethanol technology.

In a study made by Phillips, R. et al. [7], they hypothesized that several of the capital limitations associated in the production of ethanol from wood can be diminished by repurposing a kraft pulp mill and use one of the alkaline chemical produced (green liquor) to pretreat the raw material. They identify 5 major advantages: 1. the availability of the pulp and paper mills that are closed, 2. the location of those mills and the supply of the raw material (growth, harvest, and delivery), 3. the skilled workforce is in place, 4. environmental permitting easy to obtain, and 5. equipments and infrastructure available (power generation and waste water treatment is available).

They also claim that there is a worth financial return by repurposing a kraft pulp mill to ethanol production, and that it is possible to reduce capital requirement by almost 50% compared to corn grain to ethanol plants. But, additional plant modifications and the reduction of sugar concentration can increase the requirements for the operation and cause an increase in this capital. However, repurposing seems to be an attractive technical and financial starting point for economic success.

Figure 1 shows a representation of the major operation units needed for the production of ethanol from biomass. Pretreatment is the first step in this process. Then enzymatic hydrolysis breaks down the polysaccharides into sugars that will be converted to ethanol in the fermentation process unit. Distillation will purify the ethanol product, and the energy operation unit is needed to keep the process running.

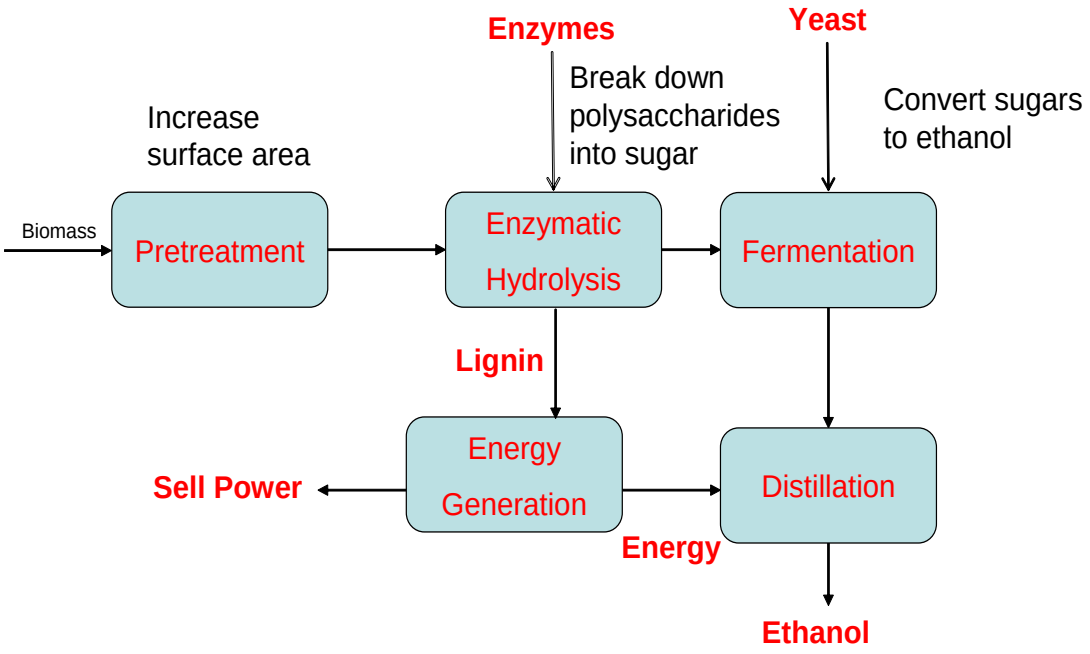

Figure 1. Major units operation in ethanol production

2. Literature Review

2.1 Pretreatment methodologies

2.1.1 Physical pretreatment

Some methods studied are: ball milling, two-roll milling, attrition milling, and steam explosion. The goal of the physical pretreatment is to reduce the particle size and crystallinity, and to increase the surface area.

According to Li et al., “steam explosion, ammonia fiber explosion and liquid hot water pretreatments have high potential, but high temperature or high pressure is required” [8].

The advantage of milling method compared with chemical pretreatment is that no byproducts are formed because the lignin fraction is not removed. However, it is not economically viable because it requires high energy demand to grind the feedstock materials and it cannot remove the lignin barrier [2].

Steam explosion is very simple and a very promising physical method that removes mainly hemicelluloses allowing the hydrolysis. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. The material is heated at high pressure steam between 20 to 50 bar and 160 to 290 °C for few minutes. However, the yields are too low (45-65% of xylose-sugars recovered) to be considered economically viable.

Limitations of steam explosion include break of a portion of the xylan fraction, partial disruption of the lignin-carbohydrate matrix, and production of compounds that may inhibit microorganisms.

In general, the physical pretreatment methods are energy demanding compared with chemical pretreatments.

2.1.2 Chemical pretreatment

Pretreatment is one of the highest cost items in the cellulosic biomass-to-fermentable sugars conversion, with a contribution estimated of 30 cents per gallon (Mosier et al, 2005). Mosier et al. reviewed various treatments (but not ionic liquids), with respect to increase in surface area, decrystallization, removal of hemicelluloses and lignin, and alteration of lignin structure.

Until now, chemical pretreatment seems to be a promising pretreatment method, but there are still some limitations. Acid, wet oxidation, solvent, metal complex, and lime are effective as pretreatment methods, but expensive considering the value of the products [9].

Hendriks and Zeeman concluded that concentrated acids, wet oxidation, solvents and metal complexes are effective, but too expensive compared to the value of glucose [10].

The selection of the cheapest and effective chemical is the key for economic success. The recovery of the chemicals must be easy and the formation of byproducts has to be very low.

Green liquor pretreatment is an alkaline method that uses relative low cost chemicals that increase the access of the enzymes to the cellulose. It can disrupt the lignin barrier and increase the delignification selectivity [11].

Figure 2 shows a schematic representation of the effect of pretreatment on the lignocellulosic material. The lignocellulosic complex is formed by a matrix of cellulose and lignin bound by hemicellulose chains. During pretreatment this matrix is broken reducing the degree of crystallinity in the cellulose and increasing the fraction of amorphous cellulose, which is the proper form for enzymatic attack.

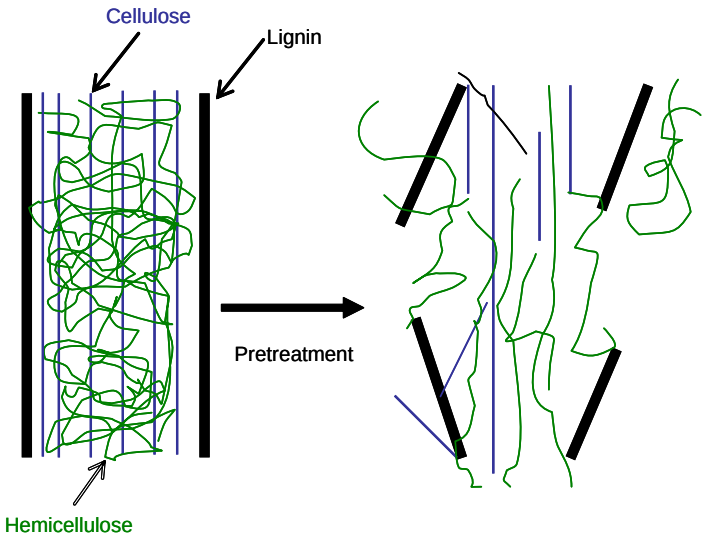

Figure 2. Schematic representation of the lignocellulosic complex

2.1.2.1 Acid pretreatment

Concentrated acid hydrolysis

Acid pretreatments will hydrolyze the hemicellulose fraction while leaving the cellulose and lignin intact in the residual solids. High concentration of acid at low temperature reduces the degradation of cellulose, but this increases the capital cost.

Acid hydrolysis has not proved to be practical because it does not give sufficient high glucose yields and/or the cost of recovering the catalyst is too high. The products obtained may be harder to ferment because of the presence of toxic substances like furfurals in the liquid phase. Also, acid recovery is required and a corrosion resistant reactor made with monel and zirconium should be used because certain metals and salts can catalyze the degradation of glucose and xylose [12].

Diluted acid hydrolysis

There is some research in the use of diluted acid hydrolysis. The acid hydrolysis solubilizes hemicellulosic sugar and release glucose, hexoses and pentoses. The main advantage of this pretreatment is the relatively low acid consumption. It has been reported that it is possible to obtain a yield of glucose between 50-60% of the theoretical value [13].

Many studies report that a small amount of lignin can be dissolved during acid hydrolysis; however, that is enough to disrupt the structure and allow the enzymes to get into the matrix. For diluted acid hydrolysis is necessary high temperatures to obtain high hydrolysis rates of cellulose to glucose that can results in decomposition of hemicellulose affecting the fermentation step, and causing corrosion in the equipments. Also, the formation of degradation products such as acetic acid, furfural, and metal ions must be removed and that increases the cost. The main reason is because high concentration of these byproducts has been reported to inhibit the yeast during fermentation and the cost of the yeast is high. So, the way to reduce costs is to increase substrate concentration.

2.1.2.2 Organosolv pretreatment

Organosolv process uses solvents like methanol, ethanol, acetone, ethylene glycol, triethylene glycol and phenol. By using an organic solvent in the pretreatment step, it protects most of the cellulose during delignification and improves the efficiency of enzymatic hydrolysis of wood. Also, in a study made by Marton and Granzon [14] using ethanol in an alkaline pulping, they claim that ethanol reduces the surface tension of the pulping liquor promoting penetration of alkali.

Other study claims that ethanol can create more mobility of kraft ions in the binary ethanol water mixture due modified solvation structure and increases in the free energy state.

They explain that the increase in the mobility results in an increase in the reactivity and an increase in the solubility of lignin. All these combinations improve the delignification rates together with the selectivity [15].

Ethanol results one of the best Organosolv pretreatment due to it has a heat capacity low of 0.62cal/g °C and heat of vaporization of 204.26 cal/g compared with water 1cal/ g °C and 540 cal/g. that means that, by combining both, it is possible to economize the heat and the solvent recovery by distillation [15]. In general, these pretreatment methods improve carbohydrates retention and lignin can be removed effectively. However, some of these substances are flammable and explosive making those difficult to handle [16].

2.1.2.3 Ionic liquid pretreatment

Li et al. pretreated wheat straw with selected ionic liquid (IL) 1-ethyl-3-methyl imidazolium diethyl phosphate ([EMIM]DEP), aiming at an efficient enzymatic hydrolysis. [EMIM]DEP has low viscosity and shows potential of accelerating enzymatic hydrolysis, plus can be recyclable. The yield of reducing sugars obtained, by treatment at 130°C for 30 min reached 54.8%, after being enzymatically hydrolyzed for 12 h. The process was evaluated using *Saccharomyces cerevisiae* and the ethanol production was 0.43 g/g glucose within 26 h. Li et al. concluded that the [EMIM]DEP is a promising pretreatment solvent for wheat straw.

An extensive and rich study of biocatalysis in ionic liquids was conducted by Rantwijk and Sheldon [17], who pointed the character of IL as "...green, high-tech still increasing rapidly, due to its near zero vapor pressure, thermal stability, and tunable properties, such as polarity and solvent miscibility behavior". Preparation and utilization of ionic liquids consist in rapidly expanding fields of research and application. Rantwijk and Sheldon point out that the first use of IL in biocatalysis were reported in 2000 [18-20]

Rantwijk and Sheldon studied the important role of purity, polarity, stability and viscosity, solvent properties and miscibility, and green aspects; including the critical issue of biodegradability, which will still require intense study and progress. They discussed the behavior of enzymes in organic liquid, the interaction of proteins with water and electrolytes, and the interaction of enzymes in aqueous ionic liquid mixtures, with leads to the understanding of the molecular engineering of IL, so the stability and activity of the enzymes is enhanced.

A substantial review of studies covering a wide range of enzymes and IL has been presented. According to Rantwijk and Sheldon, IL can be designed to dissolve enzymes without denaturation, with enzyme activity and solubility in IL being strongly dependent.

It is quite clear that there is much to be done towards the understanding of IL, and its interaction with enzymes, to reach efficient and economically justified processes.

Recent advances of biocatalysis in ionic liquids, such as transesterification, synthesis, conversion, ammoniolysis, hydrolysis, epoxidation, resolution, and oxidation,

as well as investigation of ionic liquids for protein related to the folding/refolding and the issue of toxicity have been described [21].

The disadvantages to separate solvent and catalyst from product (Li et al.) are leading to the study of alternatives such as pervaporation [22], nano filtration [23], and extraction with supercritical CO_2 [24]. Kamiya et al., experimenting with 1-ethyl-3-methylimidazolium diethylphosphate, report the development of "an enzymatic in situ saccharification process in aqueous-IL media, that eliminates the need to recover regenerated cellulose", which according to their best knowledge is the first report of a one-batch enzymatic process for the saccharification of cellulose [25]. More study is needed in this technology to elucidate the process costs and see the toxic effect on enzymes because the studies have been done on pure crystalline cellulose.

2.1.2.4 Alkaline pretreatment

In the case of alkaline pretreatment, the use of sodium hydroxide, ammonia, and ammonium sulfite has been considered. The alkaline approaches tend to have more effect on the lignin component by leaving both hemicellulose and cellulose intact. However, these standard pretreatment techniques are not economically feasible [26].

Solvation is the first reaction that occurs during the alkaline pretreatment, swelling the biomass and making it more accessible to enzymes. Alkaline pretreatment can change the structure of the cellulose to a denser and thermodynamic stable structure (Pettersen, 1984).

This technology has high effect on the increase of accessible surface area and alteration of lignin structure, less effect on hemicellulose solubilization and formation of furfural (for hemicellulose degradation), and can solubilize lignin.

2.1.2.4.1 Green Liquor pretreatment

Green Liquor is the partially recovered form of kraft white liquor (GL is sulfide rich) obtained after combustion of the black liquor in the recovery boiler in a pulp mill. It is produced by dissolving the smelts from the recovery boiler (mainly Na_2S and Na_2CO_3) in water [27].

Green liquor pretreatment has been applied to improve kraft pulping because it achieves high pulp yields and increases delignification [28]. That is why it represents an attractive method in the biofuels production [28] even though no one has ever report the use of this method to convert cellulose into ethanol. The Green Liquor usages in pretreatment conditions have important effects on chemical sorption, especially on sulfide sorption; for example, pretreatment conducted under higher temperature with a moderate Green Liquor charge benefits higher sulfide sorption.

Furthermore, an interesting possibility that may reduce the production cost is to integrate ethanol production with a combined heat and power plant or with a pulp and paper mill.

All these factors made interest the use of this alkali chemical to help delignification, to break down and solubilize the hemicelluloses, and to expose the cellulose to be enzymatic attack producing high sugars yields.

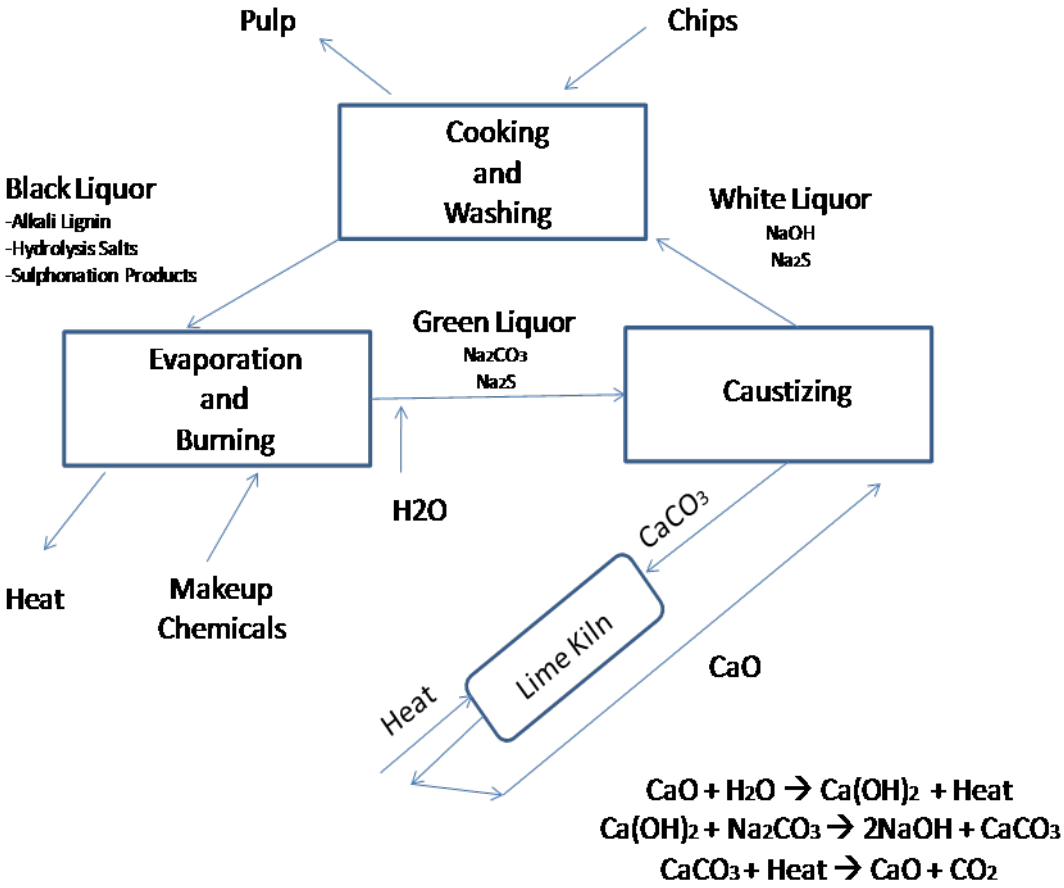

Figure 3. Green Liquor pretreatment process in a pulp and paper mill

2.2 Variables in Green Liquor pretreatment

Temperature

According to Ban and Lucia, temperature is an important factor for lignin dissolution. Concentration of GL and the temperature in the pretreatment influence the removal of lignin [29]. Also, it has been reported that high temperature helps in the sorption of sulfide concentration in chips and favor pulping selectivity due to reduction in liquid viscosity, increasing penetration rate.

