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Optimal delignification conditions of *Pinus patula* sawdust as a crucial stage in obtaining bioetanol

Condiciones óptimas de deslignificación del aserrín de *Pinus patula* como etapa crucial en la obtención de bioetanol

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Abstract

The growing of the transport sector in demand for energy highlights the need to take action to improve efficiency and develop energy alternatives. Much of the materials with high cellulose content are likely to be used as raw material for the production of biofuels. They are generated as waste in productive processes of crops, forestry, and industrial sectors. The residue used in this case comes from the species of *Pinus patula*. It is a prior delignification stage crucial to obtain bioethanol, by allowing access to the cellulose and achieve transformation. In this work, we studied the best conditions to achieve the delignification of *Pinus patula* sawdust cultivated in the department of Cauca reaching the best conditions of delignification using 12 % sodium hydroxide, a reaction time of 60 min using 30 mesh (600 µm), which yielded percentages of cellulose between 63 and 72 % and rates of samples hydrolyzed between 10 and 15 %.

Keywords: sawdust; lignin; *pinus patula*; bioethanol; delignification; pretreatment.

Resumen

El peso creciente del sector del transporte en la demanda de energía pone de manifiesto la necesidad de emprender acciones para mejorar la eficiencia y desarrollar alternativas energéticas. Una gran parte de los materiales con alto contenido en celulosa son susceptibles de ser utilizados como materia prima para la producción de biocarburantes, se generan como residuos en los procesos productivos de los sectores agrícola, forestal e industrial. El residuo utilizado en este caso proviene de la especie de *Pinus patula*. La deslignificación es una etapa crucial previa a la obtención de bioetanol, al permitir acceder a la celulosa y lograr su transformación. En este trabajo se estudiaron las mejores condiciones para lograr la deslignificación del aserrín de *Pinus patula* cultivado en el departamento del Cauca, alcanzando las mejores condiciones de deslignificación al emplear hidróxido de sodio al 12 %, un tiempo de reacción de 60 min y uso de malla 30 (600 µm), lo cual permitió obtener porcentajes de celulosa entre 63 y 72 % y porcentajes de muestra hidrolizada entre 10 y el 15 %.

Palabras clave: aserrín; lignina; *pinus patula*; bioetanol; deslignificación; pretratamiento.

Introductión

The strategy for energy diversification in transport contemplates different possibilities when using different types of energy production. Biofuels are fuels of vegetable origin that have similar characteristics and can be mixed in a low proportion with fossil fuels to be used in conventional combustion engines. In addition, they do not contain sulfur, one of the main causes of acid rain (Negro et al., 2000). To obtain alcohol from lignocellulosic residues, three consecutive processes must be carried out, such as basic hydrolysis, enzymatic hydrolysis, and fermentation of the obtained carbohydrates.

The chemical composition of biomass varies among species, this mainly consists of cellulose, lignin, and hemicellulose. Most species also contain about 5 % of the third portion of smaller molecular fragments called extractive (Fengel and Wegener, 1989). The crystalline cellulose is the majority fraction of the lignocellulosic biomass. It is composed of long chains of D-glucose molecules linked by β-bonds (1-4) which in turn are grouped into superior structures of great crystallinity, which hinders their hydrolysis and conversion to fermentable sugars. Lignin is the second most abundant biopolymer of nature after cellulose and is a complex formed by a three-dimensional polymer of phenylpropane residues bound by ether bonds and carbon-carbon bonds, it's found around the cellulose microfibrils in an amorphous state (Negro *et al*., 2000). The hemicellulose is formed by polymers of sugars of five carbon atoms (mainly xylose). This fraction is easily hydrolyzable since it does not present a crystalline structure; however, xylose is a difficult sugar to ferment to ethanol (Negro *et al*., 2000).

The aim of the pretreatment of lignocellulosic substrates is to achieve the decomposition of the polymeric components and to form monomeric sugars and thus increase the enzymatic conversion of the cellulose fraction to increase the digestibility of the material for microbial and enzymatic bioconversion and obtain a high yield of ethanol production (Zilliox any Debeire, 1998). Therefore, pretreatment is essential for the removal of lignin, the reduction of cellulose crystallinity and the increase in porosity of the material.

