ANALYSIS OF HYDROLYSIS YIELDS BY USING DIFFERENT ACIDS FOR BIOETHANOL PRODUCTION FROM BRAZILIAN WOODS

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In this work, the wood’s hydrolysis yields using different mineral acids H2SO4, HNO3, H3PO4, and CH3COOH were analyzed in order to identify the better for ethanol production levels. Twelve Brazilian biomasses, including native and exotic species in the form of wood chips were prepared to produce ethanol and cellulosic fibers simultaneously. For fermentation, a savage line of Saccharomyces cerevisiae strain was chosen. Income related to hydrolysis efficiency, ethanol yields, fibrous fractions efficiency, starch and glucose levels plus delignification efficiency were tested and proved in laboratory. From the results it is possible to join the production of ethanol and cellulosic fibers simultaneously from wood chips, and because of the complexity of the material used as samples is possible to isolated several chemical derivatives that can be developed and contribute to enrichment of further studies. The acids tested showed to be potentially utilized in hydrolysis processes as well.

Palavras-chaves: Acid hydrolysis, Ethanol production, Wood.
1. Introduction

There is a considerable potential to modernize the use of biomass fuels to produce convenient energy vectors such as electricity, gas and automotive fuels, while the traditional uses of biomass is preserved. When produced in sufficient and sustainable way energy from biomass brings many environmental and social benefits compared with other fossil fuels. Some geopolitical factors related to the security of oil supply, high prices and serious environmental concerns, like global warming, are essential to sustainability through alternative fuels such as ethanol (COPOLLA et al. 2009). Using ethanol instead of petroleum derivatives is advantageous in many aspects (KRISHNA et al., 2001 and ZHU et al. 2010). The development of new technologies for treatment of lignocellulose materials for ethanol production is well regarded by the world, both economically and environmentally, as compared to fossil fuel production.

Today, ethanol is one of the most important alternative fuels that can be obtained through the fermentation of sugars using a biological catalyst, such as yeast. Processes for producing ethanol using yeast have been extensively investigated. In an attempt to minimize the production costs, the use of cellulosic materials, which includes agro-industrial residues and wood waste, appears as important source of carbohydrates for fermentation of biomass (SUN, 2002 and CHENG, 2004).

Wood is a complex substance of polymeric character. Some 40% to 50% by weight of dry wood consists of cellulose, which is a fiber of great commercial value. The cell walls consist of polysaccharides, lignin and holocellulose. The holocellulose is a mixture of short-chain polymers such as arabinose (C₅H₁₀O₅), galactose (C₆H₁₂O₆), glucose (C₆H₁₂O₆), mannose (C₆H₁₂O₆) and xylose (C₅H₁₀O₅). Lignin is a complex polymer of substituted phenols that act as ligands of cellulose fibers (FENGEL and WEGENER, 1989). Cellulose can be hydrolyzed by the action of mineral acids, unfolding into glucose. This glucose can be fermented by yeast to produce alcohol. In nature, these processes are carried out by fungi and bacteria, which secrete enzymes that break down the cellulose molecules, which are called cellulases, and also by yeasts that ferment sugars into alcohol. The technological process of producing ethanol from cellulosic material can be schematically divided and three stages: extraction of sugars from the raw material, fermentation of sugars, distillation and rectification of fermented alcohol contained in the fermented wine. In most cases a pre-saccharification hydrolysis is included in the procedure to remove the more easily hydrolysable polyoses before the main hydrolysis of cellulose. The greatest potential for utilization of glucose is fermented to produce ethanol, but other valuable products can also be derived from glucose wood (LEWIN; GOLDSTEIN, 1999; MARTÍN et al. 2003).