The first compound to be thermally affected is the hemicellulose. According to Tjeerdsma, deacetylation is the first step of degradation and then acetic acid is released which acts as a depolymerization catalyst enhancing polysaccharide decomposition [30]. For this reason, temperature plays an important role in the preservation of the carbohydrates. The effect of temperature depends on the type of wood chips, the liquid, and the pressure applied.

Liquor to wood ratio

According to Klevinska and Treimanis, this factor influences pulping selectivity and delignification [29]. Higher liquor to wood ratio (GL concentration in the pretreatment liquor) increases the sulfide content in the chips and increases liquor adsorption rate.

The increase in GL charge results in a decrease of pulp yield and kappa number [28].

Time

According to Ban and Lucia, longer pretreatment times (more than 1 hour) are not necessary to achieve higher sulfide sorption. During pretreatment with GL, 80% of the total chemicals are absorbed in the chips between 15 to 30 min; that is why long pretreatment times are not necessary [29].

Alkalinity

According to Ban and Lucia, "when alkali is sorbed into the chips, it undergoes a neutralization process with sugar acids; thus, it is continuously consumed during the pretreatment and results in a higher sorption" [31].

High alkalinity results in a decrease of pulp yield, a decrease in the residual lignin, and an increase in the sugar yield after enzymatic hydrolysis.

Sulfidity

Sulfidity is the ratio of Na_2S to the total titratable alkali (TTA) expressed as percent.

$$\%Sulfidity = \frac{Na_2S}{NaOH + Na_2S} \cdot 100$$

Typically a US pulp and paper plant runs between 24-28% sulfidity. Higher sulfidities help to prevent loss of cellulose and improve delignification during cooking.

Pretreatments at short time and lower temperature generate a decrease in the sulfide composition of the chips. Wethermann suggested the concept of xylophilicity to explain the effect of sulfide and its affinity to wood [29].

Delignification increases when the levels of sulfide in the chips are increased. High sulfide content minimizes lignin condensation reactions which is a barrier to degrade lignin polymer [31].

After pretreatment, sulfide is mostly adsorbed into the chips. Sulfide displays xylophilicity that means it is selectively adsorbed. Also, the chemically bonded form of sulfide suggests that it can react with lignin [31].

Repurpose concept of a pulp and paper mill

The use of GL in pulping has several advantages. It has been used because it improves conventional kraft pulping because it can reduce the use of cooking and bleaching chemicals, it produces better pulps qualities, and improves delignification [11].

The reason to use this alkaline solution to produce ethanol is because GL is cost efficient; it is accessible in pulp mills, has higher delignification selectivity, and carbohydrates stabilizes.

2.3 Enzymatic Hydrolysis

Enzymatic hydrolysis is probably the most promising technology to break cellulose into fermentable reducing sugars.

After pretreatment, the enzymes bind the surface to catalyze the reaction. Cellulose is hydrolyzed using cellulases which are large proteins with molecular weight between 30,000 and 60,000, and size of 30 to 200 angstroms. However, approximately 20% of the pore volume of the wood internal surface area (without pretreatment) can be accessible for the cellulases [32].

That is why pretreatment plays a big roll to remove the lignin and increase the pore volume. Then, cellulases can catalyze the breakdown of cellulose into glucose for fermentation into ethanol.

Enzymatic hydrolysis is the best hydrolysis method due to no degradation components of glucose formed. It would be preferable to use enzymes at high temperatures to make use of the increased rate of reaction. However, enzymes are proteins and undergo basically permanent denaturation at temperatures above what they have in their natural environment. Above the critical temperature, there is a fast rate of loss of activity. For fungi, the optima enzymatic hydrolysis temperature is between 45-50°C.

One term used to describe the activity of the cellulose enzyme is filter paper unit (FPU). This is the amount of sugar produced per unit time using 50 mg of Whatman filter paper strip. The assay states dilutions of the cellulases needed to release 2mg of reducing sugars in 1 hour at 50°C and pH 4.8 [33].

Commercial cellulases can be obtained aerobically from *Trichoderma reesei* which releases a combination of cellulases. β-glucosidases can hydrolyze formed cellobiose into two molecules of glucose; that is why it is essential to complement the action of the cellulases [34]. More research is needed to improve the process of developing cheaper and better enzymes.

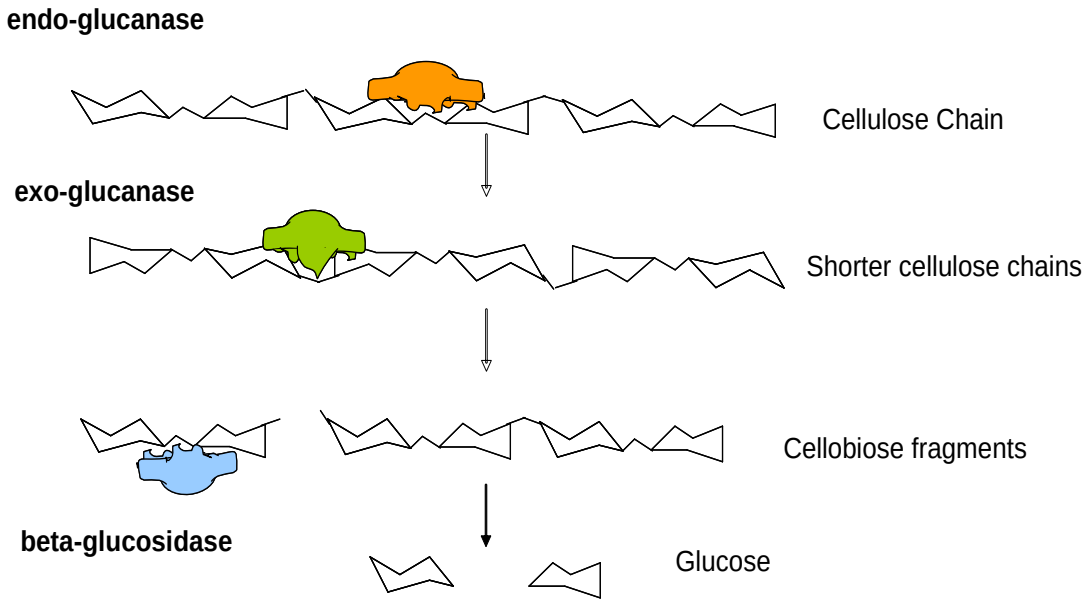

Figure 4. Representation of the mechanism of enzymatic hydrolysis

Figure 4 shows a representation of the enzymatic hydrolysis mechanism. The most widely accepted theory is that enzymatic hydrolysis occurs due to the synergistic action of three cellulase enzymes. Synergisms mean that the cellulase system has higher combined activity than the sum of the activity of individual enzymes.

The three cellulase enzymes are: endoglucanases, exoglucanases and β-glucosidases. The endoglucanases attack the interior of the amorphous cellulose polymer in a random style, generating oligosaccharides and new chain ends. Exoglucanases removes cellobiose units from the non reducing ends of cellulose chains. They are absorbed in the crystalline and amorphous substrates liberating either glucose or cellobiose as main products. Apparently exoglucanases can also attack the microcrystalline cellulose by peeling cellulose chains. β-glucosidases hydrolyze soluble cellobiose to glucose [32, 35].

2.4 Analytical Methods

2.4.1 Ion Exchange chromatography technique

Ion chromatography analyzes analyte molecules based on coulombic (ionic) interactions. The way it works is explained as follows: a buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material (a resin or gel matrix made with charged functional groups). The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge and retains the ionic compounds. A stoichiometric chemical reaction occurs between ions in a solution and a solid substance carrying functional groups that can fix ions as a result of electrostatic forces.

Carbohydrates are separated by anion chromatography (retains anions using positively charged functional group) at high pH and detected by pulsed electrochemical detection.

Electrochemical detection is used to measure the current resulting from oxidation or reduction of analyte molecules at the surface of a working electrode [36].

During oxidation reactions, electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the electrochemical cell. When a single potential is applied to the working electrode, the detection method is DC amperometry. Pulsed amperometry, which employs a repeating sequence of potentials, is the technique employed for carbohydrate analysis, and is a reproducible and sensitive method for the detection of all carbohydrates of molecular weight up to ten thousand.

When a substance reaches the detector flow cell, a signal is produced (gives a waveform for each analyte) that is proportional to the concentration of the substance.

The exact quantity of each substance can be calculated by determining the peak area and by means of a previously acquired calibration curve (quantitative analysis) [36].

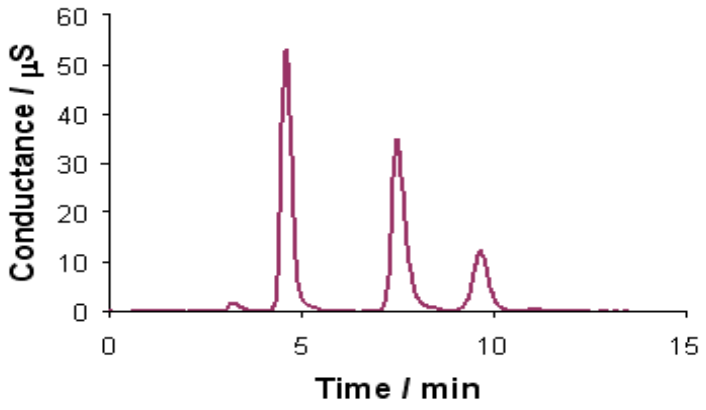

Figure 5. Representation of an Ion Exchange Chromatography signal

2.4.2 ToF-SIMS technique

Some analytical methods can give information about the chemical composition of the upper surface to determine some properties such as chemical and physical [37].

Secondary ion mass spectrometry (SIMS) is the most sensitive of all surface analysis techniques that can investigate molecular and elemental composition of the surface with submicron lateral resolution.

This is a versatile technique with great potential for simultaneous analysis of organic and inorganic constituents on wood surfaces at an analysis depth of 1 nm. It is able to detect elements present in the parts per billion ranges [37]. Normally the primary ions (Bi_3+, C60+, or Ar+) are pulsed at 10ns with a dose of 10^{12} ions/cm^2 and energy of 15 keV.

Characteristic secondary ions originating from lignin, carbohydrates, extractives, and metals are suited for assessment of their location and spatial distribution in wood tissues. SIMS has been successfully applied for chemical characterization of pulp fibers and paper surfaces, and, more recently, for analysis of extractives in wood tissues.

SIMS instruments based on sector mass analyzers have been available since the 1950s, and were used exclusively for inorganic and elemental analysis until the late 1970s.

It was a that time that SIMS methods for the examination of organic surfaces and the determination of organic compounds were revealed, and more sophisticated instruments such as ion microprobes, ion microscopes, and time of flight secondary ion mass spectrometry (ToF- SIMS) instruments were developed [38].

ToF-SIMS has the following advantages: i) greatly improve sensitivity (using a low-dose pulsed beam of primary ions to eject secondary ions) due to the greater transmission of ions through the ToF mass spectrometer, ii) mass assignment accuracy (the ability to define the mass of a spectral peak) equivalent to quadrupole instruments, but with a wider mass range and the ability to measure the entire mass spectrum for a single pulse of incident ions without scanning the mass analyzer; and iii) improved lateral resolution (the ability to resolve adjacent features) for greater imaging capability [38].

ToF-SIMS uses a pulsed primary ion beam to desorb and ionize species from the outermost atomic layers of the sample surface. That desorption from the surface is the result of a "collision cascade" initiated by the primary ion contacting the sample surface. The resulting secondary ions are accelerated (by applying a high voltage potential) into a mass spectrometer, where they are mass analyzed by measuring their time-of-flight from the sample surface to the detector because they travel at different velocities depending on their mass to charge ratio. So, each primary ion pulse can generate a mass spectrum which is obtained for the influx times of the secondary ions in the detector. Images can be obtained by accumulating a mass spectrum at every pixel as the primary ion beam is scanned across the sample surface.

2.4.3 Gel Permeation Chromatography (GPC) /size exclusion chromatography (SEC) technique

The basic principle of this type of chromatography is the separation of molecules in a solution on the basis of their sizes or hydrodynamic volumes with respect to the average pore size of the packing (using a macroporous gel) generating different retention times (elution volumes) that can be converted into molecular weight by using a calibration standards or molecular weight sensitive detectors [39].

The sample is introduced into a stream of mobile phase that pass through a bed of a stationary phase. This stationary phase consists of small polymeric or silica-based particles that are porous and semirigid to rigid. Sample molecules that are smaller than the pore size can come into the stationary-phase particles (for diffusion) and, consequently, have a longer path and longer retention time than larger molecules that cannot enter the pore structure. Larger molecules are expelled and, consequently, are rapidly carried through the system. On the other hand, very small molecules can go in almost every pore they come across and, therefore, elute last. The extent to which molecules can go into the pores is controlled by their sizes and shapes. Also, the porosity of the packing material can be altered to exclude some molecule sizes. **Figure 6** shows a representation of this mechanism of molecules separation [39].

SEC is generally used to separate biological macromolecules and to determine molecular weight distributions of polymers and average molecular weights of soluble oligomers and polymers.

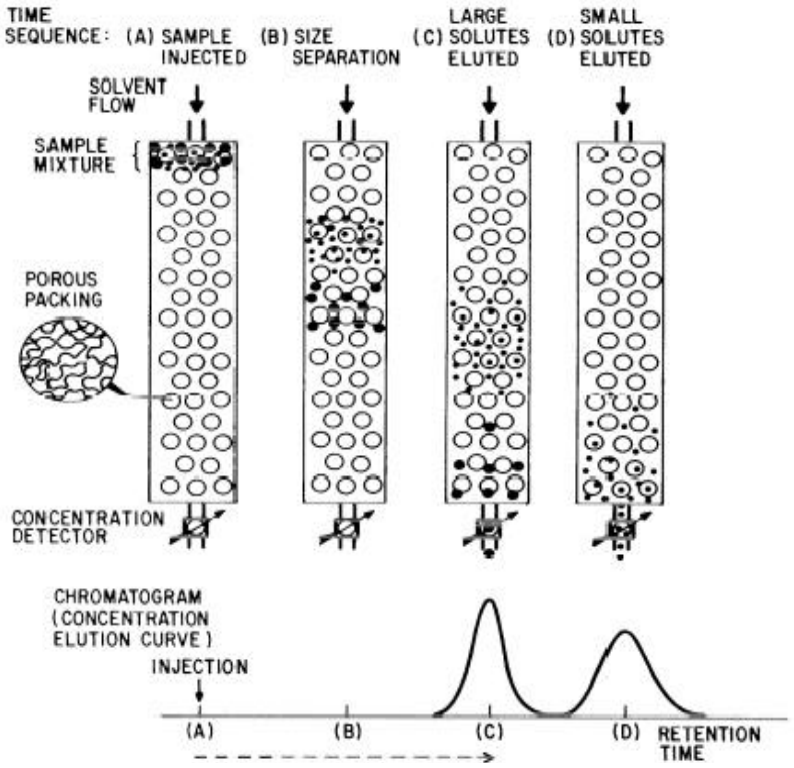

Figure 6. Representation of the molecules separation in SEC

3. Experimental

In this work is examined different pretreatments used to process wood-to-ethanol and also is provided results from a novel pretreatment process. Mixed hardwood chips, a dominant bioenergy source used nowadays in the Western Hemisphere, for the new pretreatment process were pretreated with modified green liquor under alkaline conditions. The efficacy of this new type of pretreatment was evaluated based on the yield of cellulose to glucan.

Different conditions of sulfidity and alkalinity were analyzed together with different enzyme activities.

Also, different concentrations of ethanol were used in one case of green liquor pretreatment together with different enzymes activities. At the end is shown an economic analysis of all the cases studied to compare production and profit costs and see which method is more economically attractive for ethanol production.

3.1 Methodology

Preparation of the Chips before Pretreatment: Mixed Southern Hardwood chips composed mainly of oak and the rest of gum were received from a local mill. The chips were storage at (35°F) in sealed plastic bags to prevent moisture loss and fungal spoilage. To perform the analysis, the chips were screened to remove barks, knots, and fines.