Different types of pretreatment methods have been investigated by several researchers such as a steam explosion, diluted sulfuric acid treatment, alkali treatment, sulfur dioxide, extraction with organic solvent, subcritical and supercritical water, biological treatment using fungi, among others (Liu and Wyman, 2005). Acid pretreatment studies have been carried out using sulfuric acid (Hsu, Guo, Chen, Hwang, 2010), hydrochloric acid (Marcotullio and Krisanti, 2011), phosphoric acid (Geddes *et al*., 2010) and nitric acid (Zhang, Lu, Liu, Wang, and Zhang, 2011). In several scientific studies, it has been reported that acid pretreatment increases the hydrolysis of hemicelluloses and portions of amorphous cellulose and also increases the recovery of hemicelluloses monomers in the liquid fraction and digestible cellulose in the solid fraction (Linde, Jakobsson, Galbe, and Zachhi, 2008, Sassner, Martensson, Galbe, and Zacchi, 2008, Yang and Wyman, 2008). Pretreatment with dilute acid (0.5-1.5 %, temperature above 160 °C) has proved to be a more favorable method for industrial applications by providing percentages of yields of sugars such as xylose between 75-90 % obtained from hemicelluloses. Sodium hydroxide, potassium hydroxide and calcium hydroxide are usually used for pretreatment with alkalis. Alkaline hydrolysis usually involves the cross-linking of intermolecular ester bonds crossed with the xylan group of hemicelluloses and other components and the porosity of the lignocellulosic biomass increases with the removal of cross-links (Tarkov and Feist, 1969). The efficiency of the alkaline hydrolysis depends on the substrate and the treatment conditions, being this more effective in biomasses with low lignin content such as agricultural residues. Sodium hydroxide increases the internal surface of cellulose, decreasing the degree of polymerization and crystallinity and breaking the structure of lignin (Taherzadeh and Karimi, 2008). Authors have reported that carrying out an oxidative pretreatment with lime to poplar plant material at 150 °C conditions for 6 hours, efficiently removes 77.5 % of the lignin present in pieces of wood and also increases yield to obtain glucose (Chang, Nagwani, Kim, and Holtzapple, 2001).

In the department of Cauca, there are more than 14,000 hectares of forests cultivated with conifers and Myrtaceae, located in the municipalities of Popayán, Cajibío, Sotará, and Timbío. The species *Pinus patula* has great potential for forest exploitation (Gallo, Sarria-Villa, and Palta, 2012, Gallo and Sarria, 2013). The aim of the pretreatment of lignocellulosic materials is to remove the lignin barrier and modify the crystalline structure of cellulose. Among the pretreatments, the most used is the one carried out with sodium hydroxide under different conditions (Sánchez and Clavijo, 1982, Camacho, Gonzales, Jiménez, and Moya, 1987). The pretreatment seeks to facilitate contact between the cellulose material and the enzyme, to increase the contact area between the solid and the solution; breaking the lignin seal that surrounds cellulose molecules in most cellulosic residues and decreasing the orientation effect of cellulose molecules (Mojica, Peñaloza, and Retamoso, 1984). The effectiveness of the alkali is attributed to the dissolution of the hemicelluloses and part of the lignin, accompanied by a swelling of the cellulose, saponification of the intermolecular ether bonds and a reduction in the degree of polymerization of the cellulose. With this method, no changes are observed in the degree of crystallinity of the cellulose molecule (Cunningham and López 1994).

Among the degradation compounds of lignin are r-cumaric acid, 4-hydroxy-benzaldehyde, and ferulic acid. In addition to the aforementioned compounds, catechol, vanillin, syringaldehyde, vinylic acid, and low molecular weight polymers are also formed during the basic hydrolysis of lignin, which reacts to produce crosslinking and condensation, reducing the reactivity and value of lignin. Once the hydrolysis of lignin occurs, the hydrolysis process is carried out, which can be enzymatic or chemical, in order to obtain oligosaccharides of short chains and glucose (Sucklin, 1990). Once the hydrolysis is done, the fermentation process is carried out where the glucose is transformed into ethanol and carbon dioxide, obtaining an ideal fermentation with a conversion of 95 %, but due to the formation of some minor secondary products such as propanol, butanol, methanol, glycerol, and lactic acid, among others, a conversion of 94.5 % is obtained (Lima, 2000). For the high production of bioethanol, it is necessary to carry out hydrolysis of the lignin to obtain high cellulose yields. Lignin influences the accessibility to cellulose. Some plant materials that are used to obtain ethanol, such as cotton stems, they can have up to 30.52 % lignin (Shi, Sharma-Shivappa, Chinn, and Howell, 2009), rice straw (10-15 %) (Zhang and Cai, 2008). Pretreatment of plant material to remove lignin can increase the amount of cellulose in the process. The use of ammonium liquor for the treatment of soybean straws has allowed quantities of cellulose of 42.55 %, 6.97 % of hemicellulose and 12.32 % of lignin. Therefore, compared to the content of the plant material without treatment, there is an increase in the cellulose of 70.27 % and decrease in hemicellulose and lignin by 41.45 % and 30.16 % respectively (Xu, Wnag, Jiang, Yang, and Ji, 2007; Moncada *et al.*, 2016).

