REUSE OF WOOD WASTES FOR SECOND GENERATION ETHANOL PRODUCTION

REUTILIZAÇÃO DE RESÍDUOS DE MADEIRA PARA PRODUÇÃO DE ETANOL DE SEGUNDA GERAÇÃO

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Abstract. This paper reports a production of ethanol by using wood residues using a method for identification of compounds dispersed in fermented nitric cellulosic hydrolysate liquor. It was used twelve different species collected in south regions of Brazil as biomass. High performance liquid chromatography (HPLC) employing analytical proton-exchange technique was used. The fermentation ability plus ethanol yields by using *Saccharomyces cerevisiae* were investigated. Standard compounds identified in analysis were: fructose, lactic acid, acetic acid, glycerol, glucose and ethanol. The yeast showed good ethanol productivities in ranges between: 3.00 g/L *Cedrelinga catenaeformis* and 0.76 g/L using *Ocotea porosa*, respectively; after 8 h of fermentation essay. All residues demonstrated possibility of second generation ethanol production, from cellulose, emerging this discards feasible when used as biomasses. Finally, this paper contributes to the sustainable production of biofuels through the process monitoring and optimization, contributing to the renewable energies generations research area.

Keywords. Wood residues, nitric acid hydrolysis, second generation ethanol, HPLC.

Resumo. Este artigo relata a produção de etanol utilizando resíduos de madeira como também um método para identificação de compostos químicos dispersos no licor fermentado de celulose hidrolisada com ácido nitríco. Como biomassa, foram utilizadas doze diferentes espécies coletadas em regiões do sul do Brasil. Foi utilizada cromato-

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grafia líquida de alta eficiência (HPLC) associada à técnica analítica de troca de prótons. Foi investigada a capacidade fermentativa e mais o rendimento da produção de etanol celulósico utilizando a levedura *Saccharomyces cerevisiae.* Os compostos identificados na análise foram: frutose, ácido lático, ácido acético, glicerol, glicose e etanol. As leveduras mostraram boa produtividade de etanol na faixa de: 3,00 g/L utilizando *Cedrelinga catenaeformis* e 0,76 g/L usando *Ocotea porosa*, respectivamente, após um ensaio de fermentação de 8 h. Todos os resíduos demonstraram possibilidade de produção de etanol de segunda geração a partir da celulose, tornando estes descartes utilizáveis como biomassa. Finalmente, este trabalho contribui para a produção sustentável de biocombustíveis através do monitoramento e otimização do processo, colaborando para a área de pesquisa em geração de energias renováveis.

Palavras-chave. Resíduos de madeira, hidrólise com ácido nítrico, etanol de segunda geração, HPLC.

INTRODUCTION

Concerns about the depletion of fossil fuel resources and climate changes attributed to anthropogenic carbon dioxide emissions are driving a strong global interest in renewable, and carbon-neutral energy sources, as well as chemical feedstock's derived from plant sources (Doherty et al., 2011, Zhu and Pan, 2010).

One of more important renewable energy source is biomass. It offers many advantages over petroleum-based fuels aspects (Demirbas et al., 2009; 2011). Also, ethanol production from biomass is one way to reduce both consumption of crude oil and also environmental pollution (Balat et al., 2011).

Dilute acid hydrolysis is one of the pretreatment methods for converting cellulose biomass to ethanol (Xie et al., 2011).

Martin et al. (2006) highlighted the importance of *Saccharomyces cerevisiae*, the micro-organism which has traditionally been used for production of alcoholic beverages and ethanol, and sugars such as sucrose and glucose as substrates. This microorganism also has the ability to produce ethanol from lignocellulosic material. As not requiring oxygenation and the relative tolerance to ethanol and fermentation inhibitors, which are generally compounds formed in the chemical and/or physical treatment given lignocellulosic material prior to fermentation (Santos et al., 2010a).

Brazil is a major ethanol producer, mainly utilizes sugarcane for ethanol production while United States and Europe mainly uses starch from corn, or wheat and barley (Hartemink, 2008; Wen et al., 2010). Ethanol levels in order from 7.8 to 17.5% have been reported using sugar cane silage without the use of additives (Freitas et al., 2006).

However, during the ethanol production, a wide variety of degrading compounds from hydrolysis are released. Most of these compounds possess inhibitory activities reducing biochemical conversions, yields and efficiency (Carrasco et al., 2010). Therefore, an efficient analytical approach is increasingly needed to qualify and quantify these degradation compounds for understanding their roles in the bioconversion processes (Xie et al., 2011).