All natural lignocellulosic material and most refined must be submitted to a pretreatment which cellulosic biomass becomes susceptible to hydrolytic enzymes. Generally, the yield of hydrolysis, without the phase of pre-treatment, 20% of theoretical yield, while the yield after pre-treatment is often greater than 90% of theoretical yield. It is assumed that the limited effectiveness of current enzymatic processes with soft wood is due to the relative difficulty of performing the pre-treatment of these materials. A study was conducted on the pre-treatment (MCMILLAN, 1994 and YANG et al 2006). The hydrothermal treatment using
water at temperatures of 170 °C to 220 °C has been applied for pretreatment of lignocellulose material aimed at the solubilization of lignin, hemicellulose and providing these polymers for subsequent enzyme treatment leading to production of glucose and other saccharides that may be used in bioprocesses of commercial interest (CHUM et al., 1990; ZHU et al. 2010).

Hydrolysis of cellulosic biomass in mineral acid is one of the major methods employed to separate lignin and monosaccharides from lignocellulosic biomass, however, this process is strongly dependent on the acid concentration and temperature. It has been reported that at low mineral acid concentrations, high temperature is required to achieve significant hydrolysis, thus requiring high energy loading. Although a high mineral acid concentration can significantly reduce temperature or the energy requirement, higher than 20% concentration of mineral acids would be needed. The process does not provide a promise for substantial industrial gains (Sasaki et al., 1998 and Iranmahboob et al., 2002; and Kakanson, Ahlgren, 2005) because of the difficulties in reusing and recycling the mineral acid. Therefore, establishment of an effective and environmentally benign hydrolysis process is of great significance to the conversion and the utilization of lignocellulosic biomass.

During hydrolysis, however, are toxic compounds produced derivatives of furfur (2-furaldehyde and 5-hydroxymethyl-2-furaldehyde), degraded phenolic compounds and organic acids that can often inhibit or undesirably affect the performance of biocatalysts in bioprocesses on interfering ferment ability when in concentrations above 1% in weight (WOICIECHOWSKI et al., 2000). The control of the hydrolysis process aims to minimize the concentrations of toxic compounds, currently; different treatments are being used for improving the capacity of fermentation of hemicellulose hydrolyses (PALMQVIST et al., 1999, CARDONA et al., 2010; XAVIER et al., 2010; ZHU et al., 2010).

The aim of this study was investigate the hydrolysis yields using mineral acids: sulfuric (H₂SO₄), nitric (HNO₃), phosphoric (H₃PO₄), and acetic (CH₃COOH), in order to find which hydrolysis process is more advantageous for Brazilian wood. Also, a theoretical projection was carried out to determine the ethanol yield produced on liters per 100 kg of biomass, as well as the delignification and samples fibrous fractions efficiency. The samples used in this paper consisted in chips from twelve Brazilian species of wood, among them some native and exotic species in the form of wood chips and sawdust. These biomass were utilized because they are considerable of low commercial value and currently burned in boilers to steam generation and power cogeneration. This research contributes by generating new information about the performance of acid hydrolysis of Brazilian wood species that had no been until now studied, which is important to identify new biomass and new acids for hydrolysis with potential for ethanol production.

2. Materials and Methods

2.1 Raw materials

The lignocellulosic waste used in this study was supplied by furniture companies of the city of São Bento do Sul, localized in Santa Catarina State. The raw material consists of chips and sawdust obtained directly from the industrial process. The samples analyzed consisted of particles with 0.2 to 2.7 mm of diameter, (90% or more particles pass through a
40 mm sieve). All lignocellulosic materials used in this research were collected in the moment that the timber passed through the machining process. The wood chips were packed in containers of 5 kg each, and then stored. The chips selected for testing were those which passed through 40 mesh sieve mesh.

The samples were obtained from twelve different species of trees native and exotic species, including: *Hymenolobium petraeum*, *Tabebuia cassinoides*, *Myroxylon peruiferum*, *Nectandra lanceolata*, *Ocotea catharinensis*, *Cedrela catenaeformis*, *Cedrela fissilis* Vell., *Ocotea porosa*, *Laurus nobilis*, *Balfourodendron riedelianum*, *Pinus Elliottii*, and *Paulownia imperialis*.