Materials and méthods

Samples processing

Sawdust samples from *Pinus patula* were subjected to a physicochemical analysis to determine humidity, cellulose, hemicellulose, and lignin. To carry out the hydrolysis process of the lignocellulosic material, an experimental design of factorial type 2^2 was carried out, varying the concentration of sodium hydroxide (w/v) and the hydrolysis time (min). The results were analyzed using the statistical program Statgraphics Centurion XVI.

Physical treatment

Sawdust samples were obtained, using an electric saw (manual Skill 3 ¼ HP), then passed through an electric mill (Retsch - Germany SK100) and sieved using 30 and 40 mesh sieves. Samples were stored in a dry place to proceed with the respective analyzes.

Physicochemical analysis

In the physicochemical analysis, the percentage of humidity existing in the sawdust samples of *Pinus patula* was initially determined.

Determination of humidity of sawdust samples

The moisture content of *Pinus patula* sawdust was determined by weight difference. A 1,000 g sample was placed in the oven (Fisher scientific isotemp oven) at 105 °C previously ground and passed through a 30-mesh sieve to constant weight.

Determination of hemicellulose by the neutral detergent and acid detergent method of Van Soest

In a second step, with the use of the neutral detergent and acid detergent methods proposed by Van Soest (Moncada, Cardona, Higuita, Vélez, and López-Suarez, 2016), the content of hemicellulose was determined for the respective samples. Each test was carried out in triplicate. Reflux equipment with 500 mL flat-bottom balloons was used and one liter of neutral detergent solution containing lauryl sodium sulfate GR (Reagent Grade) was prepared. Ethylenediaminetetraacetic acid (EDTA) GR, sodium borate decahydrate GR, disodium phosphate anhydrous GR and ethyl ether GR. 1,000 g of milled sample was weighed and passed through 30 mesh sieve and deposited in the balloon, then 100 mL of neutral detergent solution, 1.6 mL of naphthalene and 0.500 g of sodium sulfite were added. The reflux was heated and maintained for 60 min after boiling. Then the solution was placed with the sample suspended in a funnel of coarse porosity and filtered under vacuum, washed with hot water and then acetone. The funnels with the sample were dried at 105 °C overnight and after cooling in a desiccator they were weighed to calculate the percentage of hemicellulose (w1). Then 1.0 L of an acidic detergent solution containing sulfuric acid GR 1.0 N and 20.00 g of cetyl trimethyl ammonium bromide GR was prepared. To start the procedure, 1,000 g of sawdust were weighed and placed in a 500 mL balloon, 100 mL of acidic detergent solution and 1.6 mL of naphthalene were added, then it was refluxed for 60 min, then filtered through a funnel of coarse porosity previously weighed and washed with hot water (90 - 100 ºC) and then with acetone. The sample was dried at 105 °C in an oven, cooled in a desiccator and weighed (w2). The difference between the fiber content by the neutral detergent method (w1), and acid detergent (w2) is the percentage of hemicellulose present in the sample. The determinations of the two major components of sawdust were made separately, using the methodologies implemented in the paper industry that are mentioned below (ASTM, 1985).

Determination of cellulose by the Kurschner and Hoffer method

1,000 g of dry sawdust screened by a 40 mesh was weighed, placed in a 250 mL balloon adding to this sample 20 mL of 95 % ethyl alcohol GA (Analytical Grade) and 5.0 mL of concentrated nitric acid GR. It was refluxed in a water bath for a time less than 1 hour, then the solution was decanted on a funnel of coarse porosity previously tared, the procedure described above was repeated once more and finally, third digestion was carried out with 100 mL of distilled water for 1 h. At the end of this time, it was filtered in a funnel of coarse porosity, previously tared and the residue was washed several times with hot distilled water, then washed with a saturated solution of sodium acetate and again with hot water. It was dried at 105 °C until constant weight and the weight obtained was registered. Then the percentage of cellulose in the dry base was calculated.