Generous efforts have been extended towards the analysis of degradation products in biomass hydrolysates, with varying degrees of success. High performance liquid chromatography (HPLC) is a method frequently used in the analysis of these degrading products in the prehydrolysate or even in the hydrolysis liquor of lignocellulosic biomass. Although gas chromatography (GC) coupled with flame ionization or mass spectrometry detection has been quite successful in identifying a large variety of or-

ganic degradation products in lignocellulosic biomass (Klinke et al., 2002; Karagöz et al., 2004). Also, the implementation of (GC) methodologies for quantitative work has suffered from inherent complexities of derivatizing samples of unknown composition.

Liquid chromatography (LC) methods, employing post-column (UV) or refractive index detection have historically suffered from incomplete resolution of analytics. As result, (LC) analyses of degradation products in hydrolysate samples have typically employed multiple chromatographic modes and detection strategies, and the choice of which depends on analytic class. Aliphatic acids have been determined using high-performance anion-exchange chromatography with (UV) or conductivity detection, ion-exclusion chromatography with (UV) detection or electrophoretic methods (Xie et al., 2011). In contrast, (LC) analyses of aromatic acids, furans, phenolic and aldehydes have typically been accomplished using reversed-phase chromatography with refractive index (Luo et al., 2002) or mass spectrometry detection (Persson et al., 2002; Chen et al., 2006; 2009).

The aim of this study was to use a high performance liquid chromatography (HPLC) analysis employing proton-exchange technique for mapping short-chain organic acids, monosaccharide's, glycerol, plus the ethanol fractions present in hydrolysate liquor of second generation ethanol using wood wastes. Twelve different species in form of wood chips were tested, and also diluted nitric acid treatment for hydrolysis and *Saccharomyces cerevisiae* yeast for fermentation essay was employed. Even showing lower ethanol productivities than sugarcane, the use of these products for energetic purposes showed to be feasible, however studies using these biomass are still lacking due to the material anisotropy and the complexity of compounds dispersed in hydrolysis liquor.

MATERIALS AND METHODS

Wood chips were collected in industries of transformation during machining-processes. Samples were original from furniture-centers: Santa Catarina and Paraná states, regions of south Brazil. Among analyzed samples are: *Hymenolobium petraeum*, *Tabebuia cassinoides*, *Myroxylon peruiferum*, *Nectandra lanceolata*, *Ocotea catharinensis*, *Cedrelinga catenaeformis*, *Cedrela fissilis Vell*, *Ocotea porosa*, *Laurus nobilis*, *Balfourodendron riedelianum*, *Pinus Elliotti* and *Brosimum spp.* a mix of hardwoods and softwoods, but here all were tested separately.

The testes were realized two times using duplicate, and for the final results the mean value was considered. For statistic treatment, the experimental error, variance analysis and significance test were used.

After collected, samples were cataloged and packed in containers of 5 kg capacity, then stored. Wood chips were then milled using in laboratory by a centrifugal mill (Zenith ZTM-86). Samples tested passed through 0.6 mm mesh sieve.

Samples lignin, cellulose, hemicelluloses fractions, plus the basic density of species were demonstrated in (Table 1). Density of samples was determinate according to Tappi (T258) method. Klason lignin was determined according to Tappi (T222). Holocellulose (cellulose + hemicelluloses) and cellulose contents were determined using Tappi (T202) method.

Table 1. Physicochemical properties of samples.