Analysis of Wood: The moisture was determined by drying at 105 °C wood samples in an oven overnight. The amount of extractives was determined using a standard Soxhlet method (TAPPI method T 204 cm-97) with a solution of benzene-ethanol (2:1 v/v) for 24 hours. Then, with the extractive free samples was possible to determine the acid soluble lignin and acid insoluble lignin using a two steps acid hydrolysis (according to TAPPI methods: UM250 and T222 om-98, respectively). The acid soluble lignin is measured by UV-Vis spectroscopy (205 nm wavelength).

Green Liquor Pretreatment: Different concentrations of Green Liquor (GL) pretreatment were used. **Table 1** shows the concentrations of these chemicals.

Table 1. Different concentrations of chemicals used in GL Pretreatment

%Na_2S	%Na_2CO_3
0	100
12.5	87.5
25	75
37.5	62.5

Wood chips (900 wet grams in each study) were introduced in a digester with a liquid/wood ratio of 4:1, a temperature of 160°C, a pressure of 70 psig, and an H factor of 400. Two different total titratable alkali (TTA, a measure of alkalinity of the total Na_2S + Na_2CO_3 + NaOH) percentages (12% TTA and 16% TTA) were used at different sulfidities (%S, percent of sulfidity) percentages (0%S, 12.5%S, 25%S, and 37.5%S) as shown in **Table 1**.

The role of sodium carbonate using green liquor pretreatment has been reported to act as a buffer. Its absence can cause an increase in the H factor (cooking time and temperature as one variable) originating longer pretreatment times.

For the pretreatment using 12%TTA and 25%S, different ethanol concentrations were applied (5%, 10% and 20% of ethanol based on the total liquid). The same chip samples were used and introduced in the same digester with the conditions: liquid/wood ratio of 4:1, a temperature of 160°C, a pressure of 90 psig, and H factor of 400. After the pretreatment, the pulp was washed overnight to separate the lignin removed.

Then, the pulp was disintegrated in a refiner, screened to separate fibers and rejects, and the fibers collected were fluffed.

Analysis of Pulp: After the pretreatment, the pulp was washed overnight to separate the lignin removed. Then, the pulp moisture was analyzed and the yield was determined.

The pulp was defibrillated in a refiner and screened to separate the fibers and the rejects. The fibers were collected and put in a centrifuge to eliminate part of the water and after that, the pulp was fluffed.

Enzymatic hydrolysis, quantification, and identification of sugars: Two oven dry grams of fluffy samples were hydrolyzed using the enzymes: Cellulase, Xylanase, and β-glucosidase (in a weight ratio of 10:3:3, respectively, received from Novozymes). Together with the enzymes, a buffer solution at 5% consistency was added in each sample to maintain a pH of 4.8, and fucose solution as an internal standard.

In each study case was used different enzymes activities (5, 10, and 20 FPU/grams of pulp). The enzymatic hydrolysis was performed in a water bath at 50°C for 48 hours. After that time, the tubes were placed in a centrifuge and the liquid was filtrated under vacuum. The amount of monosaccharide present of the liquid part was determined.

Quantification and identification of sugars: Four standard sugars (glucose, fucose, xylose, and mannose) at different dilution factors were used to determine the amount of

sugar present in the pulps analyzed. The samples were analyzed using an Ion Exchange Chromatography System (Dionex ICS-3000).

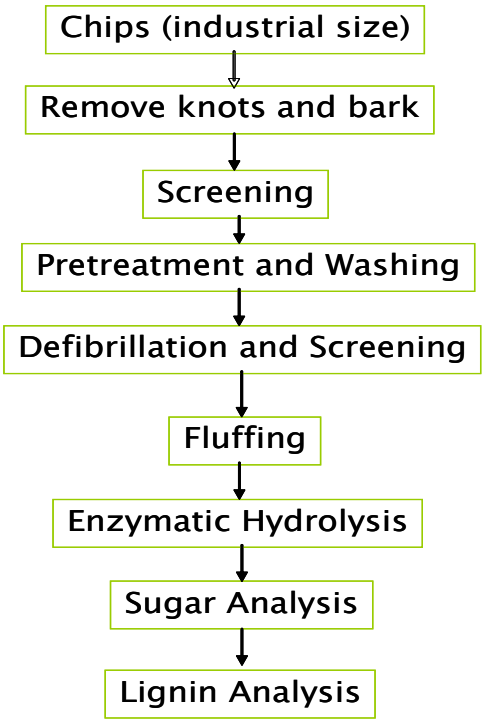

Figure 7. General Methodology

3.2 Experimental procedure for lignin analysis

- *ToF SIMS*

The pulp samples to be analyzed (storage in glass fluoroware containers) were dried under vacuum and mounted on a Si or SiO_2 wafer by using a double sticky tape (double coated tape) to obtain a sample surface as flat as possible.

Then, the samples were analyzed in a surface spectroscopy static SIMS in which secondary ions are ejected only from the top most atomic layer (10 – 20 Å) and the mass resolution (>10,000 m/Δm).

The equipment has the following components: an ultra high vacuum ($P<10^{-9}$ mbar), a pulsed ion gun, electron flood gun for charge compensation, liquid metal ion source (Bi), primary focusing units, and pulsing systems.

The surface ion images were generated and the ion count is recorded in a heat scale (each ion image has its own heat scale bar). More counts per pixel means better signal to noise ratio and results in a visually clearer image.

A complete mass spectrum is acquired and stored at each pixel. After data acquisition, images were generated using only the peak (or groups of peaks) of interest.

- *Size exclusion chromatography (SEC) technique*

*Sample preparation of black liquor solution obtained after GL pretreatment:

Lignin from the black liquor solutions obtained from 16%TTA, 0%S and 37.5%S GL pretreatment were isolated by acidic precipitation as follows:

1. The liquors were first filtered through a Whatman #4 filter paper on Bucker funnel and diluted 10 times by adding water.
2. The liquors were neutralized with 2 M H_2SO_4, until a pH of ~6.0 was reached.
3. The solutions were then stirred vigorously for one hour.
4. The liquors were further acidified to a pH of 3.0 and frozen at -20°C.

5. After thawing the solutions, the precipitates were collected through centrifuge and washed twice with cold water.
6. The precipitates were freeze-dried overnight.
7. The lignin samples were obtained and dried in the vacuum drier at a temperature of 40°C.

Then, it was performed acetobromination with 2.5 ml of acetyl bromide and glacial acetic acid (8:92, %v/v) added to 10 mg of lignin samples and let it at 50°C for 2 h on continuous stirring, as a derivatization method before the size exclusion analysis. The residue was dissolved with 5 ml of tetrahydrofuran (THF) and analyzed in the chromatographer.

GPC of samples were done on size exclusion chromatographic system (Waters system) equipped with a UV detector set at 280 nm and a differential refractive index detector (RI). The analysis were carried out at 30°C using THF as the eluent at a flow rate of 0.7 ml/min. 200 µl of the sample dissolved in THF (1 mg/ml) was injected into the column. The system was calibrated with polystyrene standards in the molecular weight range of 890-1.86 x 10^6 g/mol, and Millenium 32 GPC software was used for data processing.

*Sample preparation for the pulps:

Extractive free pulps obtained from 16%TTA, 0%S and 37.5%S GL pretreatment were vacuum dried prior the pulverization. For pulverization, 2 mg of pulp were placed in the homogenizer device (with milling balls made of zirconium dioxide) at 300 rpm for 20 hours.

Then, the pulp was treated (by benzoylation in ionic liquid) mixed with ionic liquid (38mg) in a bottle and vortexed until all solid particles were dispersed, and heated at 80°C for 2 hours until the solution became clear. Pyridine (230µl, 2.6 mmol) was added to the solution, vortexed and allowed to cool at room temperature. The benzoyl chloride (280µl, 2.4mmol) was added in one portion and vortexed until a homogeneous white paste was formed. The sample was left at room temperature for 3 hr. Deionized water (5 ml) and ethanol (15 ml) was added, the mixture was vigorously shaken, and vortexed for 5 min. The solid was filtered off through a sintered funnel, washed with further ethanol and purify with methanol (stirred without heat overnight), then the solid was filtered off to give a white powder. Afterward, the powder of each sample was dissolved with 5 ml of THF and analyzed in the chromatographer.

4. Results and Discussion

4.1 Effect of Green Liquor Pretreatment

4.1.1 Effect of different sulfidities in the sugar yield by using green liquor pretreatment at 12%TTA and 16%TTA.

After GL pretreatment, the oven dry (OD) weight of the pulp obtained was determined and compared with the OD weight of the chips introduced in the digester. Pretreatment yield was calculated and is shown the **Table 2**, **Table 3, and Figure 8** for 12%TTA and 16%TTA, respectively. The formula used is:

$$\%\,pretreatment\;\; yield = \frac{OD\_weight\;\; pulp}{OD\_weight\;\; chips} * 100$$

The following formula was used to determine the OD weight for wood and pulp.

$$OD\_weight = \frac{(wet\_weight) * (100 - \%moisture)}{100}$$

Table 2. Pretreatment yield results using 12%TTA

Sulfidity, %	TTA, %	Yield, %	pH
0	12	93.3	9.56
12.5	12	81.8	9.53
25	12	80.5	9.54
37.5	12	77.9	9.66

Table 3. Pretreatment yield results using 16%TTA

Sulfidity, %	TTA, %	Yield, %	pH
0	16	90.0	9.29
12.5	16	81.7	9.60
25	16	79.7	9.70
37.5	16	74.2	9.45

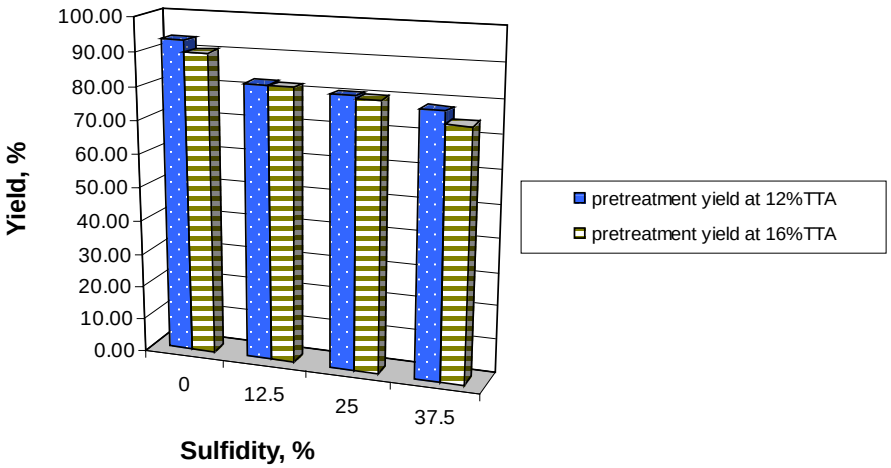

Figure 8. Pretreatment yield vs. sulfidity

Lignin content was analyzed in the pulps after pretreatment and the amount of lignin removed with respect to the amount present in the wood chips was calculated. The results are shown in **Table 4** and **Table 5**.

Sulfidity is the most important parameter analyzed with respect to overall material yield. The results illustrate that an increase in sulfidity reduces the pretreatment yield principally due to the removal of lignin as is shown in **Table 4** and **Table 5**. The formula used to calculate lignin removal is:

$$\%lignin\ removed = \left(\frac{(\%lignin\ in\ wood) - (\%lignin\ in\ pulp)}{(\%lignin\ in\ wood)} \right) * 100$$

Sodium sulfide aqueous is characterized as an equilibrium system in which S^{-2} can react with H_2O to yield equimolar concentration of hydroxide and hydrosulfide anions. The chemical equation is shown below:

$$Na_2S \quad + \quad H_2O \quad \leftrightarrow \quad NaOH \quad + \quad NaSH$$

The activity of the HS^- ions can increase the release of the lignin by causing a breakup of the lignin complex into smaller ensembles. Then, these ensembles can diffuse out of the fiber wall [40].

Table 4. Lignin content of pulps at 12%TTA

Sulfidity, %	TTA, %	ASL	AISL	Total Lignin, %	Lignin removed%
0	12	2.6	20	22.6	2.3
12.5	12	2.3	20	22.3	3.6
25	12	2.3	20	22.3	3.6
37.5	12	2.2	20	22.2	4.2
	WOOD	3.1	20	23.1	

Table 5. Lignin content of pulps at 16%TTA

Sulfidity, %	TTA, %	ASL	AISL	Total Lignin, %	Lignin removed%
0	16	2.6	20	22.6	2.3
12.5	16	2.8	20	22.8	1.6
25	16	2.4	20	22.4	3.0
37.5	16	1.8	20	21.8	5.6
	WOOD	3.1	20	23.1	

Sulfidity is a measure of the sulfide content of the environment and is related to delignification enhancement (sulfide helps delignification).

The results suggest that the increase in sulfidity and total titratable alkali helps delignification, obtaining more lignin removal using 16%TTA and 37.5%S, 1.38% further than by using the same sulfidity at 12%TTA.

The effect of sulfidity was analyzed not only based in the lignin removed after pretreatment but also based in the sugars yield after enzymatic hydrolysis (EH).

Enzymatic hydrolysis of the pulps was performed and the weight loss of the pulp obtained after enzymatic hydrolysis was determined. The formula used to calculate the weight loss is shown below:

$$\%Weight\ loss = \frac{(grams\ initial\ \ pulp - grams\ \ pulp\ after\ EH)}{grams\ initial\ \ pulp} * 100$$

The results are summarized in **Table 8**. The liquid fraction obtained was analyzed using the Ion chromatography. The chemical composition of the hardwood chips is shown in **Table 6,** and the total mass balance for all the study cases is shown in **Table 7**.

Table 6. Chemical Composition of hardwood chips

Sample #	Glucan, %	Xylan, %	Mannan, %	Total Sugar, %	Lignin, %	Extractives	Balance
1	59.7	13.5	0.5	73.8	23.1	1.9	96.9
2	59.7	13.5	0.7	73.8	23.1	1.9	96.9

Table 7. Total mass balance for Green Liquor pretreated chips

Sulfidity, %	TTA, %	Glucan, %	Xylan, %	Mannan, %	Total Sugar, %	Lignin, %	Extractives, %
0	16	44.0	11.0	0.5	55.5	22.6	0.5
0	12	42.0	11.5	0.4	53.8	22.6	0.4
12.5	16	46.6	11.4	0.4	58.3	22.8	0.8
12.5	12	44.6	11.3	0.5	56.4	22.3	0.8
25	16	48.7	11.9	0.4	61.1	22.4	1.8
25	12	47.5	11.5	0.4	59.4	22.3	1.7
37.5	16	49.6	12.6	0.4	62.5	21.8	1.3
37.5	12	47.6	12.1	0.6	60.2	22.2	1.2

Table 7 shows that by using 37.5%S and 16%TTA the chemical composition of the pulp is higher in glucan and xylan and the lignin amount is lower compared with the other pretreatments.

The weight loss of the pulps after enzymatic hydrolysis was calculated and the results are plotted in **Figure 9**, **Figure 14**, and **Figure 19**. The weight loss can give an idea about the carbohydrates yield that can be obtained.

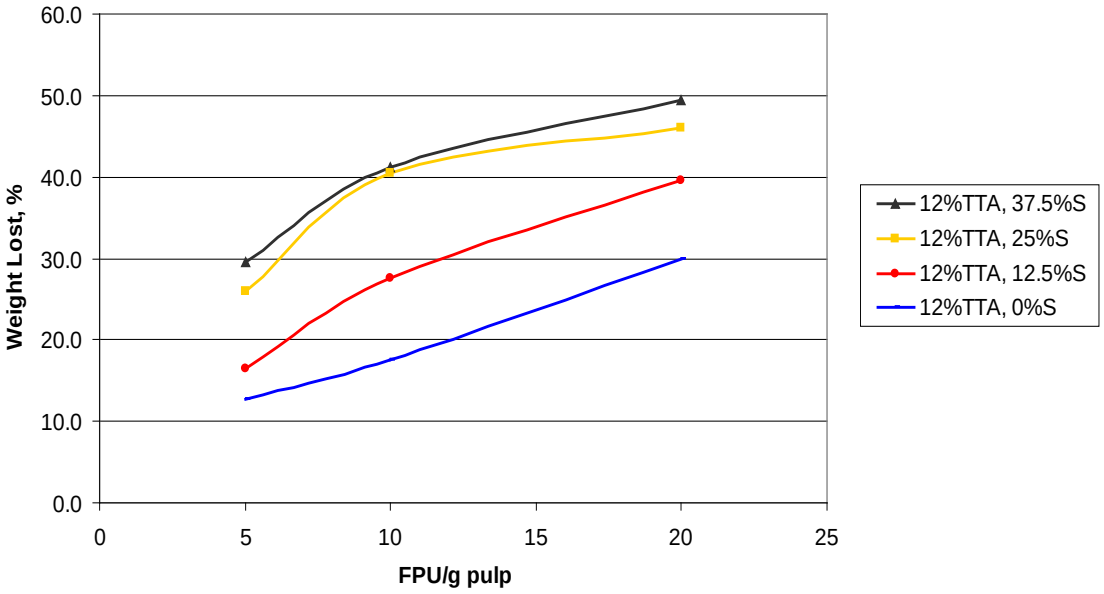

Figure 9. Weight loss vs. FPU/g pulp using 12%TTA

Analyses of glucan, xylan, and mannan yield after enzymatic hydrolysis from pulps pretreated using GL as a function of its alkalinity and sulfidity were performed. The formulas used to calculate the yields are:

$$\%glucan\ yield = \left(\frac{grams\ glucan\ in\ \ filtrate\ after\ EH}{grams\ glucan\ in\ wood} \right) * 100$$

$$\%xylan\ yield = \left(\frac{grams\ xylan\ in\ \ filtrate\ after\ EH}{grams\ xylan\ in\ wood} \right) * 100$$

$$\%mannan\ yield = \left(\frac{grams\ mannan\ in\ \ filtrate\ after\ EH}{grams\ mannan\ in\ wood} \right) * 100$$

$$\%carbohydrates\ yield = \left(\frac{grams\ (glucose + xylose + mannose)\ in\ \ filtrate\ after\ EH}{grams\ \ pulp} \right) * 100$$

The results are shown in **Figures 10-17**. The total carbohydrates (the sum of glucose, xylose, and mannose) were also determined and plotted for each study case. The results are shown in **Figure 13** and **Figure 18**.