### 2.2. Chemicals

The chemical used in this work are all from commercial sources. In this research was used acetic acid (CH$_3$COOH), nitric acid (HNO$_3$), sulfuric acid (H$_2$SO$_4$) and phosphoric acid (H$_3$PO$_4$). The intention of use different acids is to identify which acid one can perform the most efficient hydrolysis action on wood particles, having higher affinity to break cellulose and starch present in the samples into d-glucose, for subsequent fermentation.

### 2.3 Yeast

For this study the yeast utilized was savage *Saccharomyces cerevisiae* strain line, cultivated in microbiology laboratory. This yeast was chosen because it is a microorganism widely used today in the production of ethanol and biotechnological processes. The yeast was cultivated in agar-malt slant. The agar slant consisted of malt extract (3 g/L), yeast extract (3 g/L), peptone (5 g/L), agar (20 g/L) and distilled water (up to 1 L). Before use as an inoculum for the fermentation, the culture was aerobically propagated in 500 ml flasks in a shaking bath at 30 °C for 48 h and then separated by centrifugation. The liquid consisted of yeast extract (3 g/L) glucose (10 g/L) and distilled water.

### 2.4 Determination of d-glucose

The samples were sieved in (20 mesh) in an amount of 10g each, and then subjected to a treatment. In this treatment it was used 150 ml distilled water (H$_2$O) at concentration of dilute sulfuric acid 1:3 (volume-based) after this submitted to reaction time for 2 h using a heating plate. The relationship between dry weight and volume of sample solution (1:30) were established on the preliminary studies cited by Yang 2006, which sought to preserve the maximum integrity of shredded wood chips. After the treatment applied to chips with shredded diluted acidic solution was determined the hydrolysis fraction which corresponds to the glucose concentration (cellulose, starch and glucose hydrolyzed). This d-glucose sugar was identified using a blank test by replacing the acid solution diluted by distilled water.

### 2.5 Efficiency of fibers and delignification

The fibrous fraction of lignocellulosic material is the result of the sum of lignin and holocellulose (composed of cellulose and hemicelluloses). The cellulosic fibers are feasible for manufacture of paper and it’s possible to obtain fibrous fractions efficiency after acid or alkaline delignification (pretreatment). In acid ways the samples were treated with a solution
composed of glacial acetic acid (50%), hydrogen peroxide (40%) and distilled water. The alkaline treatment was a solution of sodium hydroxide (NaOH) at 5% concentration. Both treatments were kept in water bath until complete individualization of cellulosic fibers and other anatomical elements. Then, the cellulose fibers were washed on fine-mesh sieve (20 meshes) and dried at 105 ± 3 °C until constant weight. The ratio between the dry weight of cellulosic fibers and dry weight of initial sample of shredded wood chips provided the yield on cellulosic fibers, according to methodology adapted by Krishna et al. (2001)

The procedure for determining the cellulose fibers consist in taking 10.0 g of samples before hydrolysis occurs and applying to it a solution of 100 mL of H₂O and acetic acid (CH₃COOH), diluted to 72% and 2% hydrogen peroxide (H₂O₂, 40 volumes). The proceedings were kept in water bath for 1h at 70 °C ± 5 and washed exhaustively with H₂O and dried in electric oven at 105 °C.

The efficiency of cellulosic fibers was calculated from the formula: (1)

(1) Efficiency of cellulosic fibers (%) = Initial weight - Final weight × 100

Initial weight

2.6 Hydrolysis and fermentation process

The hydrolysis process was carried out in stainless steel autoclave with a total capacity of 20 liters at a pressure of 7 kg/cm². Operating parameters were used in other research work to carry out this process according to Taherzadeh (2001). Added to the amount of 150 g of sawdust each sample timber separately and 400 mL of distilled water in acid concentrations of 82%. After this process the samples received a suspension of calcium carbonate (CaCO₃) to stabilize the pH between 6.0 and 7.0 and were kept on stirring for a period of 2 hours. After this process, the liquid was filtered and then received a further addition of acid (nitric, sulfuric acetic or phosphoric) until the pH was lowered to 5.0 this procedure consisted of hydrolysis of the material. After 2h, the solution was filtered again on paper filters to remove small particles. These hydrolyzed samples were subjected to fermentation.