Hard/ Soft-wood	Species name	Chemical (%)			Physical (g/cm^3)
		Celulose	Hemiceluloses	Lignin	Density
Hardwood	*Hymenolobium petraeum*	42.2 ± 0.2	27.2 ± 0.3	28.4 ± 0.1	0.67 ± 0.1
Hardwood	*Myroxylon peruiferum*	41.1 ± 0.5	25.4 ± 0.3	27.3 ± 0.2	0.61 ± 0.2
Hardwood	*Tabebuia cassinoides*	44.2 ± 0.4	29.4 ± 0.4	25.6 ± 0.3	0.99 ± 0.4
Softwood	*Nectandra lanceolata*	45.4 ± 0.2	30.1 ± 0.6	23.6 ± 0.3	0.50 ± 0.2
Hardwood	*Ocotea catharinensis*	44.7 ± 0.2	27.5 ± 0.1	27.7 ± 0.3	0.62 ± 0.2
Hardwood	*Cedrelinga catenaeformis*	40.6 ± 0.3	29.5 ± 0.2	27.2 ± 0.5	0.50 ± 0.2
Hardwood	*Cedrela fissilis Vell.*	40.4 ± 0.1	28.1 ± 0.1	29.7 ± 0.1	0.47 ± 0.1
Hardwood	*Ocotea porosa*	43.8 ± 0.2	26.9 ± 0.4	30.2 ± 0.6	0.66 ± 0.4
SoftWood	*Laurus nobilis*	46.7± 0.5	32.4 ± 0.3	20.1 ± 0.3	0.44 ± 0.2
Softwood	*Balfourodendron riedelianum*	45.1 ± 0.4	26.6 ± 0.6	22.2 ± 0.2	0.69 ± 0.6
Softwood	*Pinus elliotti*	45.3 ± 0.1	30.5 ± 0.3	22.9 ± 0.6	0.48 ± 0.5
Hardwood	*Brosimum spp.*	44.1 ± 0.2	26.5 ± 0.1	26.2 ± 0.2	0.54 ± 0.3

In hydrolysis essay, approximately 500 g of wood chips were treated with nitric diluted (70% HNO_3) diluted in 100 mL of distilled water solution at 120ºC during 2 h to hydrolyzate the cellulose and remove part of lignin, allowing to the yeast easier access to cellulosic fractions (Zhang et al., 2010). After this, cellulosic solid phase the as separated using a hydraulic press by filtering, applying to it 2 tons of pressure over an area of 200 cm^2 (Maeda et al., 2011).

Solid fractions were submitted to an additional step of partial delignification by using alkaline treatment NaOH (1.0% m/v) and 1:20 (w/v) ratio at 121 ºC during 30 min (Vasquez et al., 2007). The pH was corrected to 4.0 using NaOH with intention of not create a harmful environment for the yeast. For the fermentation essay, the yeast used, strains of *Saccharomyces cerevisiae* were originally from Chemistry Laboratory (UDESC).

The pretreatment, delignification, hydrolysis and fermentation essay activities were also were tested in this laboratory. The strain was maintained on an agar-malt slant. The agar-malt slant consisted of malt extract (5 g/L), yeast extract (5 g/L), peptone (5 g/L), agar (20 g/L) and distilled water (1 L), supplemented with (1 g/L) glucose in a flask. Before use as an inoculum for fermentation, the culture was aerobically propagated using 200 ml Erlenmeyer's.

S. cerevisiae seeds grown overnight at 30 ºC in regulate climate for 48 h with 200 rpm agitation using shaking baths until the concentration reaches approximately 3 % (v/v), then it was separated by centrifugation, always monitored by optical density OD-600nm measurements (Agilent UV–visible Spectroscopy system).

At this time, approximately 250 g of samples were inoculated and fermented separately using twelve 500 mL Erlenmeyer's. The colony formed was inoculated with 3 % (v/v) and 50 ml of pure distilled and deionized (H_2O) water. Then samples were kept stored in anaerobic conditions and regulate climate ambient (30 ºC) during 8 h period, after this HPLC analysis was realized.

In the analysis of reducing sugars, free ketones and aldehyde groups on its structure are released, thus having oxidation reaction. The non-reducing sugars (as saccharose) have such groups linked by a glycosidic bond and become reducing sugars by acidic hydrolysis to form D-D-fructopyranose and gluco-pyra-

nose. However, in this work, the analysis of reducing sugars was not focused in xylose and cellobiose, but regarded to glucose and fructose, with the aim to discover the amount produced available for fermentation by converting cellulose.

Analysis was made in a HPLC (Merck-Hitachi D-7000 IF model) with refractive index (RI) detector and column (Transgenomic ICE-ION-300). HPLC analysis was made in the Chemistry Laboratory (UNIVILLE). Ultra-pure water was used to dilute the acid concentrations of hydrolysate liquor and the eluent used (mobile phase) was 8.5 mM of sulphuric acid (isocratic).