Determination of lignin content by the Klason method

1,000 g of dry sawdust, grounded by a 40 mesh sieve, was placed in a 50 mL beaker and 15 mL of 72 % cold GR sulfuric acid was added, macerating the sample with a glass rod and keeping the container in a bath at 20ºC for two hours, shaking regularly. At the end of this time, the contents of the beaker were transferred to a 1000 mL round bottom balloon with 575 mL of distilled water until a concentration of sulfuric acid of 3.0 % was obtained. This solution was refluxed for four hours and allowed to settle for about 12 h, then the supernatant was decanted on the funnel of coarse porosity (Kimax Brand 40-60 µm) previously tared, then transferring the lignin to the funnel using hot water to completely remove the acid, then dried at 105 °C, cooled in a desiccator and weighed, to finally calculate the percentage of lignin in dry base.

Treatment of the samples

Pretreatment of Pinus patula sawdust samples with sodium hydroxide (basic hydrolysis).

To perform the basic hydrolysis, 35,000 g of sawdust of the species under study were peeled and deposited in a 304-grade stainless steel reactor having a volume of 500 mL, adding 250 mL of sodium hydroxide GR (Reagent Grade); (worked with three concentrations 9, 10 and 12 % p/v). The reactor was connected to a thermocouple and the heating was carried out until reaching a temperature of 176 ºC ± 2 which was maintained for 60 and 90 min. From this procedure, the black liquors are obtained, to which the amount of residual soda was determined by volumetric analysis. The solid residue was carefully washed with water until neutral and dried in an oven, the data is reported in Table1.

Table 1.

Data for Basic Hydrolysis of Pinus patula sawdust

Sample	W initial sample (g)	[NaOH] % p/v	Time (min)	Residual Soda (mL)	W final sample (g)	Hydrolyzed sample %
m1-30	35,005	9,0	60	0,70	26,940	12,45±0,026
m2-30	35,006	9,0	90	0,53	29,110	5,290±0,035
m3-30	35,223	10	60	1,56	24,620	20,48±0,044
m4-30	35,007	10	90	1,13	27,900	9,330±0,020
m5-30	35,004	12	60	2,53	27,660	10,11±0,026
m6-30	35,000	12	90	1,70	26,100	15,18±0,035
m1-40	35,005	9,0	60	0,60	28,240	8,220±0,017
m2-40	35,005	9,0	90	0,15	26,470	13,98±0,111
m3-40	35,140	10	60	0,36	27,900	9,680±0,044
m4-40	35,001	10	90	0,21	26,690	13,26±0,035
m5-40	35,031	12	60	1,40	25,860	16,01±0,053
m6-40	35,004	12	90	2,16	24200	21,35±0,035

Source: the authors.

Determination of residual soda in black liquors

10 mL of black liquor was taken by volumetric pipette and placed in a 250 mL Erlenmeyer flask, 50 mL of distilled water and 10 mL of a 10 % barium chloride solution were added, then the mixture was titrated with hydrochloric acid. GR 0.25 N up to a pH of 8.3 potentiometrically and the consumed volume of acid was reported to perform the respective calculations and establish the appropriate concentrations of sodium hydroxide during the basic hydrolysis.

Determination of the permanganate number (residual Lignin)

For the determination of the effectiveness of the basic hydrolysis, as for the degree of delignification obtained, the determination of the "Permanganate Number" was made, for which 1,000 g of the treated sample was taken and placed in a 600 mL beaker, which contained 200 mL of distilled water and a magnetic stirrer. By means of a plate with agitation, the sample is mixed and it is verified that there are no lumps. In a 250 mL Erlenmeyer flask, 25 mL of GR 0,100 N potassium permanganate and 25 mL of 0.280 N sulfuric acid are deposited, this mixture is added to the beaker content and a stopwatch is started, 25 mL of ferrous sulfate was immediately measured ammonia GR, 0.100 N, being deposited in the same Erlenmeyer that previously contained the acid and permanganate solutions, likewise, 100 mL of distilled water was measured in a graduated cylinder. The reaction was suspended at three (3) min by adding the contents of the Erlenmeyer flask in the beaker, the agitation was suspended after 15 s. It was then filtered through a Buchner funnel and the filtrate was titrated with a 0.100 N potassium permanganate solution until a pale violet color was obtained. The volume spent during the titration was recorded and the percentage of residual lignin in the already hydrolyzed samples was calculated with the data obtained.