The efficiency of the hydrolysis process was defined according to the methodology applied by Taherzadeh (2001) and is determined as the percentage of starch which was removed from the chips and turned into glucose. Was considered the conversion factor (100%), assuming that 100.0 g of starch produces 100.0 g of D-glucose.

The formula used was defined as: (2)

(2) Hydrolysis Efficiency (%) = D - Glucose concentration after hydrolysis

Cellulosic concentration in samples

After the hydrolysis 10.0 g of biomass samples were fermented using twelve Erlenmeyer’s with capacity of 200 ml in anaerobically conditions. The samples were inoculated with approximately 10 g/L of strain savage line of Saccharomyces cerevisiae strain colony formed and 100 mL of pure distilled water. The samples are submitted to fermentation during 48 hours anaerobically. For distillations was used a mini-scale distillatory device which one yielded 10 mL of ethanol per sample After produced 1.000 mL of alcohol, using
an Alcoholic Strength Table (ratio temperature and density of the mixture) passing 30 minutes for ethanol stabilization in alcoholometer of Gay-Lussac, was founded the alcohol 70° g/L content of the prepared mixture. As this minimum quantity of produced ethanol considerate not sufficient for industrial production, the ethanol levels by samples were estimated in calculus by Liters per 100Kg of biomass, as explained in the next topic.

2.7 Estimative of ethanol efficiency

For the estimative calculus of ethanol yields in liters per 100 kg of samples the results can be obtained by multiplying the concentration of fermentable glucose by the conversion factor from glucose to ethanol (0.56) according to Sun et al. 2002. This factor was obtained by considering the normal levels of fermentation (90%), distillation (95%), density of ethanol at 25 °C (0, 785 g / cm³) and the molecular weight of glucose (180 g) and ethanol. Therefore, the results obtained by calculus in this search are considerate as potential estimative of fermentable glucose conversion in ethanol considering that only thru chromatographic and spectrometry methods and techniques is possible to obtain the results of ethanol concentrations.

3. Results and Discussion

3.1 Delignification and fibers efficiency

The delignification and the fiber efficiency obtained for the twelve species are showed in Figure 1.

Figure 1: Delignification and cellulosic fibers efficiency by species.

Lignin is one of the major components in lignocellulosic material; however, the amount of produced lignin by the industry could be greater according to Oliet et al (2001).

A study on the yield and structure changes of lignin from the promising acids hydrolysis process is necessary (XU, 2006).
The means of delignification and fiber efficiency were of improve the ethanol yields and use the fibers commercially, respectively, which was as expected for wood (Zhang et al, 2010). As can be observed, the fiber yield and the delignification efficiency are related, with some exceptions.

Lignin represents one of the main obstacles to using lignocellulosic materials in biotechnological applications based on biomass cellulose. It is generally accepted that cellulosates absorb irreversibly to the lignin in lignocellulosic materials, thus decreasing the hydrolysis efficiency (STUTCLIFFE, 1986; TATSUMOTO, 1988). The efficiency of cellulotic fibers is very important for its use in composite materials and material made of wood fibers which are mostly used in civil construction and other diversified applications. As showed in Figure 1, it is possible to save cellulotic fibers. Lignin previously removed can increase the ethanol production because lignin acts as a fermenter inhibitor for S. cerevisiae. The use of delignification before fermentation is very important to achieve better results on ethanol production.

Conversion of biomass into high-valued chemicals has also attracted significant attention. Lignin can be converted to valuable products, such as carbon fiber, phenols and adhesives (Uraki et al., 1997) and (Pan and Sano, 2000).