Technical data used in this work: Acquisition Method (Acid lactic-ion 300); Column Type (RP18); Pump A (Type: L-7100); Solvent A: (HAc 1%); Solvent B: (H_2SO_4 8.5mM), Solvent C: (Methanol); Solvent D: (CAN); Method Description: (Acid Lactic determination using column Transgenomic ice-ion 300); Chromatography Type: (HPLC Channel: 2); Peak Quantitation: (AREA); Calculation Method: (EXT-STD).

Additional parameters employed in HPLC analysis were as follows: samples were injected using an auto-sampler and the injection volume was 0.5 µL/min. The column-temperature was maintained at 30 ºC.

Proton-exchange technique was used for mapping ethanol, glycerol, lactic acid, acetic acid, glucose and fructose quantities. At this time, 100 mL of hydrolysate liquor from the twelve samples were stored at - 4 ºC during 30 min until preceded the HPLC analysis. Before analysis samples were diluted (1:1) using ultra-pure water and then filtrated using a Millipore membrane filter 0,45 µm (VWR Scientific, Suwanee, GA, USA) and transferred to a vial (vial auto-sampler specific for chromatography).

Calibration curves were used from series: 4663 for glycerol, and series: 4731 for other compounds. (Figure 1) shows calibration for ethanol, recalling that the equations of straight lines of equipment calibrations are as follows (Eq.1):

$$(Eq.\ 1)\ y = (1 / a).\ x$$

Legend: y = the surface area; x = the concentration of standards is the slope of the straight; a = angular coefficient.

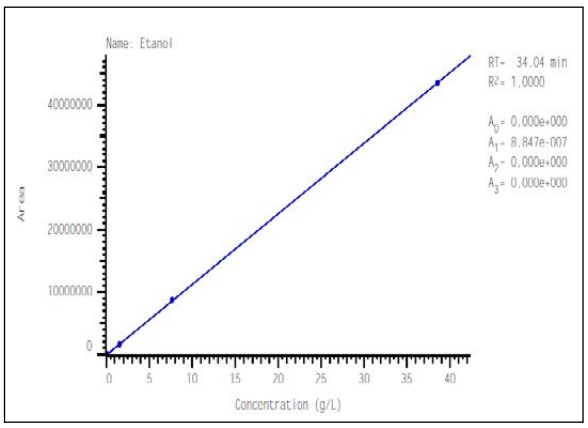

Figure 1. HPLC ethanol calibration Table 2.

Table 2. Retention times (RT) ratios calibration for compounds identification and isocratic eluent.

Compounds identification	Eluent (H_2SO_4) isocratic	
	Retention Times (RT) (min)	Mobile phase (mM)
Glucose	14.93	8.0
Fructose	16.12	8.0
Lactic-acid	20.74	8.0
Glycerol	21.70	8.0
Acetic acid	24.00	8.0
Ethanol	34.04	8.0

Recalling were the line pass through the origin, the linear coefficient (b) in the equation of the line is equal to zero. (Table 2) demonstrates the retention times (RT) ratios of calibrations for compounds mapping and the isocratic eluent used.

Results shows produced compounds after 8 hours fermentation period and results are given in g/L. The analysis curves were demonstrated in (Fig. 2), (Fig. 3), (Fig. 4), (Fig. 5) and results are shown in (Table 3) and (Table 4). The (Fig. 6) shows standard mapped compound's dispersion in hydrolysate liquid after fermentation essay. The (Table 5) and (Table 6) shows the statistic analysis made.

RESULTS AND ANALYSIS

The (RT) of compounds were automatic generated by the HPLC with higher precision and sensitivity in an intensity scale between 0-150 mV range. All (RT) were here given in minutes (min). A total time of 40 min is needed to evaluate the compounds using flow injection of 0.5 µL/min, and it was maintained equal until the analysis finish. Also, all compounds remained inside the initial ranges of calibration.

Spectrum separations in curves among compounds were satisfactory and they appeared in specific peaks with good resolution. Recently specialized literature published some efforts which resulted in good contributions to chromatography techniques (Chen et al., 2009; 2010; Matías et al., 2011).

The separation among the compounds in hydrolysate using (ICE-ION) column and proton-exchange technique, isocratic sulfuric acid 8mM (mobile phase) and ultra-pure water (1:1) was efficient and was possible to identify all standard compounds simultaneously. Good cellulose-to-ethanol conversion was obtained by nitric hydrolysis with positive glucose consumption by the yeast in fermentation essay. Also, the yeast showed good ability to produce fuel in pH (4.0) environment.