Results and Discussion

All the analyses that were carried out to determine the sawdust components of the *Pinus patula* species under study were done in triplicate. Table 2 shows the averages of the data obtained for moisture, cellulose, lignin, and hemicellulose. These values are also presented for conifers.

Table 2.

Results of the Bromatological Analysis of Sawdust

Determination (%)	*Pinus patula*	*Conífer**]
Humidity	12,10 ± 0,20	-
Cellulose	54,75 ± 0,15	40 – 45
Lignin	27,98 ± 0,11	25 – 30
Hemicellulose	7,80 ± 0,10	20

Díaz (1986).
Source: the authors.

Básic hidrólisis

All determinations were made in triplicate, having a total of 36 samples. Immediately after performing the basic hydrolysis of all the samples, the residual soda was determined, or analysis of residual alkalinity to the black liquors, in order to know how much sodium reacted in the process. The samples were carefully washed with distilled water until neutral, then dried in an oven for further analysis. Figure 1 shows the stainless steel reactor in which the basic hydrolysis was carried out, also a sample of black liquor and sawdust after the hydrolysis.

Figure 1. Reactor for carrying out basic hydrolysis, black liquor and sawdust after hydrolysis
Source: the authors.

The data reported in Table1 show the results obtained after the basic hydrolysis of *Pinus patula* samples sieved by 30 mesh and by 40 mesh. In the data reported in Table 1, it can be seen that of the samples sieved by a 30 mesh, The one with the highest percentage of hydrolysis is the one treated with 10 % sodium hydroxide, but in general there is a non-sequential behavior. It was observed that with 30 mesh, 10% sodium hydroxide and 90 min, percentages of the hydrolyzed sample are obtained lower than with a time of 60 min. Similarly, 9 % of sodium hydroxide and 90 min were observed for mesh 30, a higher percentage at 60 min. The opposite occurs for mesh 30 % - 12 % sodium hydroxide, where higher percentages are obtained at a time of 90 min. For a mesh 40, hydroxide concentrations of 9, 10 and 12 % higher yields are observed at high times of 90 min. Taking these results into account, it is shown that small particle sizes facilitate hydrolysis at intermediate concentrations and shorter reaction times (Singh, Shukla, Tiwari, and Srivastava, 2014). A high correlation (0.993) was observed between the hydroxide concentration and the percentage of hydrolyzed samples to a mesh 30 and 90 min time. High correlation (0.976) was also observed between the hydroxide concentration and the percentage of hydrolyzed sample at mesh High correlation (0.976) was also observed between the hydroxide concentration and the percentage of hydrolyzed sample at mesh High correlation (0.976) was also observed between the hydroxide concentration and the percentage of hydrolyzed sample at mesh 40 and 60 min time.

From this Table, it can also be concluded that the percentage of hydrolysis for the samples sieved by mesh 40 increases as the concentration of sodium hydroxide increases and the reaction time increases. The percentage of sample hydrolyzed in the basic pretreatment represents a fraction of the sample that was extracted, which consists of soluble carbohydrates (pentoses, xyloses, etc.), resins, lignin, hydrolyzed hemicellulose during the pretreatment and products of cellulose degradation. It is estimated that cellulose was not hydrolyzed in this stage since to achieve it, more severe conditions are needed, such as temperature above 180 ºC and reaction time not less than two hours (Mojica *et al*., 1984; Díaz, 1986). Adequate pretreatment involves the breaking of the hydrogen bonds of the crystalline cellulose, the breakdown of the hemicellulose-lignin matrix, and finally, an increase in the porosity and surface area of the cellulose for the subsequent enzymatic hydrolysis (Mood *et al*., 2013). It was observed in the results of Table 4, an increase in the average percentage of cellulose from 8.67 to 10.23 % after performing the pretreatment, when compared with the cellulose content reported in Table 1, which indicates that the Pretreatment using the alkaline solution increases cellulose availability for subsequent hydrolysis. To increase the digestibility of cellulose, large amounts of hemicellulose must be removed because the hemicellulose covers cellulose fibrils, limiting its availability to perform enzymatic hydrolysis (Singh *et al*., 2014).