Non-cellulose fractions such as lignin and hemicelluloses (accounting for 50–55% of dry weight of wood and straw) are only burned to produce process energy (Vila et al., 2003).

From the results obtained delignification levels using acetic acid and hydrogen peroxide as explained in session 2.5 showed good yields of delignification in a rate of 70,0% to 93,33%.

The efficiency of cellulosic fibers produced stay between 70,0% and 97,40% demonstrating high levels stimulating delignification pretreatment for use of fibers.

The amounts of glucose and hydrolyzed cellulose and starch obtained and hydrolysis efficiency by each one of used acids in this research are illustrated in Figures 2 to 5. Starch is naturally present in lignocellulosic material intermolecular composition. As explained before, the hydrolysis is the transformation of cellulose and starch into D-glucose for subsequent fermentation to ethanol. There is a direct proportion relationship between starch, cellulose and ethanol: the greater the amount of fermentable (convertible) starch and cellulose, greater the volume of alcohol produced. The results will be commented after the figures.
Figure 2: Hydrolysis efficiency using CH₃COOH.

Figure 3: Hydrolysis efficiency using HNO₃.
Dilute acid hydrolysis can convert wood hemicellulose extract to monomeric sugars. The resulting hydrolyzate is rich in monomeric sugars, especially in xylose. After a certain process of centrifuge – neutralization – centrifuge and membrane filtration, those solid and fermentation inhibitors can be nearly eliminated and then the hydrolyzate can be used as fermentable media to yield bio-ethanol and other valuable products (Liu et al., 2009).

In literature the most used for hydrolysis is sulfuric diluted acid at (82%) Ruofei (2010). But the price of acid elevates the process costs and also sulfuric acid demands a
expansive anti-corrosion equipment (IRANMAHBOOB et al., 2002).

The use of different acids can provide practically the same or better hydrolysis results in substitute to sulfuric acid.

The concentrations are according to Iranmahboob et al. (2002) and Wooley et al. (1999) using a diluted sulfuric acid hydrolysis for mix wood biomass.

According the results, the most efficient species in hydrolysis levels using acetic acid were: Paulownia imperialis 32.62%, Hymenolobium petraeum 31.35%, and Ocotea catharinensis 27.55%. The hydrolysis efficiency levels was maintained between 21.47% and 32.62%, D-glucose rates between 11.20% and 17.20% and hydrolyzed cellulose between 10.17% and 17.20%. Acetic acid provides good rates of hydrolysis transformation.

Laurus nobilis 33.44% and Balfourodendron riedelianum 32.49% followed by Hymenolobium petraeum 28.50% are the species most efficient hydrolyzed using nitric acid (HNO₃). D-glucose rates between 10.0% and 15.0% and hydrolyzed cellulose and starch between 9.0% and 15.85%. Nitric acid also showed good rates of hydrolysis transformation.

In sulfuric acid hydrolysis the most efficient species are: Balfourodendron riedelianum 27.03%, Nectandra lanceolata 26.06% and Myroxylon peruiferum 24.07%. The hydrolyzed cellulose and starch levels are among 5.4% and 12.9%, D-glucose levels among 6% and 14.4%. Lowest rates of hydrolysis transformation were demonstrated using sulfuric acid.

On the other hand, the species which demonstrated the largest yields of D-glucose using all acids were: Myroxylon peruiferum, Nectandra lanceolata and Balfourodendron riedelianum.

Figure 5 shows the more efficient species to obtain higher rates of hydrolysis by using phosphoric acid (H₃PO₄), between them: Balfourodendron riedelianum 26.79% Paulownia imperialis 24.89%, Nectandra lanceolata 24.70%, this means that the starch and cellulose present in these specimens was transformed successfully into D-glucose. Hydrolyzed cellulose and starch rates are between 8.10% and 12.69% and D-glucose rates 9.0% and 14.10%. Phosphoric acid showed good rates in hydrolysis efficiency.