In this work, fructose levels appear in ranges of: 0.034 g/L in *Cedrela fissilis Vell.* and 0.198 g/L in *Nectandra lanceolata* species.

Lactic-acid production was mapped in a range of: 0.053 g/L using Cedrelinga catenaeformis and 1.898 g/L using Pinus elliotti. Acetic acid was identified: Laurus nobilis 1.522 g/L and Myroxylon peruiferum 1.623 g/L. This compound presence can affect the ethanol production because contributes to create a toxic enviroment for the yeast (Costa et el., 2008).

Acetic and lactic microbial activities were common found in Brazilian sugar cane production plants, in rates of: 6.84 mMol/L and 3.48 mMol/L, which could represent great diminutions if great quantities of cellulosic ethanol are produced due to the contamination (Moreira et al., 2008; Costa et al., 2008).

In this work, the yeast was manipulated under sterilized conditions, with asepsis of fermentation. However the highly lactic acid production seemed to be linked with the presence of homofermentative and heterofermentative bacterias present in the samples used.

The increase in the number these bacterias was correlated to lactic acid producing, also, can reduce the final pH, increase in content of lactic acid and decrease the production of effluents during material storage (McDonald et al., 1991). However, the effects on yeast growth and aerobic stability are variable (Driehuis & Wikselaar, 2000).

Furthermore, inoculants containing the heterofermentative bacterium that converts the lactic acid in acetic acid, 1,2 propanediol, propionic acid, carbon dioxide and traces of ethanol (Oude et al. 2001), have shown effectiveness in controlling the growth of yeasts and increased aerobic stability.

Chemical and microbial additives have been used in order to prevent the growth of yeasts in traditional samples storage; however, few have been tested in order to control the ethanol production (Pedroso et al., 2007; Souza et al., 2008). In this work, the fermented liquid concentration remained at pH 4.

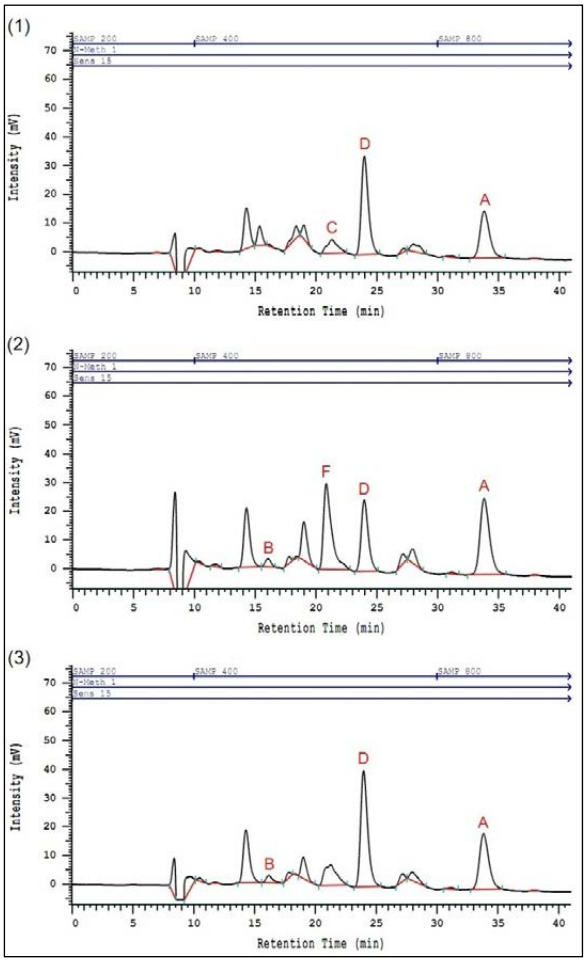

Figure 2. Legend: (1) *Hymenolobium petraeum*. (2) *Myroxylon peruiferum* (3) *Tabebuia cassinoides.* Compounds identification: (A) Ethanol (B) Fructose (C) Glycerol (D) Acetic acid (E) Glucose (F) Lactic acid. Column, Transgenomic (ICE-ION 300); injection flow rate, 0.5 µL/min; mobile phase, 8.0 mM (H_2SO_4) eluent.