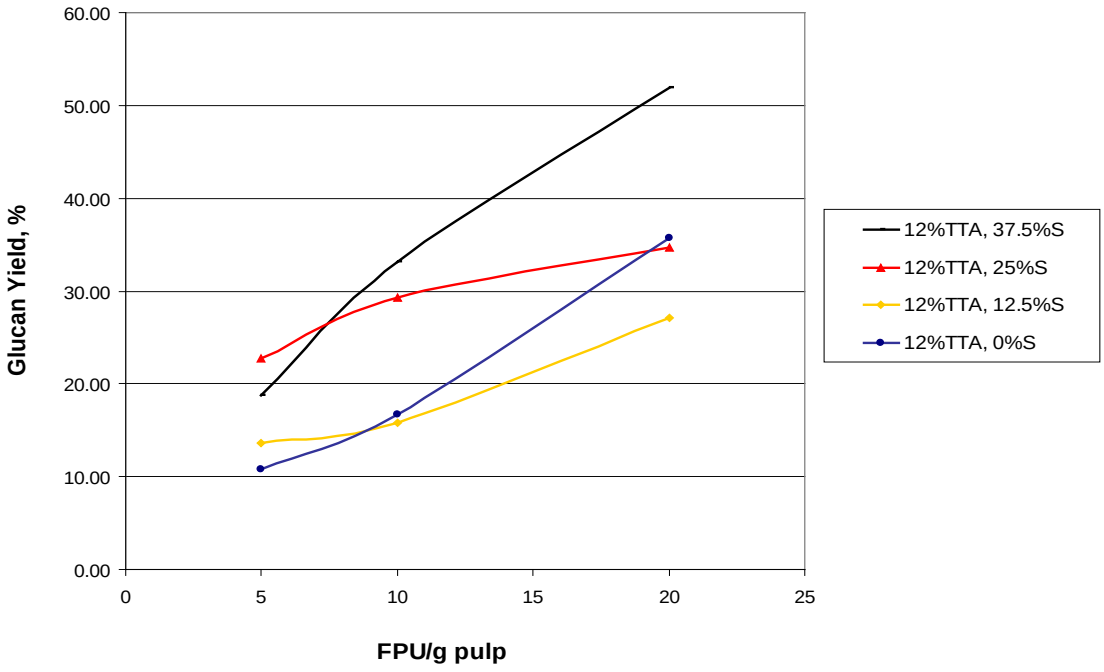

Figure 10. Glucan yield vs. FPU/g pulp at 12%TTA

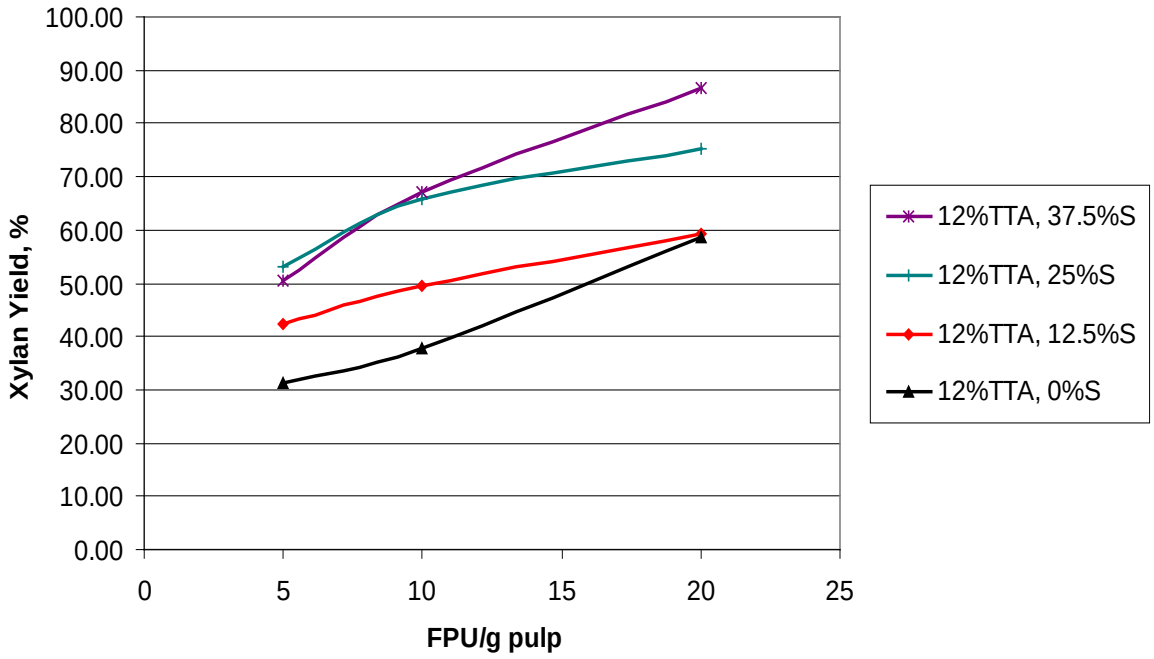

Figure 11. Xylan yield vs. FPU/g pulp at 12%TTA

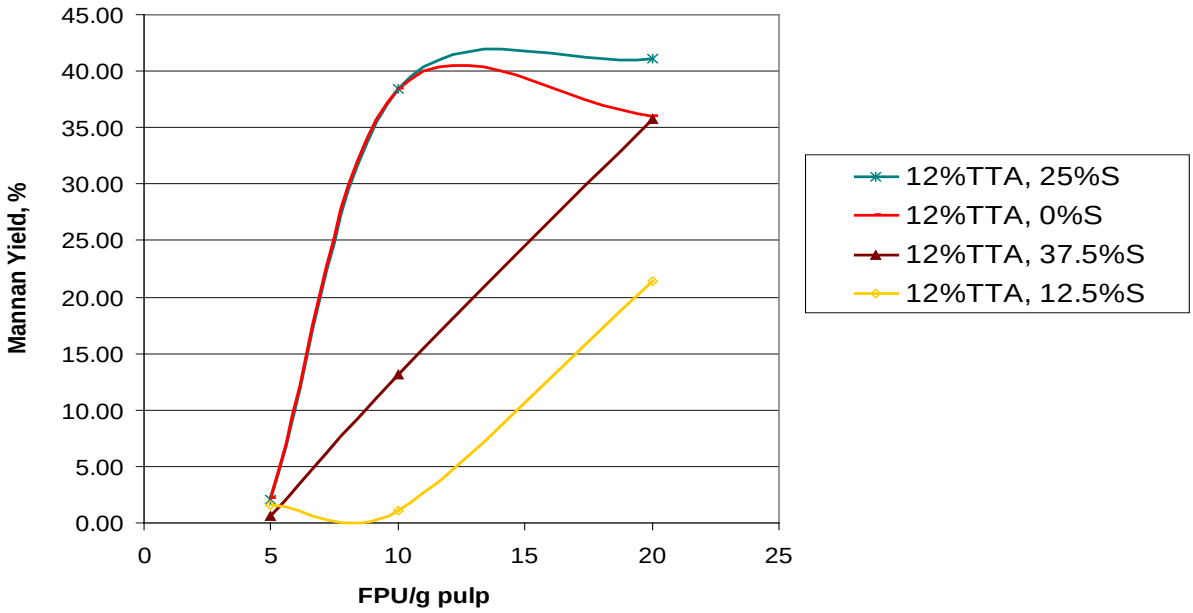

Figure 12. Mannan yield vs. FPU/g pulp at 12%TTA

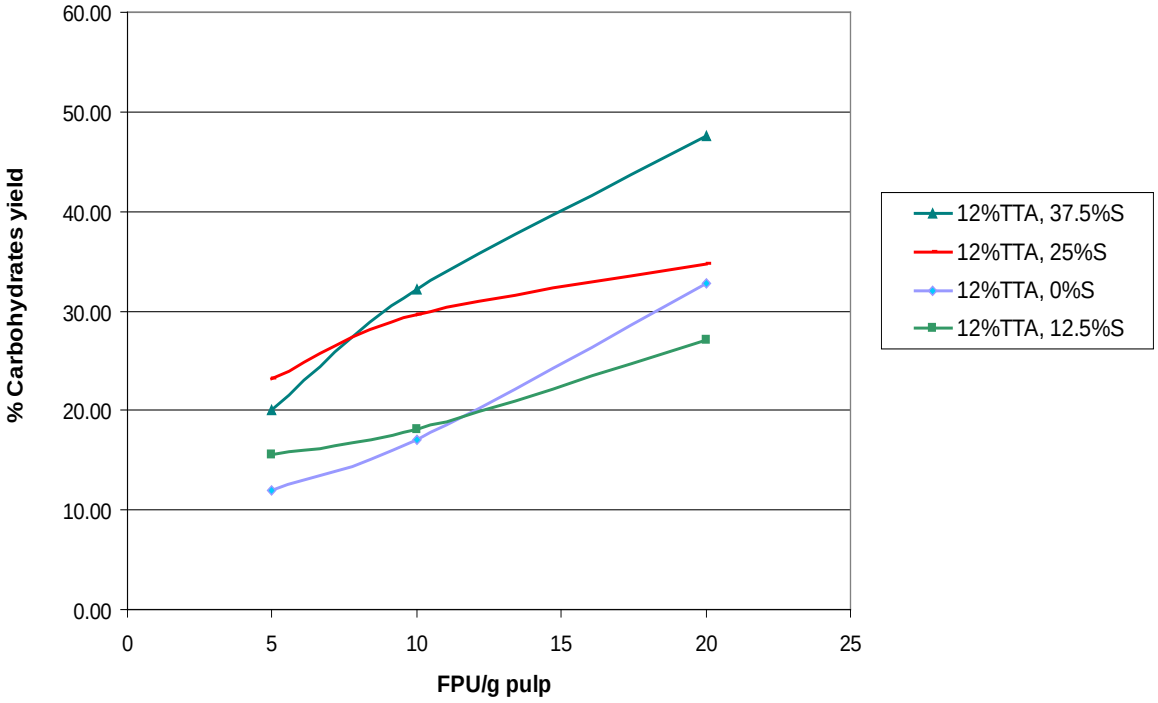

Figure 13. Carbohydrates yield vs. FPU/g pulp at 12%TTA

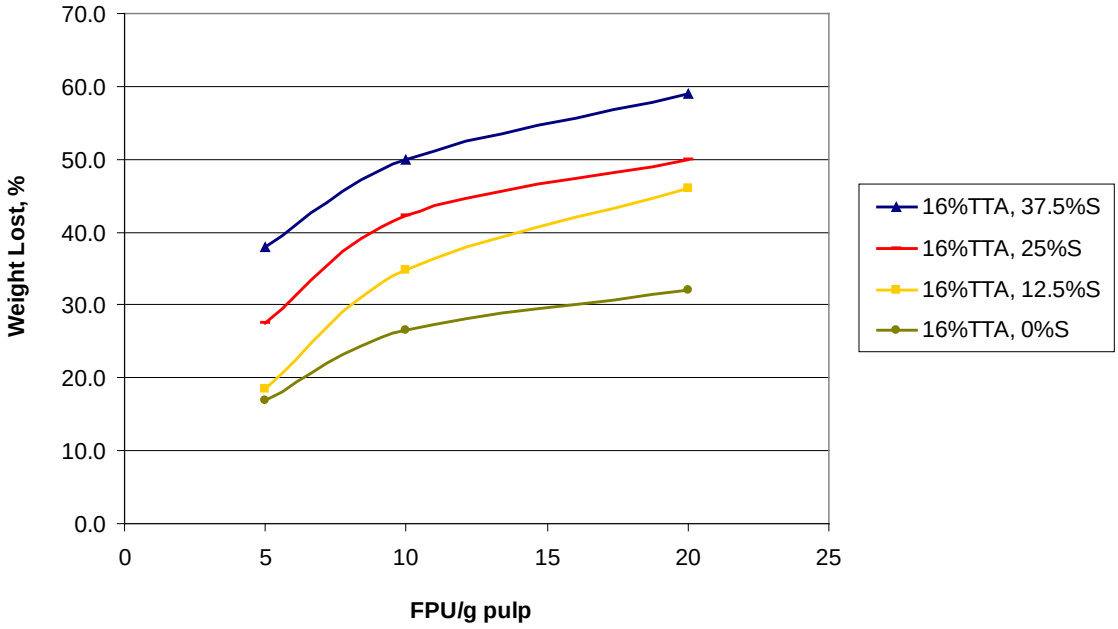

Figure 14. Weight loss vs. FPU/g pulp using 16%TTA

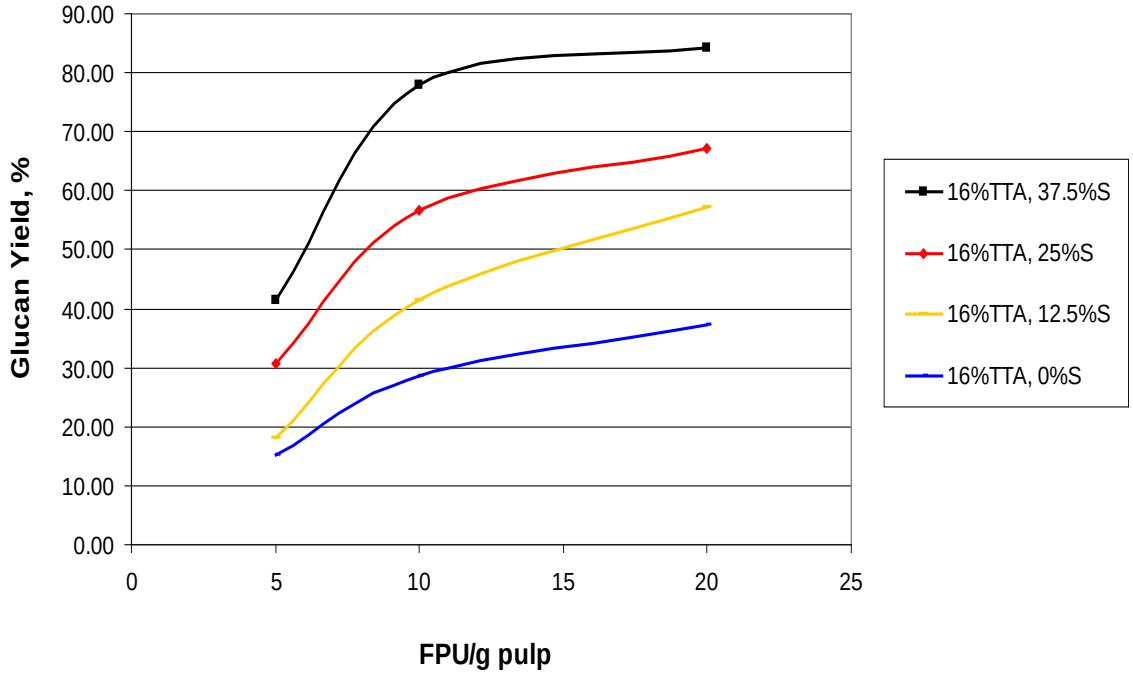

Figure 15. Glucan yield vs. FPU/g pulp at 16%TTA

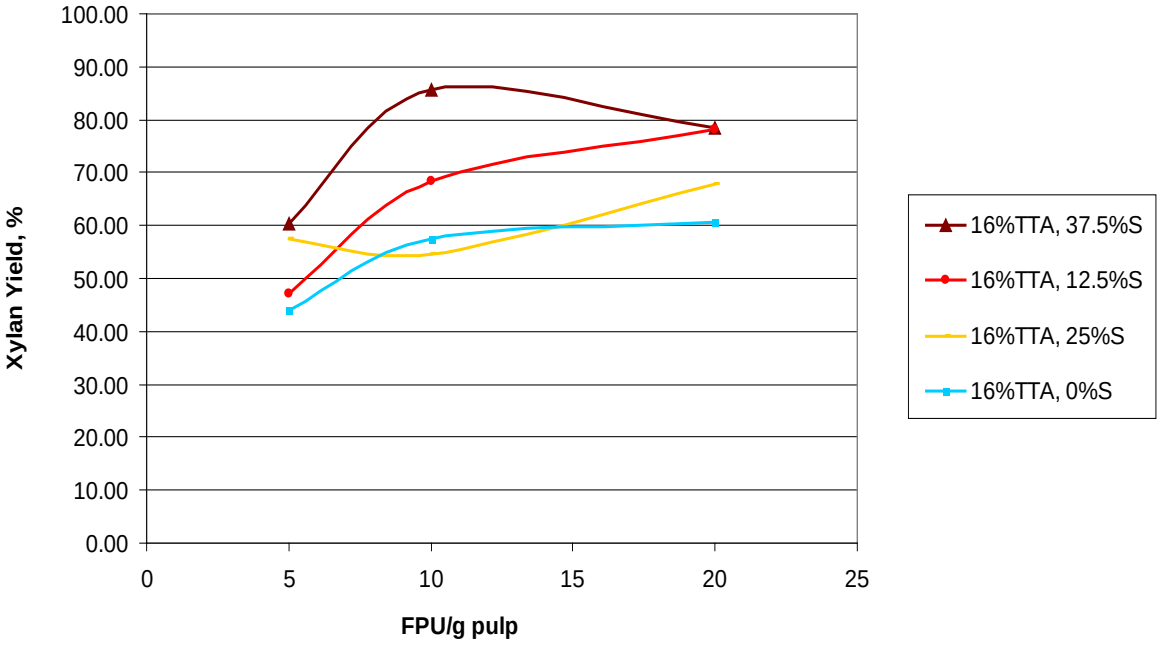

Figure 16. Xylan yield vs. FPU/g pulp at 16%TTA

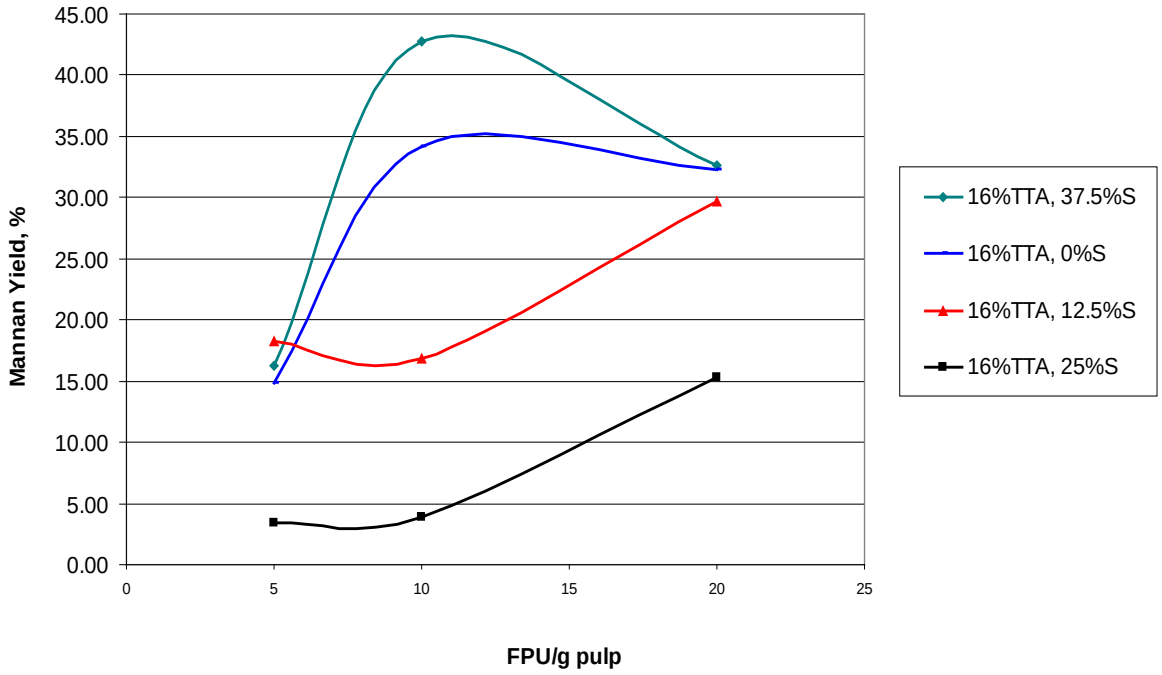

Figure 17. Mannan yield vs. FPU/g pulp at 16%TTA

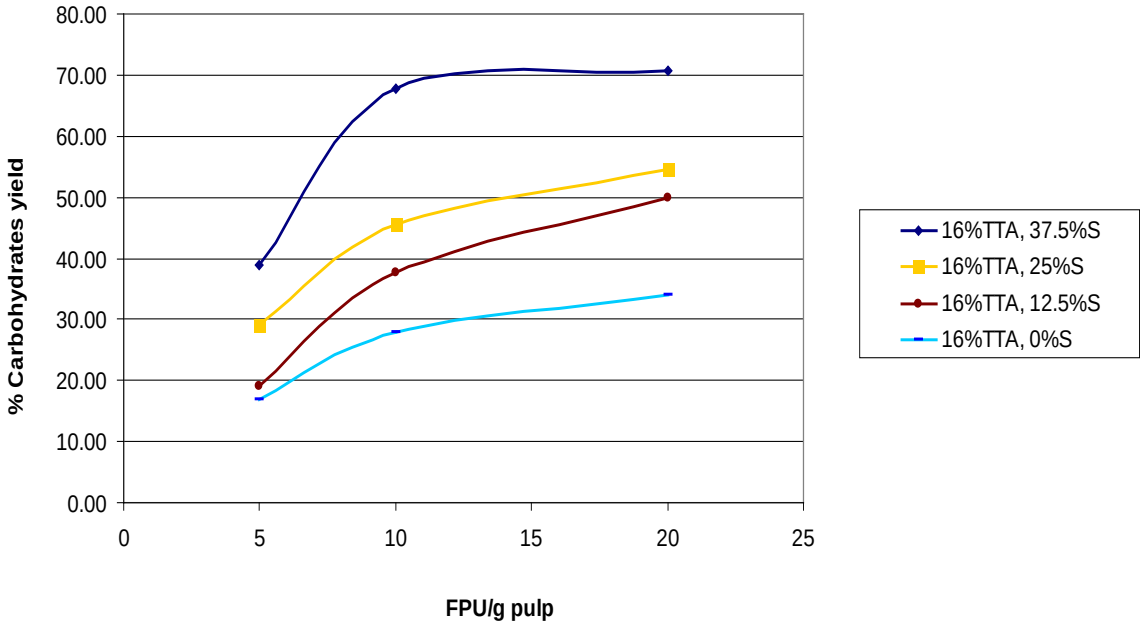

Figure 18. Carbohydrates yield vs. FPU/g pulp at 16%TTA

By using different sulfidities it was possible to notice that by increasing it enhanced higher carbohydrates yields. Hydrosulfide ions HS^- can remove part of the hemicellulose but not cellulose because the cellulose fraction is mainly susceptible for hydroxide attack.

The weight loss after enzymatic hydrolysis can give an idea about the total carbohydrates yield that is possible to obtain. For this reason, the weight loss was compared with the amount of carbohydrates yield. In general, by increasing the sulfidity, the weight loss of the pulp increases. The results are summarized in **Table 8**.

The carbohydrates yield was calculated based on the sum of glucose, xylose, and mannose obtained. As it is shown in **Figure 10**, **Figure 11,** and **Figure 12,** the yield of xylan by using 12%TTA is higher compared with the glucan and mannan yield. However, by using 16%TTA**, Figure 15, 16 and 17** shown that there is an increase in the glucan yield compared with 12%TTA.

Comparing the alkalinities used, the results suggest that 16%TTA produced an increase on carbohydrates yield. Also, with high enzyme activity (20 FPU/g pulp), higher carbohydrates yields were obtained (70.6%).

Table 8. Weight loss and Carbohydrates yield results

Sulfidity, %	Active Alkali, %	FPU/g pulp	Weight Lost, %	Carbohydrates Yield, %
0	12	5	12.7	12.0
0	12	10	17.5	17.0
0	12	20	29.9	32.7
0	16	5	16.9	16.8
0	16	10	26.5	27.8
0	16	20	32.0	33.9
12.5	12	5	16.5	15.5
12.5	12	10	27.5	18.1
12.5	12	20	39.5	27.1
12.5	16	5	18.6	19.2
12.5	16	10	34.9	37.8
12.5	16	20	46.0	49.8
25	12	5	26.0	23.1
25	12	10	40.5	29.6
25	12	20	46.0	34.6
25	16	5	27.5	29.1
25	16	10	42.2	45.6
25	16	20	49.9	54.6
37.5	12	5	29.5	20.1
37.5	12	10	41.2	32.2
37.5	12	20	49.5	47.6
37.5	16	5	38.0	38.8
37.5	16	10	50.0	67.8
37.5	16	20	59.0	70.6

In general, comparing the results obtained for weight loss and carbohydrates yield (**Table 8**), it is possible to observe a approximation between those values. So, weight loss is a good experimental procedure to give an approach on the carbohydrates yield results.

Increasing the sulfidity together with an increase in the activity of the enzyme gives a general increase in the trend of carbohydrates yields. Thus, an increase in carbohydrates yields during Green Liquor pretreatment is closely related with an increase in delignification. Once the lignin barrier is partially destroyed during pretreatment, the cellulase enzymes have more space to get into and hydrolyze the cellulose into sugars.