All acids tested showed good levels of hydrolysis efficiency. The result’s obtained of hydrolysis efficiency remained according to the literature for some hardwoods and softwoods and also for some tested acids.

Starting for the determination of starch and cellulose levels in hydrolysis and glucose levels for species presented in Figures 2 to 5, according to the main results, it’s possible now to calculate the estimate capacity of ethanol production for species in different types of acid used.

3.2 Estimation of ethanol production capacity by species

The Figures 6 to 9 shows the estimate production of ethanol yields by using acetic, nitric, phosphoric and sulfuric acids. In this work, hydrolysis, fermentation and sequent distillation processes were performed in laboratorial scale and resulting in minimum quantities. The calculus of the estimate ethanol production given in liters per 100 kg of
biomass was explained in item 2.7. The results will be discussed after the graphics.

**Figure 6:** Estimation of ethanol production capacity by species in CH$_3$COOH.

**Figure 7:** Estimate capacity of ethanol production by species in HNO$_3$.

**Figure 8:** Estimate capacity of ethanol production by species in H$_3$PO$_4$. 
According to the results of acid hydrolysis performed using acetic acid, the *Hymenolobium petraeum* 9.24 liters, *Ocotea catharinensis* 8, 12 liters and *Paulownia imperialis* with 9, 63 liters which one demonstrate the best result in ethanol levels (Figure 6). The ethanol rates stay between 6,32 L/100 kg and 9,63 L/100 kg of biomass.

Hydrolysis performed using nitric acid, showed good results due to HNO₃ reaction and affinity to breaking cellulose into D-glucose, the specimens: *Hymenolobium petraeum, Laurus nobilis* and *Balfourodendron riedelianum* excelled showing yields of hydrolysis sometimes even better using sulfuric (H₂SO₄) and hydrochloric acid (HCl) as showed in Figure 7. The ethanol rates stay between 5,60 L/100 kg and 9,85 L/100 kg of biomass. *Balfourodendron riedelianum, Paulownia imperialis* and *Tabebuia cassinoides* were the efficient species in estimate capacity of ethanol production using phosphoric acid H₃PO₄ (Figure 8). The ethanol rates stay between 5,04 L/100 kg and 7,89 L/100 kg of biomass.

Using sulfuric acid H₂SO₄, *Balfourodendron riedelianum, Nectandra lanceolata,* and *Myroxylon peruiferum* are the most efficient producers of ethanol (Figure 9). The ethanol rates stay between 6 L/100 kg and 12,9 L/100 kg of used biomass.

4. Conclusion

In this study was verified that the acid hydrolysis represent an interesting alternative for production of both, energy and chemical compounds from Brazilian wood wastes. Results showed that it is possible to produce ethanol from lignocellulosic waste and to maintain the wood’s fibrous fractions simultaneously. *Saccharomyces cerevisiae* yeast showed to be very efficient in production of ethanol, with an estimate maximum production of 12, 94 Liters per 100 kg of biomass using *Balfourodendron riedelianum* specimen and sulfuric acid. In general, all tested acids demonstrate great and similar efficiency in breaking the starch and cellulose into D-glucose molecules, indicating that these acids are all feasible for use in hydrolysis process. The distillatory process occurred in laboratorial scale and due to the minimum quantities of ethanol produced was necessary to use calculus to estimate a large scale of ethanol production. The delignification levels using acetic acid and hydrogen peroxide are feasible for lignin production to generate energy and for high cellulosic fibers efficiency.
yields, which can be used technologically. So, the most efficient lignocellulosic specimens tested in estimation ethanol production were *Hymenolobium petraeum*, *Laurus nobilis* and *Balfouriodendron riedelianum*, all of them are very promissory specimens if used as biomasses for ethanol conversion. All acids also showed to be potential used for biomass hydrolysis.

6. References