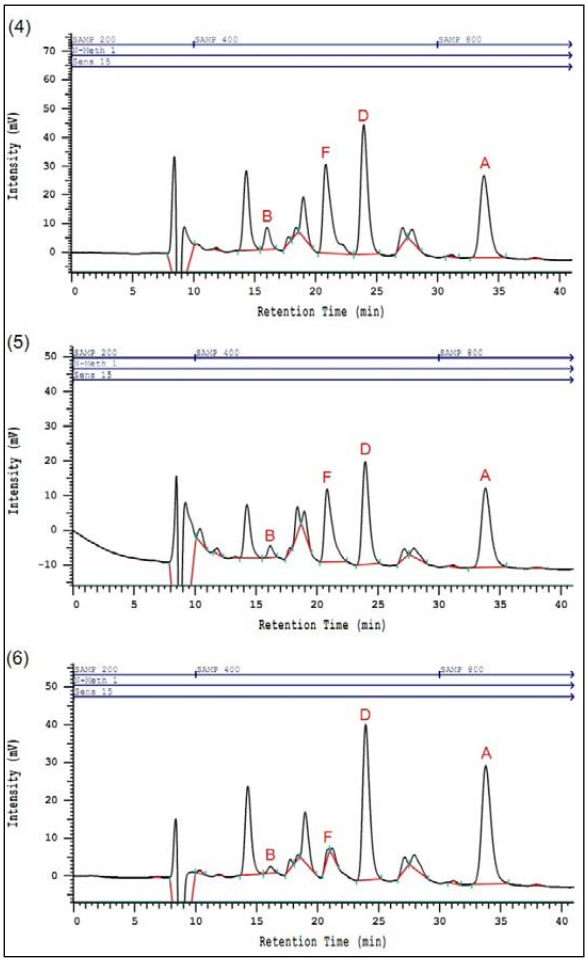

Figure 3. Legend: (4) *Nectandra lanceolata* (5) *Ocotea catharinensis* (6) *Cedrelinga catenaeformis*. Compounds identification: (A) Ethanol (B) Fructose (C) Glycerol (D) Acetic acid (E) Glucose (F) Lactic acid. Column, Transgenomic (ICE-ION 300); injection flow rate, 0.5 µL/min; mobile phase, 8.0 mM (H_2SO_4) eluent.

Table 3. Quantitative results of the compounds mapped in nitric hydrolysate liquor by using HPLC employing the proton-exchange technique.

	Species	A - Ethanol		B - Fructose		C - Glycerol		D - Acetic acid		E - Glucose		F - Lactic acid	
		min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]
1	*Hymeno lobium*	33.81	1.56	*n.d	*n.d	21.29	0.26	22.97	*n.d	*n.d	*n.d	*n.d	*n.d
2	*Myroxylon*	33.81	2.55	16.04	0.07	*n.d	*n.d	23.96	1.62	*n.d	*n.d	20.84	*n.d
3	*Tabebuia*	33.81	1.81	16.16	0.07	*n.d	*n.d	23.96	*n.d	*n.d	*n.d	*n.d	*n.d
4	*Nectandra*	33.79	2.71	16.01	0.19	*n.d	*n.d	23.95	*n.d	*n.d	*n.d	20.83	1.77
5	*Ochotea*	33.80	2.18	16.16	0.07	*n.d	*n.d	23.95	*n.d	*n.d	*n.d	20.83	1.13
6	*Cedrelinga*	33.77	2.99	16.12	0.04	*n.d	*n.d	23.93	*n.d	*n.d	*n.d	20.85	0.05

[a](min): Retention Times (RT) [b](g/L): concentration *n.d: not detect

Using robust cellulosic ethanol production (SPORL), lodge pole pine as biomass and an adapted strain of *Saccharomyces cerevisiae,* it was possible to produce ethanol in the range of 0.81 g/L/h and 2.0 g/L/h using different methods over 4 and 24 hours of fermentation in an undetoxified run (Tian et al., 2010). In this work *S. cerevisiae* produced ethanol in ranges of 3.00 g/L/h and 0.76 g/L/h after 8 h fermentation.

Using spruce wood chips; more viable strains were able to consume nearly 2.0 g of glucose per gram of initial biomass during the first 8 h (Brandberg et al., 2004). In this work a similar result was obtained, about 1.8 g to 2.5g.