Comparing 0%S and 37.5%S by using 12%TTA, a mass balance can illustrate the sulfidity effect. In the case of 0%S the yield obtained was 93%. By using a base of 100 grams of chips, it represents 93 grams of pulp after pretreatment with a composition of 27.4 grams of lignin and 65.5 grams of carbohydrates. After enzymatic hydrolysis with an enzyme activity of 20 FPU/g pulp, it was possible to obtain 21.4 grams of sugars (glucan+ xylan+ mannan). In the case of 37%S, the yield obtained was 78% which in a base of 100 grams of chips, it represents 78 grams of pulp after pretreatment with a composition of 20.7 grams of lignin and 56.2 grams of carbohydrates. After enzymatic hydrolysis with an enzyme activity of 20 FPU/g pulp, it is possible to obtain 26.8 grams of sugars.

The same analysis was made by using 16%TTA, obtaining for 0%S 90 grams of pulp with a composition of 25.9 grams of lignin and 63.5 grams of carbohydrates. After enzymatic hydrolysis with an enzyme activity of 20 FPU/g pulp, it is possible to obtain 21.6 grams of sugars. By using 37.5%S, it was obtained 74.3 grams of pulp with a composition of 18.9 grams of lignin and 54.2 grams of carbohydrates. After enzymatic hydrolysis with 20 FPU/g pulp, 38.3 grams of sugars was obtained.

All these results can give us an idea about the effect of sulfidity. At higher sulfidity it was possible to obtain more sugar recovery.

4.1.2 Effect of different sulfidities in the sugar yield by using green liquor pretreatment with ethanol at 12%TTA.

It has been reported that Organosolv pretreatment using organic solvents like ethanol helps in the solubilization of lignin, and breakdown of internal lignin and hemicellulose bonds [41].

For these reasons, Green Liquor solution was prepared together with ethanol to see it effect in the recovery of sugars. **Table 9** shows the results obtained for pretreatment yield after GL pretreatment with ethanol.

Table 9. Pretreatment results using ethanol

Sulfidity, %	TTA, %	Ethanol, %	Yield, %	pH
25	12	5	80.3	9.84
25	12	10	75.7	9.90
25	12	20	71.8	10.12

Comparing the results obtained at different ethanol concentrations, it is possible to say that the increase in ethanol causes a decrease in the pretreatment yield. Lignin present in the pulp after pretreatment was determined by experimental analysis, as it is explained in the experimental procedure. The results are shown in **Table 10**.

Table 10. Lignin content in pulps with ethanol

Sulfidity, %	TTA, %	Ethanol, %	ASL	AISL	Total Lignin, %	Lignin Removed,%
25	12	0	2.3	20	22.3	3.6
25	12	5	0.3	20	20.3	12.3
25	12	10	0.3	20	20.3	12.2
25	12	20	0.0	20	20.0	13.4
	WOOD		3.1	20	23.1	

The use of ethanol in GL pretreatment shown better effect on the delignification obtaining 13.45% of lignin removal by using 20%ethanol compared with all the other cases studied.

Enzymatic hydrolysis of the pulps was performed and the weight loss of the pulp obtained after enzymatic hydrolysis was determined. The results are summarized in **Table 11.** The liquid fraction after enzymatic hydrolysis was analyzed using the Ion Exchange Chromatography equipment.

In the experiments using ethanol (see **Figure 20**), higher glucan yield (90.8%) and higher carbohydrates yield were obtained at higher concentration of ethanol (20%).

Comparing carbohydrates yield results with the pretreatment without ethanol (12%TTA, 25%S), we obtain a big difference (ca. 30%). The results suggest that ethanol affects the structure of the pore cell wall and allow the enzymes to get into the matrix improving the hydrolysis of the carbohydrates.