The amount required to effect good delignification depends on the species of the wood and other factors such as the size of the wood, but it is in the order of 12 % (w/v) of sodium hydroxide solution, which in our case achieves a greater degree of hydrolysis (McDonald, 1970, Saddler, Hogan, Chan, and Louis-Seize, 1982). According to our results regarding the values obtained from residual soda, it can be said that in all cases there is always presence of it, but it is not appropriate to work with very low values, it is more appropriate to have approximately 2 % of residual soda in a process such as the one carried out for the delignification of the sawdust of the species under study. Pretreatment studies for species such as Red Pine have yielded percentages of untreated wood from 1.8 to 3.7 % (Zhu, Pan, Wang, and Gleisner, 2009). In addition, it is a parameter to be taken into account in a productive process, since it serves as a control for the consumption of reagents and indicates whether there is an amount that can be recovered (Alkasrawi, Galbe, and Zacchi, 2002, Dowe and McMillan 2000).

Table 3 shows the results obtained from the analysis of variance (ANOVA), performed with the help of the Statgraphics Centurion XVI software. The analysis of variance was carried out to determine the statistical significance of each effect by comparing its mean square against an estimate of the experimental error; in this way, the values of the significant parameters are obtained in the basic hydrolysis process of *Pinus patula* sawdust.

Table 3.

Analysis of variance for hydrolysis of Pinus patula sawdust

Source	Sum of squares	Gl	Average Squares	Reason-F	Value-P
A:Concentration	14,2506	1	14,2506	0,68	0,5607
B:Time	19,5027	1	19,5027	0,93	0,5111
AA	29,3356	1	29,3356	1,40	0,4464
AB	50,5145	1	50,5145	2,42	0,3640
Total error	20,9157	1	20,9157		
Total (corr.)	137,833	5			

Source: the authors.

The statistical results of ANOVA presented in Table 3 show that in the basic hydrolysis process of Pinus sawdust the concentration of sodium hydroxide and time do not show a significant effect on its yield, because no factor has a P-value. less than 0.05, indicating that they are significantly different from zero with a confidence level of 95.0 %. The Pareto diagram, shown in Figure 2, shows that neither the sodium hydroxide concentration nor the reaction time significantly influences the hydrolysis percentage. A positive influence of the concentration of sodium hydroxide can be observed in the Pareto diagram, which indicates that as the concentration increases the higher the hydrolysis process performance and negative influence of the reaction time, indicating that the yield increases as the reaction time decreases

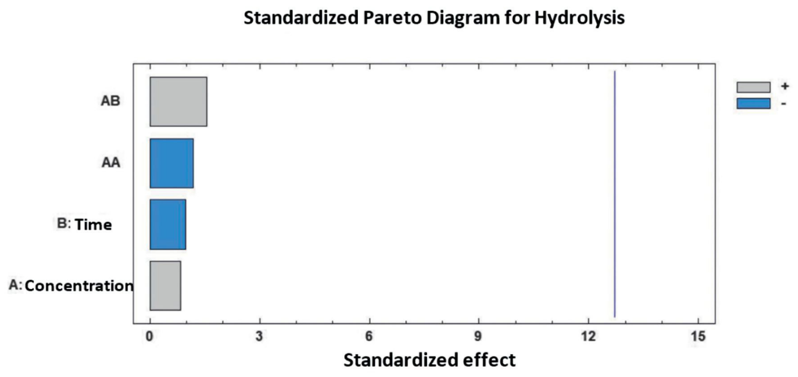

Figure 2. Pareto diagram for the hydrolysis process of Pinus patula sawdust

Source: the authors.

La Table 4 presenta la combinación de los valores de los factores, con la cual se maximiza el porcentaje de rendimiento de hidrólisis del aserrín de *Pinus patula* para alcanzar un rendimiento máximo del 18,07 %.

Table 4.

Parameters for an answer to maximize the yield percentage of Pinus patula sawdust hydrolysis

Factor	Low	High	Óptimum
Concentratión	9,0	12,0	10,2766
Time	60,0	90,0	60,0

Source: the authors.