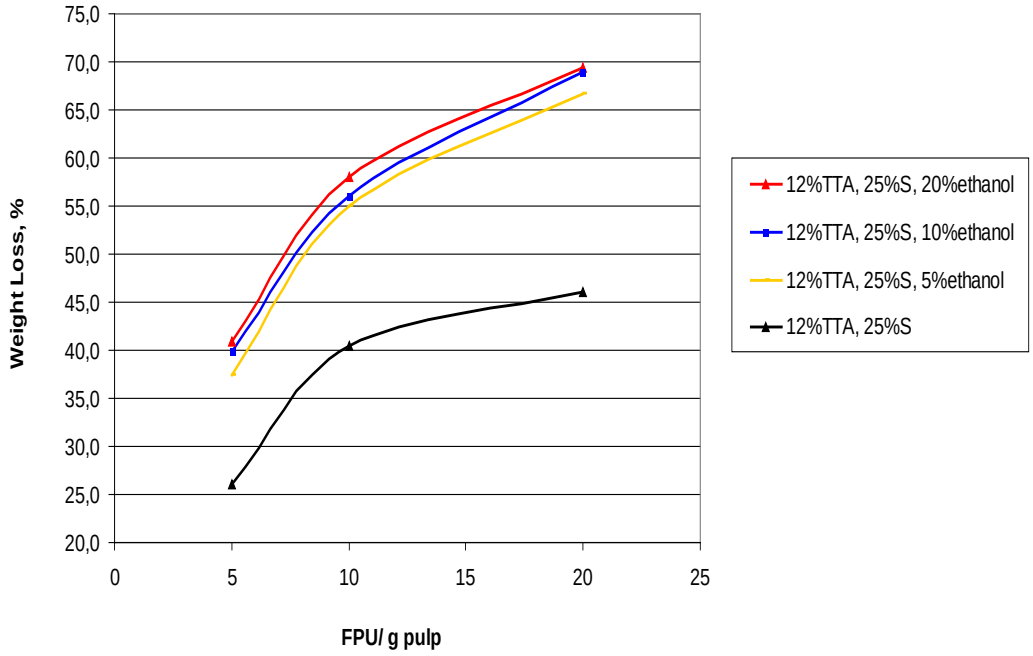

Figure 19. Weight loss vs. FPU/g pulp at 12%TTA, 25%S and ethanol

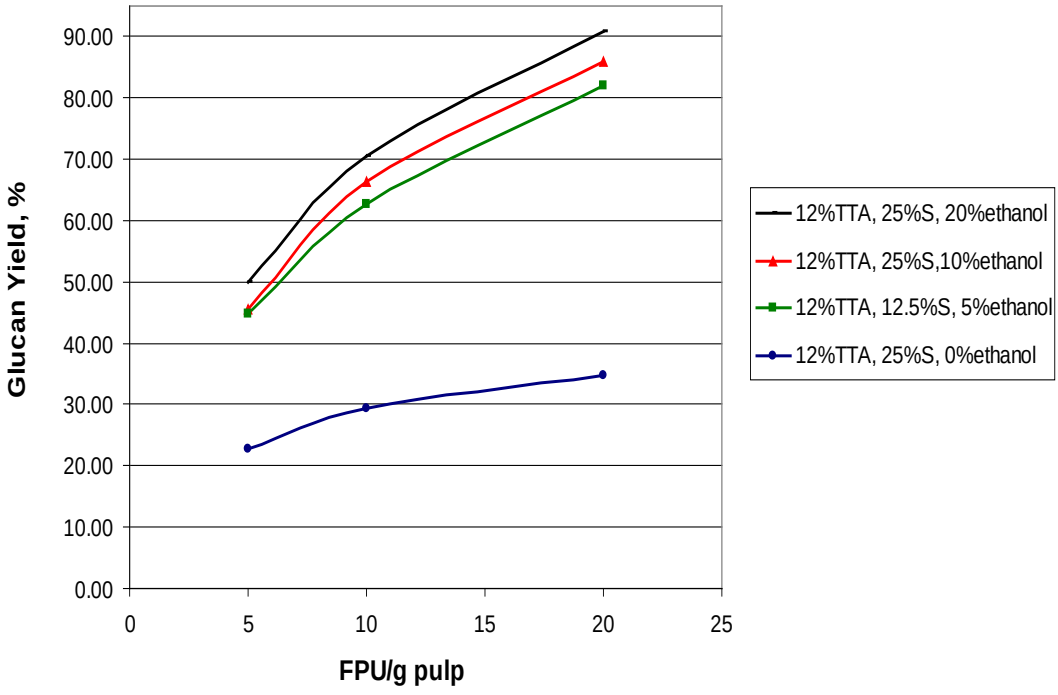

Figure 20. Glucan yield vs. FPU/g pulp at 12%TTA, 25%S and ethanol

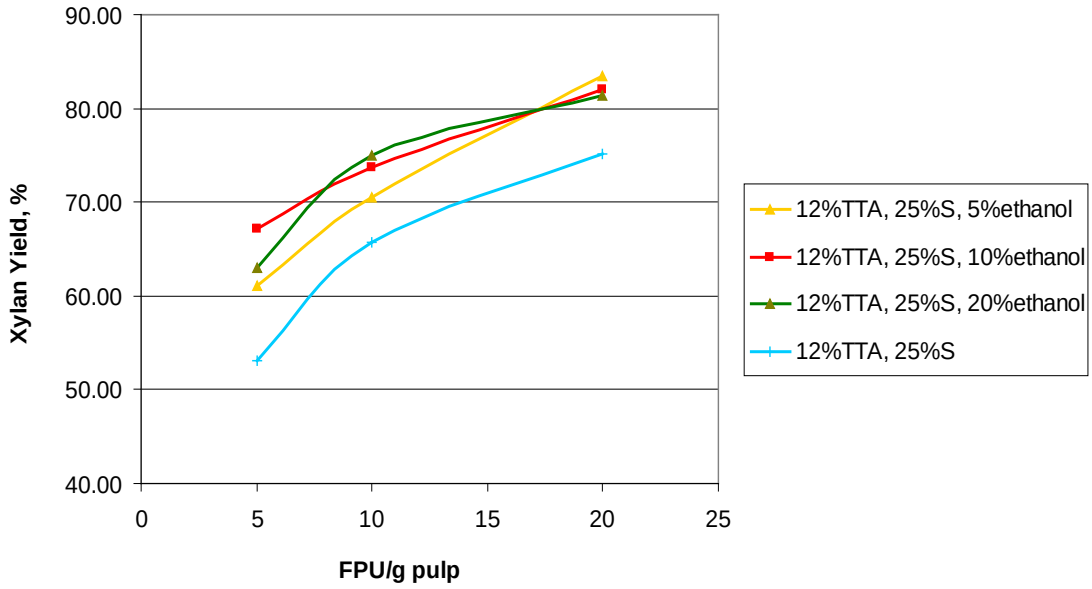

Figure 21. Xylan yield vs. FPU/g pulp at 12%TTA, 25%S and ethanol

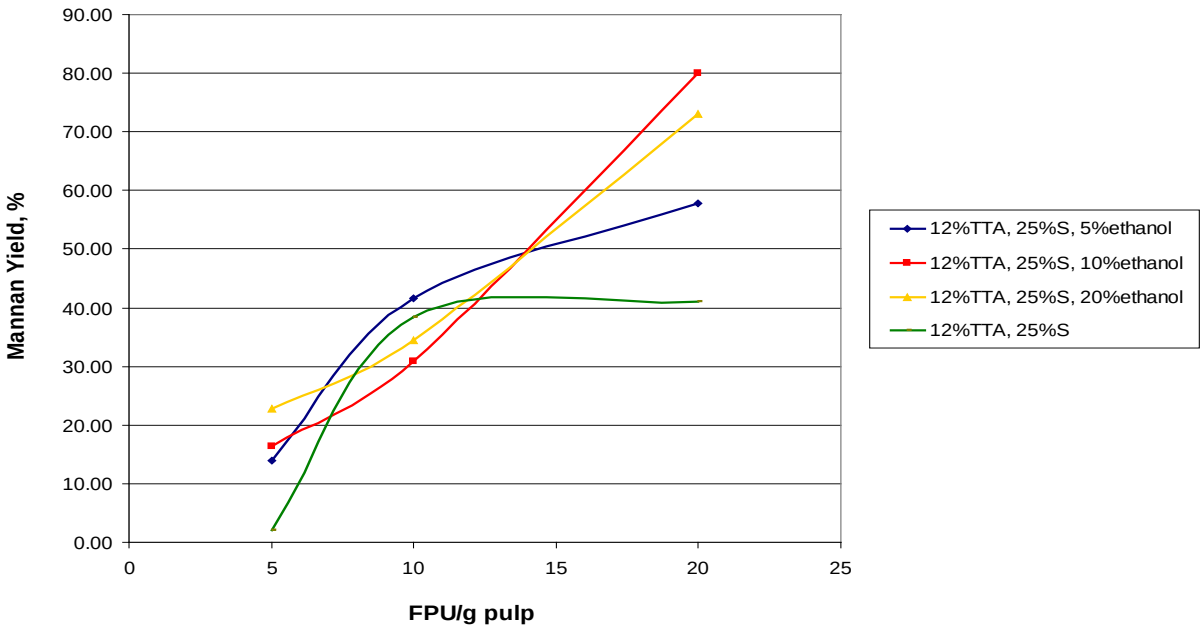

Figure 22. Mannan yield vs. FPU/g pulp at 12%TTA, 25%S and ethanol

The total carbohydrates yields are shown in **Figure 13**.

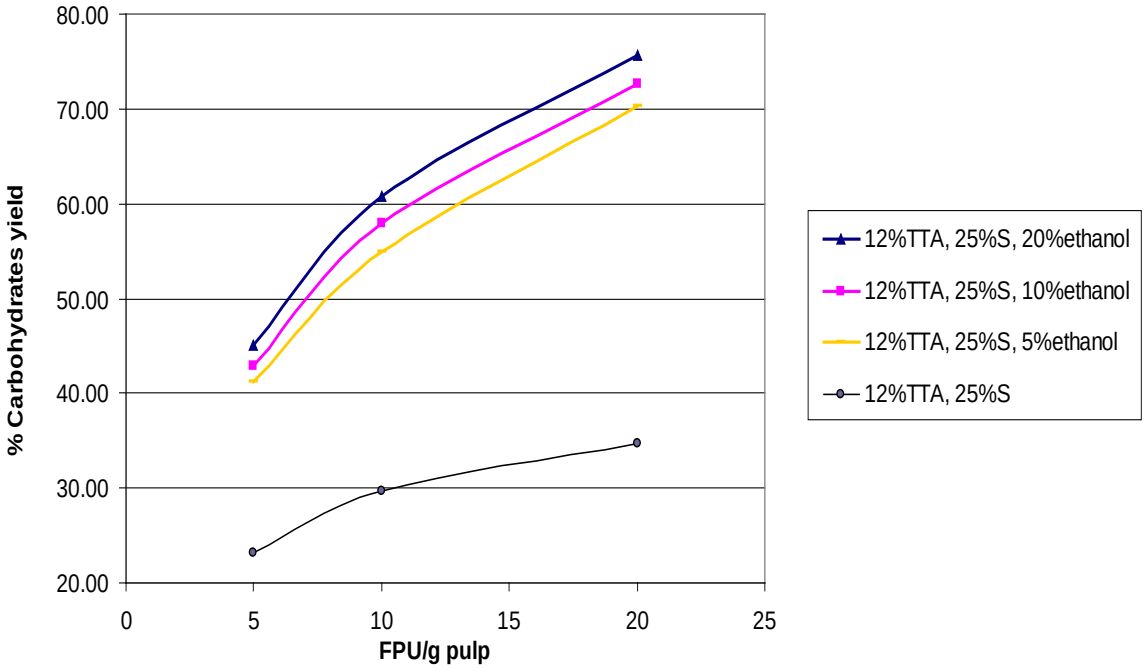

Figure 23. Carbohydrates yield vs. FPU/g pulp at 12%TTA, 25%S and ethanol

By using different ethanol concentrations, it was possible to notice that by increasing it enhanced higher carbohydrates yields.

Comparing the 12%TTA and 25%S with and without ethanol, the results suggest that the presence of ethanol in the GL pretreatment increases the percent of carbohydrates yield over 30% compared with the pretreatment without ethanol. Also, with high enzyme activity (20 FPU/g pulp), higher carbohydrates yields were obtained (75.7%).

Weight loss was compared with the amount of carbohydrates yield. The results are summarized in **Table 11**.

Table 11. Weight loss and Carbohydrates yield results for GL using ethanol

Sulfidity, %	TTA, %	Ethanol, %	FPU/g pulp	Weight Lost, %	Total Carbohydrates, %
25	12	5	5	37.4	41.3
25	12	5	10	55.0	55.0
25	12	5	20	66.7	70.3
25	12	10	5	39.9	42.9
25	12	10	10	56.0	57.9
25	12	10	20	69.0	72.7
25	12	20	5	41.0	45.0
25	12	20	10	58.0	60.8
25	12	20	20	69.5	75.7

The total mass balance for GL pretreatment using ethanol is shown in **Table 12**.

Table 12. Mass balance for green liquor pretreatment with ethanol

Sulfidity, %	TTA, %	Ethanol, %	Glucan, %	Xylan, %	Mannan, %	Total Sugar, %	Lignin, %	Extractives, %
25	12	5	49.8	12.9	0.4	63.1	20.3	1.5
25	12	10	49.8	14.0	0.7	64.5	20.3	1.1
25	12	20	50.0	14.5	0.7	65.2	20.0	1.2

In summary, higher lignin removal was illustrated in the samples pretreated with ethanol. Some research states that ethanol causes solvolysis in the green liquor solution improving delignification and preserving the carbohydrates [14]. That could explain the fact that once more lignin is removed in the pulp, enzymes can easily penetrate and hydrolyze the carbohydrates. However, to find a better explanation, more studies are needed.

A mass balance shows that at 20%ethanol, 71.7 grams of pulp is obtained with a chemical composition of 16.6 grams of lignin and 54.2 grams of carbohydrates. After an enzymatic hydrolysis using 20 FPU/g pulp, 41.0 grams of sugars was obtained.

4.2 Lignin Analysis

4.2.1 ToF- SIMS

ToF-SIMS uses a pulsed primary ion beam (Bi^+ liquid metal ion source) to bombard the surface of the pulp samples, which leads to the formation of secondary ions including both positive and negative ones. A specific area of the pulp sample was chosen for the analysis (see **Figure 24**). Then, the secondary ions resulted, were accelerated into a mass spectrometer, where they were mass analyzed by measuring their time-of-flight from the pulp surface to a detector.

The species were detected and it was possible to obtain a visualization of their spatial distribution. The images of selected constituents (total ion image 100 x 100 μm^2, 256 x 256 $pixel^2$) are shown in **Figures 26, 28, and 30**.

The images show the elemental and molecular imaging from a cross-section of pulp samples.

The mass spectrum (mass resolution: m/Δm 1314) and the secondary ion images were then used to determine the composition and distribution of sample surface constituents.

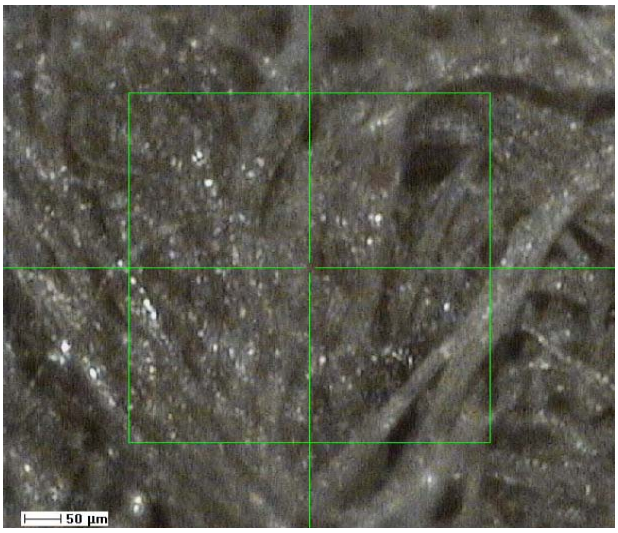

Figure 24. Fluffy pulp pellet used for ToF-SIMS analysis (Area of 500 x 500 µm)

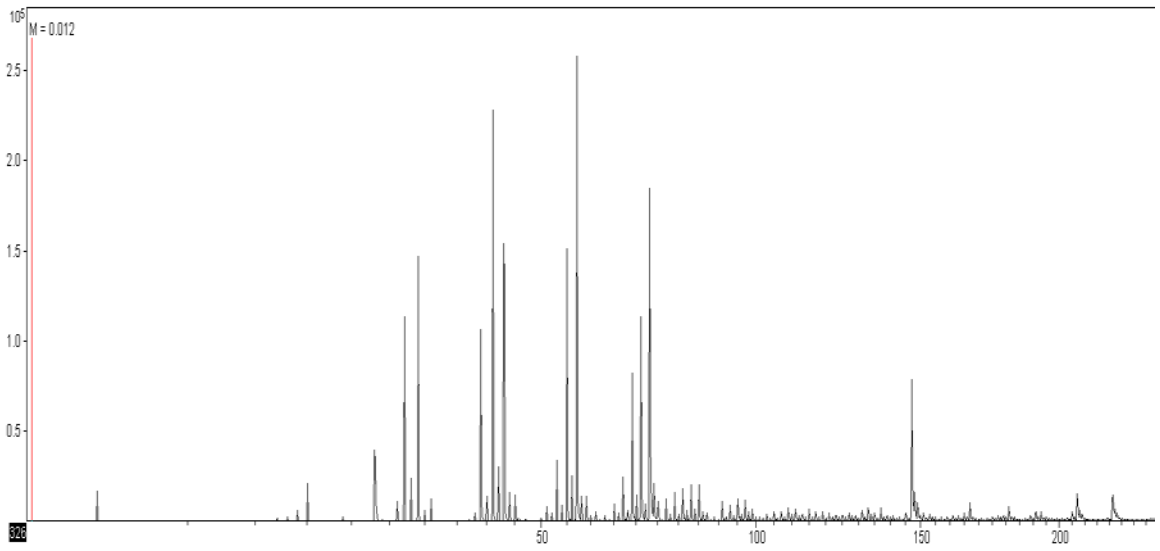

Figure 25. ToF SIMS total ion spectra from the pulp pretreated with 16%TTA and 0%S. Counts vs. mass (m/z)

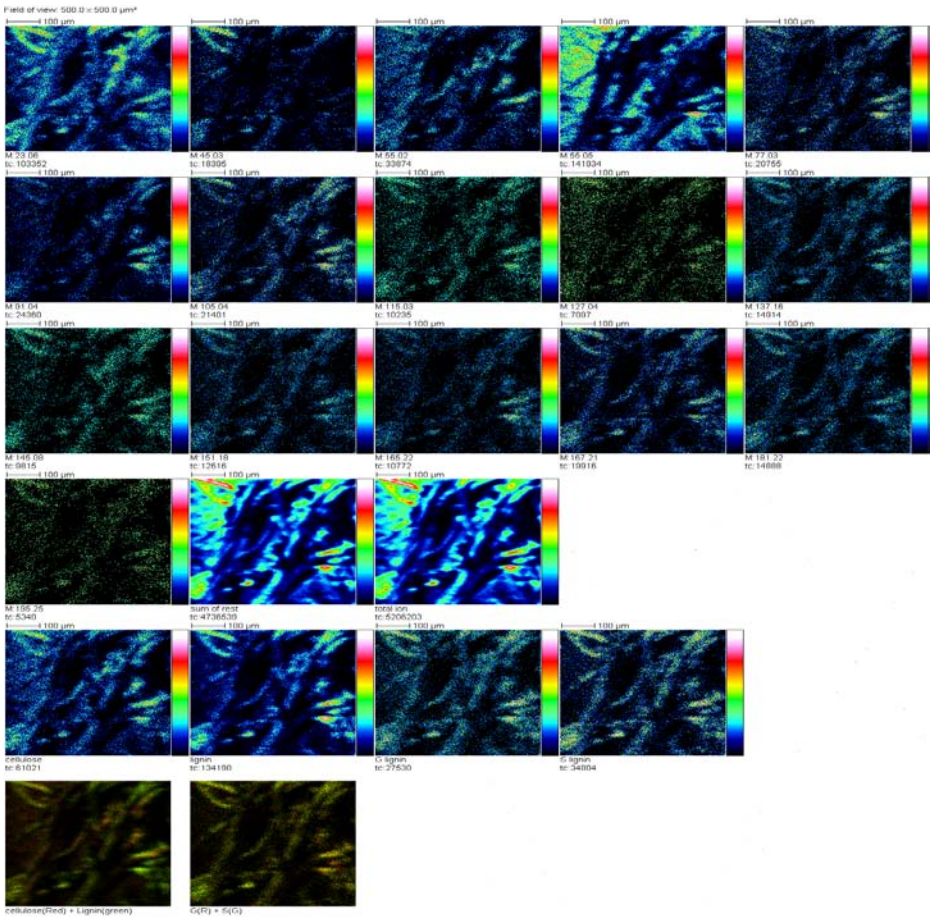

Figure 26. Imaging from a pulp sample pretreated with 16%TTA and 0%S

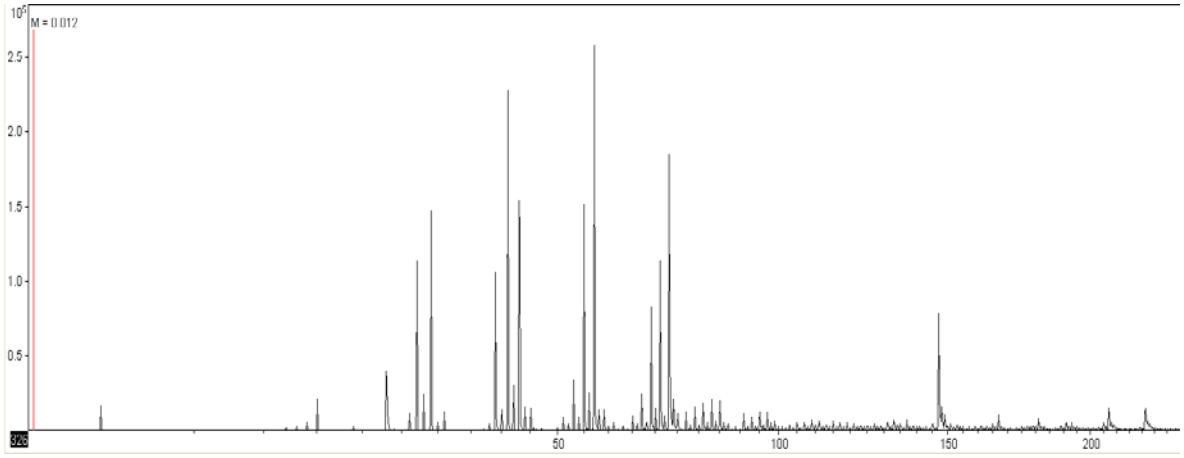

Figure 27. ToF SIMS total ion spectra from the pulp pretreated with 16%TTA and 37.5%S. Counts vs. mass (m/z)

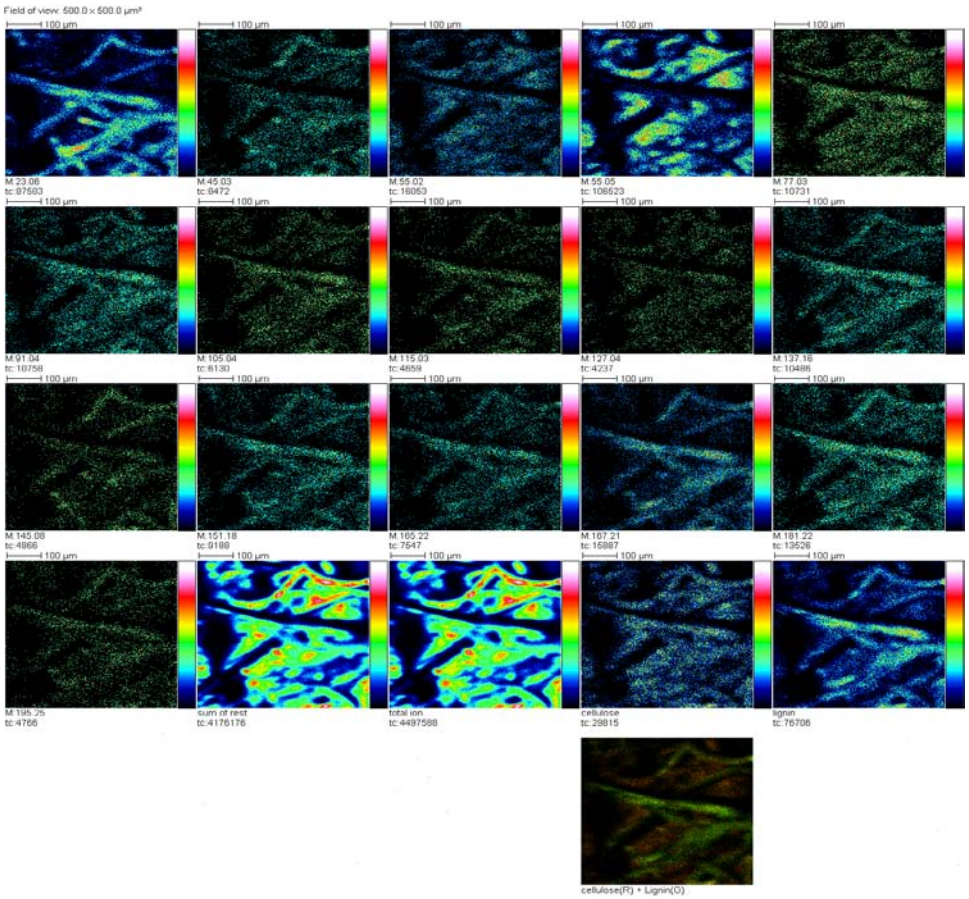

Figure 28. Imaging from a pulp sample pretreated with 16%TTA and 37.5%S

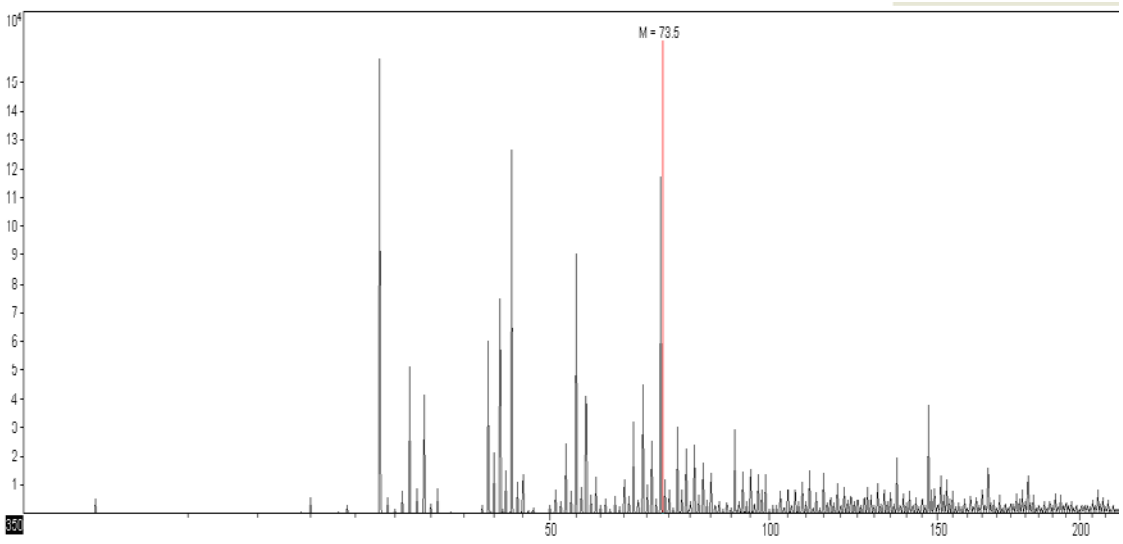

Figure 29. ToF SIMS total ion spectra from the pulp pretreated with 12%TTA,25%S, and 20%ethanol. Counts vs. mass (m/z)

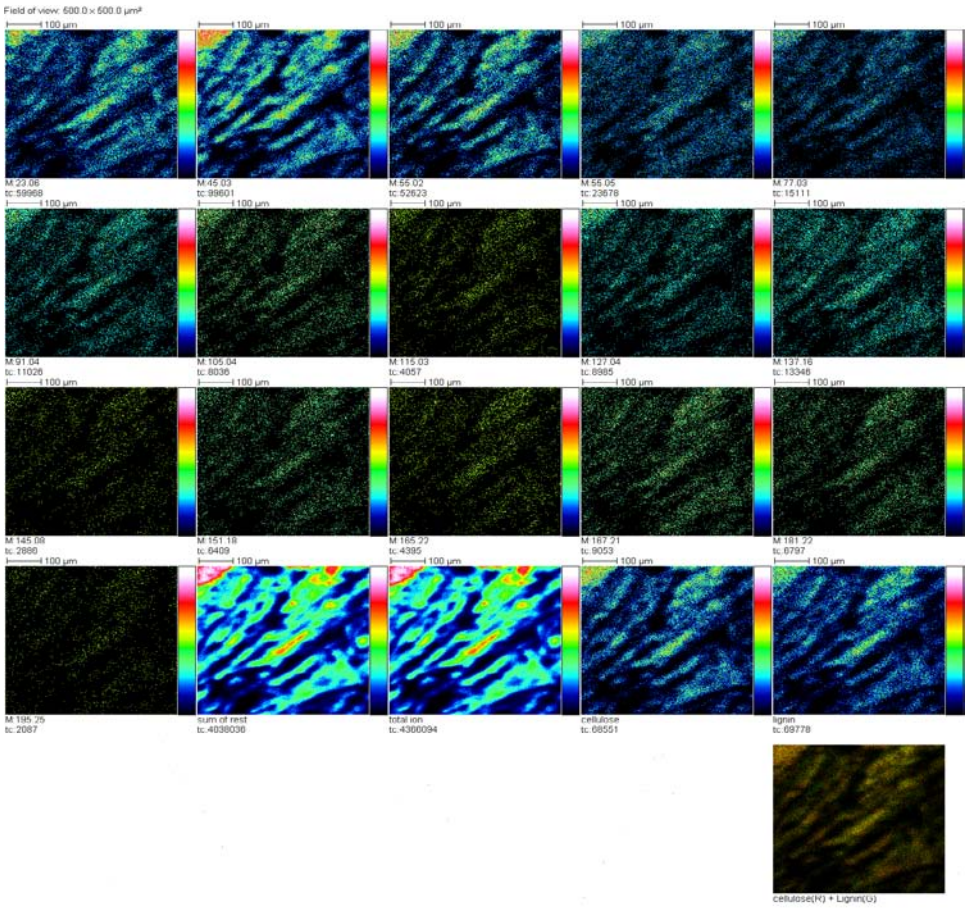

Figure 30. Imaging from a pulp sample pretreated with 12%TTA and 25%S, and 20% ethanol

The total ion image is the sum of all peaks in the spectrum of each sample analyzed.

Black/blue color indicates low intensity and red/white color indicates high intensity of ion signals. The color scale was normalized to the brightest pixel for each image. However, the signal intensity is not proportional to the amount of the species presented.

The selection of the peaks in the spectrum is based on the species presented according to **Table 13**.

Table 13. Mass/charge of some ions

Ion	Mass/ charge (m/z)
Cellulose	55,57,59
Carbohydrates	115, 127, 145
Aromatic	77, 91, 105, 119
G lignin	137, 151
S lignin	167, 181

The images show the localization and spatial distribution of lignin and carbohydrates in pulp tissues. It was possible to observe differences between lignin and carbohydrate distributions. But, the main objective of this analysis is to compare the ration between carbohydrates and lignin detected by using ToF-SIMS with the ones obtained by using Ion Exchange Chromatography (for sugar analysis) and Klason (for lignin analysis) experimental analysis. **Table 14** and **Table 15** shows the results obtained.

Table 14. Results of cellulose counts obtained by ToF-SIMS

Pretreatment	55	57	59	137	151	167	181	cellulose I (55+57+59)
0% S	26494	22174	19664	11243	9466	15292	11342	68332
	15373	13717	12776	8313	6074	12163	8155	41866
	16130	12418	14814	7976	6427	11592	8366	43362
37% S	14466	7710	9095	4705	4025	5239	5789	31271
	17931	11305	14160	5962	5102	9123	6338	43396
	18678	10227	10890	4620	3905	7837	5959	39795
25% S, 20%Eth	70013	24565	14194	5706	3908	6055	4486	108772
	65222	26789	14537	3877	3723	4203	8199	106548
	30363	14246	8742	2166	1675	2195	1467	53351

Table 15. Results of ratio between cellulose and lignin using ToF-SIMS

Pretreatment	lignin	C(I)/(C(I)+L)	average	standard dev	std%
16%TTA, 0% S	47343	0.6	0.6	0.02	4.0
	34705	0.5			
	34361	0.6			
16%TTA, 37%S	19758	0.6	0.6	0.01	2.3
	26525	0.6			
	22321	0.6			
16%TTA,25%S,20%ethanol	20155	0.8	0.9	0.02	2.3
	20002	0.8			
	7503	0.9			

Table 16. Composition of pulp obtained by Ion Exchange Chromatography

Pretreatment	cellulose	lignin	cellulose/(cellulose+lignin)
16%TTA, 0%S	55.0	22.6	0.7
16%TTA, 37.5%S	62.2	21.8	0.7
16%TTA, 25%S, 20%ethanol	64.5	20.0	0.8

Although quantitative analysis of ToF-SIMS data is not straightforward, the relative ion intensity can be used to determine the changes among different samples. It is noted that the values listed in **Table 15** are the relative ion intensities between cellulose and lignin. The general trend is that cellulose/ (cellulose+lignin) ion intensity ratio increases by increasing the sulfidity and also, by the addition of ethanol during pretreatment, in agreement with the trend obtained by Ion Exchange Chromatography method (**Table 16**). The comparison where made based on the surface of the pulps and the bulk analysis of the samples after pretreatment.

As it is possible to see, less lignin amount was detected on the surface of the pulp pretreated with ethanol compared with the results obtained by using Ion Exchange Chromatography. These differences can be explained due to lignin variations obtained for the effect of different pretreatments conditions and also for the difference on the sample (surface or bulk).

Green liquor seems to have an effect on the surface delignification that can possible explain the reduction of the barrier to allow enzymatic hydrolysis. It is important to mention that the results are depended on the sample surface that is going to be analyzed. So, different results can be obtained if the sample surface changes.

4.2.2 Gel Permeation Chromatography (GPC or SEC)

GPC was used to analyze and determine the average molecular weights distribution of lignin in pretreated wood by using an UV/vis absorbance detector and THF as the mobile phase. To obtain molecular weight (Mw) data, the samples were prepared as explained above in the experimental sample procedure.

For the lignin isolated in the black liquor after GL pretreatment, the molecular weight distribution is shown in **Figure 31**. The molecular weight was determined from the dissolved lignin obtained in the pretreatments using 16%TTA, 0%S, and 16%TTA, 37.5%S. The results are shown in **Table 17**.

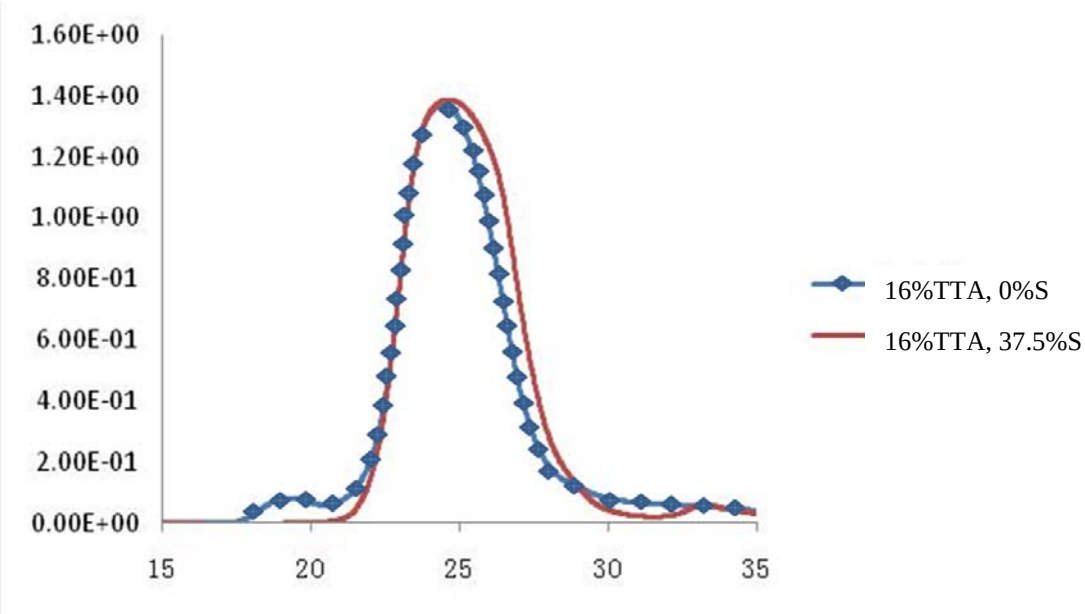

Figure 31. Molecular weight distribution of dissolved lignin
Relative intensity vs. elution time (min)

Table 17. Molecular weight of dissolved lignin samples

Sample	Mn	Mw	Mz	polydispersity (Mw/Mn)
16%TTA, 0%S	400	1500	4400	3.4
16%TTA, 37.5%S	500	1700	4600	3.0

The molecular weight of the dissolved samples remains almost the same as the sulfidity increases.

In the case of the pulp samples, the molecular weight distribution is shown in **Figure 32**. Residual lignin from the pulp pretreated at 16%TTA, 0%S and residual lignin from the pulp pretreated at 16%TTA, 37.5%S was determined. The molecular weight of the samples is summarized in **Table 18**.