The permanganate number is a measure that corroborates the degree of delignification achieved during the process because it determines the percentage of residual lignin. The residual lignin should be expressed as the volume in milliliters of potassium permanganate to oxidize an equivalent amount of it:

$$\# KMnO_4 (mL) = \frac{V \times N\ KMnO_4}{0,1} + \frac{25}{0,1} (N\ KMnO_4 - N\ FAS) \qquad (1)$$

Where:

25 = Volume of $KMnO_4$ and FAS added to the reaction mixture
0.1 = Normality of the solutions

V = Volume of $KMnO_4$ spent in the titration.
N $KMnO_4$ = Normality of $KMnO_4$.
N FAS = Normality of ferrous ammoniacal sulfate.

Table 5 shows the results of the determination of the permanganate number of the sawdust samples hydrolyzed with sodium hydroxide. It can be seen that the spent volume of potassium permanganate in the titration corresponds to the same permanganate number, this is due to the fact that during the tests we worked with solutions of equal concentrations and therefore the second factor of equation 1 is equal to zero.

In general, it can be seen that the results of # $KMnO_4$ are between 10 and 20 mL, which would correspond approximately to a percentage of residual lignin between 2.5 % and 4.8 %, according to the Table of correlation between different grade numbers of delignification and the percentage of Klason lignin present (ASTM, 1985). Due to the low percentages of residual lignin, it can be concluded that the pretreatment used, in this case, the basic hydrolysis, is adequate for the purposes to be achieved, such as the delignification of the sawdust to obtain a substrate almost free of lignin and a greater contact surface, for a subsequent enzymatic hydrolysis (Mood *et al*., 2013., Singh *et al*., 2014).

Table 5.

Data and results of # $KMnO_4$ for Pinus patula

Sample	W sample (g)	[NaOH] %	Time (min.)	V $KMnO_4$ (mL)	# $KMnO_4$ (mL)
m1-30	1.000	9,0	60	10,6	10,6
m2-30	1.000	9,0	90	10,0	10,0
m3-30	1.000	10	60	15,0	15,0
m4-30	1.000	10	90	10,2	10,2
m5-30	1.000	12	60	9,40	9,4
m6-30	1.000	12	90	11,0	11,0
m1-40	1.000	9,0	60	16,1	16,1
m2-40	1.000	9,0	90	14,7	14,7
m3-40	1.000	10	60	14,0	14,0
m4-40	1.000	10	90	14,5	14,5
m5-40	1.000	12	60	14,5	14,5
m6-40	1.000	12	90	13,5	13,5

Source: the authors.

The content of cellulose present in the samples already hydrolyzed, and that was determined to take 1,000 g of digestate sawdust, allowed to know the increase in the percentage of cellulose in the hydrolyzed samples. The results of these analyses are shown in Table 6. It could be observed for *Pinus patula* sawdust samples that almost all treatments are appropriate for a greater availability of cellulose content (Stenberg, Bollók, Réczey, Galbe, and Zacchi, 2000), obtaining the best results when samples were treated with 10 and 12 % sodium hydroxide solutions, a size 30 mesh and a time of 60 min.

Table 6.
Cellulose present after pretreatment para Pinus patula

Sample	W Sample (g)	[NaOH] %	Time (min)	% cellulose
m1-30	1.000	9,0	60	55,98
m2-30	1.000	9,0	90	60,92
m3-30	1.000	10	60	70,38
m4-30	1.000	10	90	66,38
m5-30	1.000	12	60	63,62
m6-30	1.000	12	90	72,58
m1-40	1.000	9,0	60	59,08
m2-40	1.000	9,0	90	63,18
m3-40	1.000	10	60	66,08
m4-40	1.000	10	90	62,44
m5-40	1.000	12	60	61,10
m6-40	1.000	12	90	68,63

Source: the authors.

With the results reported previously, it could be established that the samples of the *Pinus patula* species most indicated to be submitted to an enzymatic attack are those that were treated with sodium hydroxide with concentrations of 10 and 12 % but sieved by 30 mesh, due to the higher percentages of cellulose (Alegría and Muñoz, 2004).

Conclusions

The basic pretreatment is a procedure of vital application for the use of the material of interest (cellulose) since it eliminates a large part of the lignin present in a high percentage in the species under study. In general, the most favorable pretreatment for the *P. patula* species was obtained using 12 % sodium hydroxide. Between the times used, a greater delignification was achieved at 60 min and screening by mesh 30 (600 µm). This pretreatment allows to obtain a greater percentage of hydrolyzed sample and a higher percentage of cellulose, with a high yield of hydrolysis and obtaining bioethanol.

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