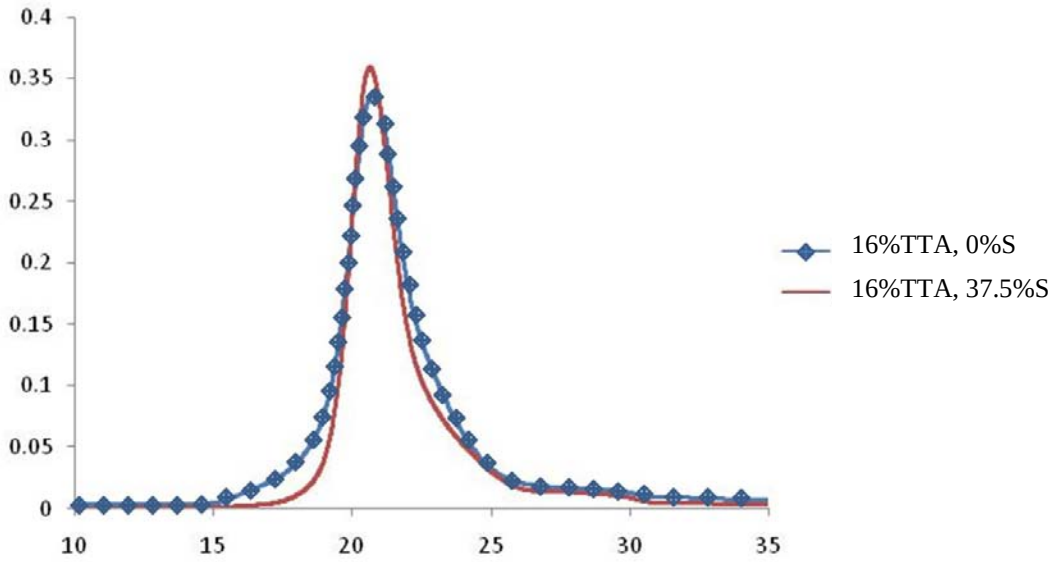

Figure 32. Molecular weight distribution of residual lignin
Relative intensity vs. elution time (min)

Table 18. Molecular weight (g/mol) of residual lignin samples in pulps

Sample	Mn	Mw	Mz	polydispersity (Mw/Mn)
16%TTA, 0%S	9000	560,000	37,000	61.8
16%TTA, 37.5%S	8000	84,000	42,000	9.4

From the residual lignin results it is possible to observe that the increase in sulfidity in the pretreatment produce a decrease in the molecular weight of the lignin (getting a Mw=84,000 for 37.5%S compared with a Mw=560,000 for 0%S).

The possible explanation is that sulfide ions degrades the lignin structure via the β-O-4 linkage and then leave small fractions in the pulp. Once the chain length of the residual lignin decreases, the molecular weight also reduces.

5. Economic Analysis

The goal of this economic analysis is to compare different pretreatment processes and identify the most economically viable. Comparison of costs based in different assumptions (**Table 20**) is explained. The model (**Figure 33**) was made in an Excel data sheet and the evaluation was focused only in the process conditions in the pretreatment and enzymatic hydrolysis that have been investigated during experimental studies. Changes in different pretreatment conditions (%TTA and %S) and enzyme dosages affected the overall process and with these data, the profit per liter of ethanol was determined. Energy loss was not considered in this analysis.

Table 19. Assumptions for the economic analysis

	Cost
Hardwood chips	$ 70 per BDt
Loss of sodium sulfide	$200 per BDt of sodium sulfide
Loss of sodium carbonate	$400 per BDt of sodium carbonate
Loss of ethanol	$ 0.65 per liter of ethanol
Fermentation chemicals	$0.08 per liter of ethanol
Sale of ethanol	$0.65 per liter

It was selected a base of 1BDt of wood. Cost of enzyme per liter of ethanol is assumed constant (0.08$/lt of ethanol) because there are great uncertainties associated with the cost of the enzymes.

Fermentation of five carbon sugars was assumed to be 70% and for six carbon sugars, 95%. Ethanol loss was varied using 2%, 10%, and 20%. The results obtained are shown in **Table 20, 21 and 22**.

As it is possible to see from the diagram in **Figure 33**, the model consists in thirteen unit operations. The first stage is wood preparation, were hardwood chips composition obtained by experimental analysis was included. Then, after cooking and washing, the composition of the pulp was incorporated in this stage. Following, the composition of the liquor analyzed by Ion Exchange Chromatography after enzymatic hydrolysis stage was included in the model.

Only with the experimental data of this three main stages and considering the pulp yield, it was possible to create a model that can give an idea for a bioethanol facility. Some of the economic drivers influenced by pretreatment are yield of both five and six carbons, cellulase activity, and enzyme loading.

For the calculation, the formulas used were:

$$tons\ of\ ethanol = \left[\left(\%glu\cos e + \%mannose\right) * 0.95 + \left(\%xylose * 0.70\right)\right] * 0.51$$

$$Kg\ of\ ethanol = 1000 * tons\ of\ ethanol$$

$$lt\ of\ \ ethanol = Kg\ of\ \ ethanol / 0.79$$

$$gallons\ of\ \ ethanol = lt\ of\ \ ethanol / 3.8$$

$$\$profit\ \ per\ liter = \$sale\ \ per\ liter - \$production\ costs$$

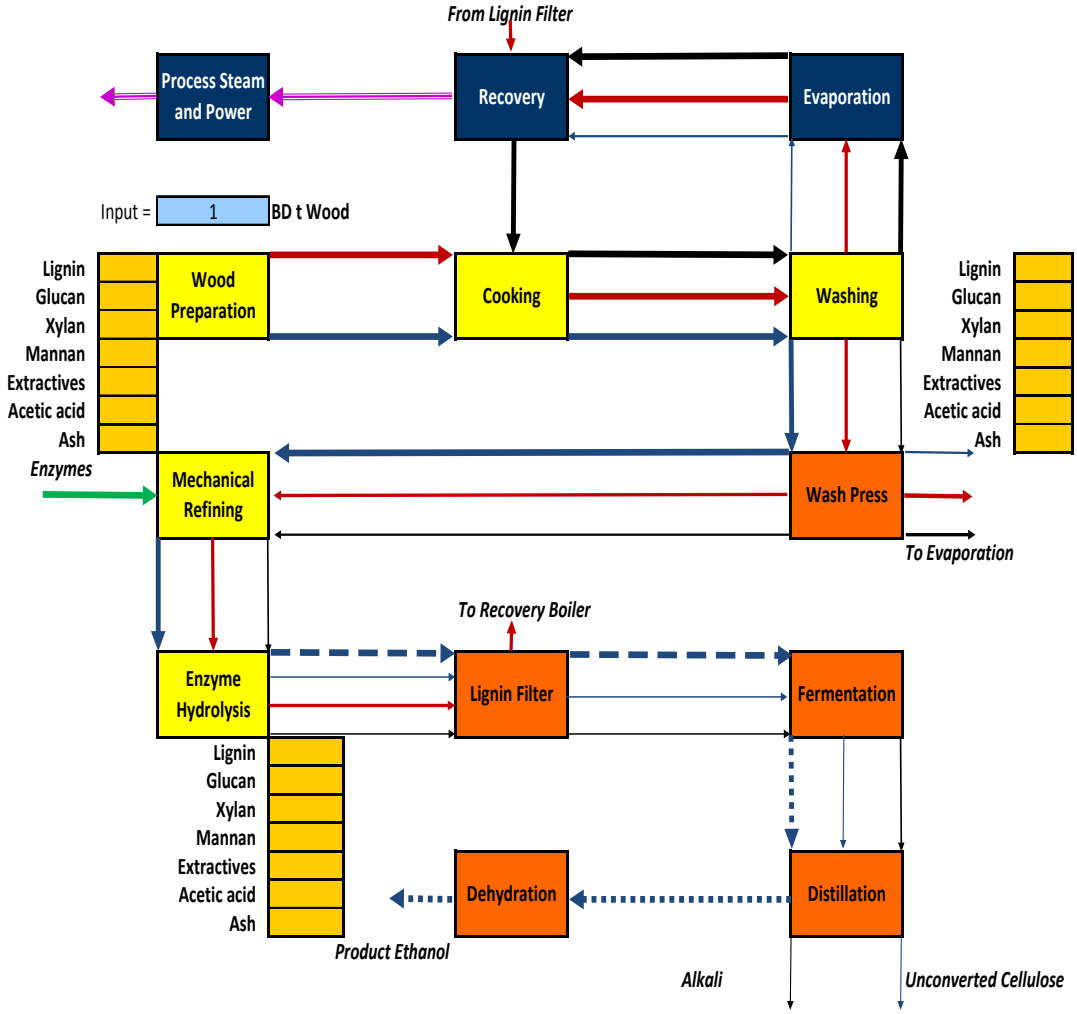

Figure 33. Model used for the economic analysis

The design generated is to differentiate between the economic performances of the different pretreatment options in this study.

Table 20. Results for economic analysis

Pretreatment	Gallons of ethanol	Production Costs, $	Sale per liter, $	Profit per liter, $
0, 16,5	27.8	0.84	0.65	-0.19
0,16,10	47.1	0.56	0.65	0.09
0,16,20	58.2	0.48	0.65	0.17
0,12,5	21.0	1.06	0.65	-0.41
0,12,10	30.3	0.78	0.65	-0.13
0,12,20	59.5	0.48	0.65	0.17
12.5,16,5	27.9	0.84	0.65	-0.19
12.5,16,10	56.6	0.49	0.65	0.16
12.5,16,20	75.4	0.41	0.65	0.24
12,5, 12, 5	22.9	0.98	0.65	-0.33
12,5, 12, 10	26.7	0.87	0.65	-0.22
12,5, 12, 20	41.0	0.62	0.65	0.03
25,16,5	40.4	0.63	0.65	0.02
25,16,10	65.5	0.45	0.65	0.20
25,16,20	78.2	0.40	0.65	0.25
25,12,5	32.7	0.74	0.65	-0.09
25,12,10	42.0	0.61	0.65	0.04
25,12,20	49.3	0.54	0.65	0.11
37.5,16,5	50.1	0.54	0.65	0.11
37.5,16,10	85.1	0.38	0.65	0.27
37.5,16,20	86.8	0.38	0.65	0.27
37.5,12,5	27.3	0.85	0.65	-0.20
37.5,12,10	44.4	0.58	0.65	0.07
37.5,12,20	66.3	0.44	0.65	0.21
				at 2% Ethanol Loss
5%eth, 12,25,5	58.4	0.49	0.65	0.16
5%eth, 12,25,10	78.6	0.41	0.65	0.24
5%eth, 12,25,20	90.7	0.37	0.65	0.28
10%eth, 12,25,5	56.3	0.52	0.65	0.13
10%eth, 12,25,10	77.2	0.42	0.65	0.23
10%eth, 12,25,20	85.9	0.39	0.65	0.26
20%eth, 12,25,5	56.1	0.54	0.65	0.11
20%eth, 12,25,10	76.2	0.44	0.65	0.21
20%eth, 12,25,20	81.6	0.42	0.65	0.23

Table 21. Results obtained using 10% ethanol loss

Pretreatment	Gallons of ethanol	Production Costs, $	Sale per liter, $	Profit per liter, $
				at 10% Ethanol Loss
5%eth, 12,25,5	58.4	0.54	0.65	0.11
5%eth, 12,25,10	78.6	0.44	0.65	0.21
5%eth, 12,25,20	90.7	0.40	0.65	0.25
10%eth, 12,25,5	56.3	0.62	0.65	0.03
10%eth, 12,25,10	77.2	0.49	0.65	0.16
10%eth, 12,25,20	85.9	0.46	0.65	0.19
20%eth, 12,25,5	56.1	0.74	0.65	-0.09
20%eth, 12,25,10	76.2	0.59	0.65	0.06
20%eth, 12,25,20	81.6	0.56	0.65	0.09

Table 22. Results obtained using 20% ethanol loss

Pretreatment	Gallons of ethanol	Production Costs, $	Sale per liter, $	Profit per liter, $
				at 20% Ethanol Loss
5%eth, 12,25,5	58.4	0.60	0.65	0.05
5%eth, 12,25,10	78.6	0.49	0.65	0.16
5%eth, 12,25,20	90.7	0.44	0.65	0.21
10%eth, 12,25,5	56.3	0.74	0.65	-0.09
10%eth, 12,25,10	77.2	0.58	0.65	0.07
10%eth, 12,25,20	85.9	0.54	0.65	0.11
20%eth, 12,25,5	56.1	0.98	0.65	-0.33
20%eth, 12,25,10	76.2	0.77	0.65	-0.12
20%eth, 12,25,20	81.6	0.73	0.65	-0.08

The economic analysis described above, based on experimental results, is a useful and easy way for comparing different pretreatment process scenarios, and to quantify the production and profit costs when some parameters are changed. The results shown that sulfidity plays an important role in the ethanol production, obtaining for the case without ethanol with 37.5%S and 16%TTA a profit of $0.27 per liter of ethanol. All cases with 37.5% Sulfidity are better than the corresponding cases at lower sulfidity. Higher sulfidity

guarantee a better production and profit. Also, all cases that use 20 FPU/g pulp are better than cases that use fewer enzymes.

In the case of the samples pretreated with ethanol, the general trend is that the increase in enzyme dosage gives better results and that all ethanol cases are among the best, when we use 20 FPU/g pulp. Efficiency of recovery of ethanol is critical to financial results as is observed in **Table 20, 21 and 22**. By increasing the ethanol loss from 2% to 20% the profit started to decrease abruptly and it is not economically viable compared with the cases without ethanol. So, the use of ethanol in the pretreatment will not bring any benefits if the ethanol loss starts to increase.

6. Conclusions

Pretreatment is needed to disrupt the crystalline structure of cellulose and allow enzymes to hydrolyze carbohydrates into fermentable sugars. Many low-cost pretreatment approaches have been tried. In general, physical pretreatment has low yields and high costs. Chemical pretreatment appears to be more economically feasible but with some limitation based on the selection of the chemical and the type of feedstock used.

Different sulfidities were studied to determine its effect in delignification and carbohydrates yield using Green Liquor pretreatment. The results obtained strongly supports that treatment with high sulfidity content enhances the hydrolytic ability of enzymes by providing easier access to the cellulose polymer matrix due to the removal of lignin. Also, it was found out that higher concentration of enzymes hydrolyze more cellulose substrate.

Green Liquor pretreatment is a highly selective pretreatment methodology for wood that maximizes the overall final yield of hydrolysable cellulose, while decreasing the levels of lignin which is a barrier to its accessibility. At 37.5%S and 16%TTA was reached 5.6% of lignin removal and a carbohydrate yield of 70.6% at 20 FPU/g pulp.

Organosolv pretreatment (green liquor with ethanol) protects cellulose during delignification. It was possible to observe that higher carbohydrates yields were obtained by using higher ethanol concentration in the green liquor pretreatment. At 20% ethanol and 20 FPU/g pulp, was reached 13.4% of lignin removal and a carbohydrate yield of 75.7%

ToF-SIMS technique was used to detect the changes in cellulose/lignin composition. The results show that with 20%ethanol, the cellulose / lignin fraction is 0.85, which is higher that the fraction obtained using Ion Exchange Chromatography (0.76). This suggests that the increase in sulfidity and also the addition of ethanol during pretreatment generates a decrease in lignin content on the surface of the pulps analyzed. So, it seems that the delignification is more pronounced on the surface.

Studies on the molecular weight distribution of lignin in two pretreated samples (16%TTA with 0%S and 37.5%S) using size exclusion chromatography confirm that an increase in sulfidity produces a decrease in the molecular weight of residual lignin due to cleavage of lignin in the β-O-4 linkage causing a decrease in the chain length.

An economic analysis was elaborated from the experimental data obtained on different pretreatment processes. Economic analysis has the advantage of identify the process economic impact of the different pretreatment approaches to see the possibility of develop a new method in the ethanol production.

The goal of these economic calculations was to have an idea about the production and profit costs. The results of this study make us have a direction about the economic drivers that must be manipulated and that influenced the pretreatment method (carbohydrates yield, enzyme activity, sulfidity in the pretreatment step). Higher sulfidity always shown better profit per liter compared with lower sulfidity. Also, higher enzyme activity resulted to be more economically viable, but just based in a fixed price for the enzymes. In the case of samples pretreated with ethanol, the profit was even better than without ethanol. However, the calculations were based in many assumptions like the percentage of ethanol loss during the process. The increase in ethanol loss resulted in a decrease in the profit that cannot be economically viable as an alternative for ethanol production.

Because an effective pretreatment should result in higher recovery of carbohydrates and high digestibility of cellulose, it is possible to conclude from the results that a GL pretreatment with a chemical composition of 16% TTA, 37.5% S, and with an enzyme activity of 20 FPU/g pulp represents a very attractive first step process toward biofuels production based on the carbohydrates yield and the economic profit.

7. Future work

More research is needed to achieve a better understanding how delignification occurs by using Green Liquor pretreatment specifically with respect to sulfidity in the solution. Below are some suggestions for future research topics:

ToF-SIMS technique in the study of sample surface needs to have more experimentation to clarify the effect of sulfidity on the surface lignin. The effect of sample preparation and compression needs to be better analyzed.

The effect of sulfidity on the ultrastructure of wood can be explained by studying the capillary structure of the chips to understand the liquid penetration process. Also, quantitative information of the substances removed during the pretreatment can equate to a better comprehension of the overall process. The mechanism of sulfide reactivity during the GL pretreatment still requires further investigation.

More experimental data are needed to clarify the basics of the phenomena of Green Liquor penetration into wood chips. The behavior of ethanol in the green liquor needs to also be explored in more detail.

Finally, to study the by-products obtained after pretreatment and see how they contribute to the overall economics and efficiency of the pretreatment process.

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