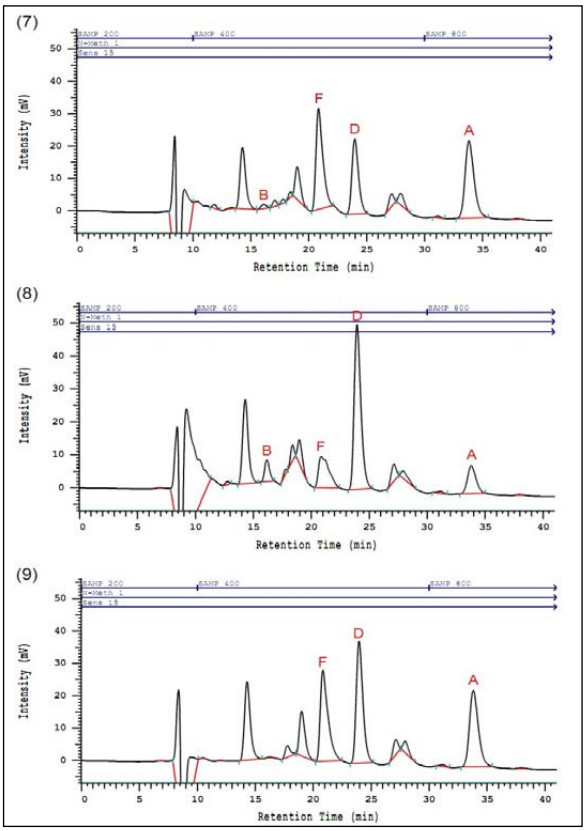

Figure 4. Legend: (7) *Cedrela fissilis Vell.* chromatogram. (8) *Ocotea porosa* chromatogram (9) *Laurus nobilis* chromatogram. Compounds identification: (A) Ethanol (B) Fructose (C) Glycerol (D) Acetic acid (E) Glucose (F) Lactic acid. Column, Transgenomic ICE-ION 300; injection flow rate, 0.5 µL/min; mobile phase, 8.0 mM (H_2SO_4) eluent.

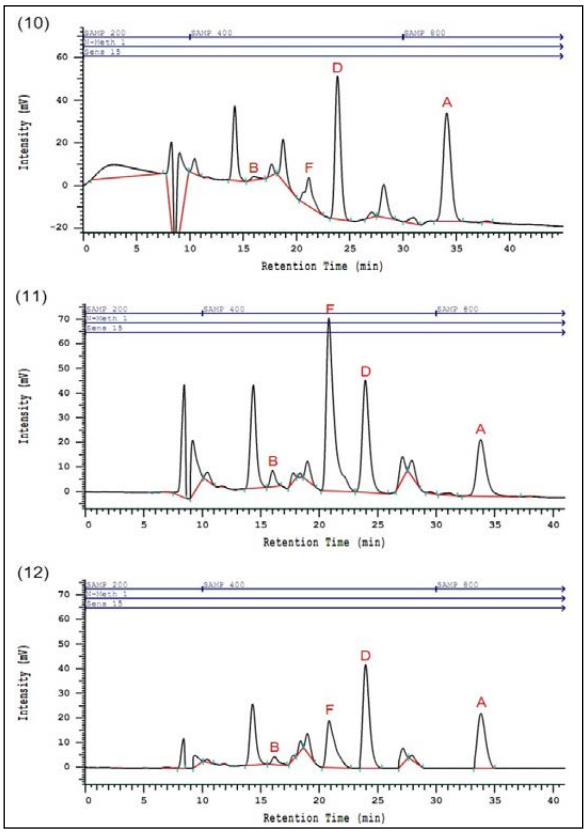

Figure 5. Legend: (10) *Balfourodendron riedelianum* (11) *Pinus elliotti* (12) *Brosimum spp.* Compounds identification: (A) Ethanol, (B) Fructose, (C) Glycerol, (D) Acetic acid, (E) Glucose and (F) Lactic acid. Column, Transgenomic (ICE-ION 300); injection flow rate, 0.5 µL/min; mobile phase, 8.0 mM (H_2SO_4) eluent.

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		min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]	min[a]	g/L[b]
7	*Cedrela*	33.81	2.25	16.11	0.03	*n.d	*n.d	23.97	*n.d	*n.d	*n.d	20.84	1.50
8	*Ocotea*	33.80	0.76	16.16	0.15	*n.d	*n.d	23.94	*n.d	*n.d	*n.d	20.84	0.69
9	*Laurus*	33.80	2.22	*n.d	*n.d	*n.d	*n.d	20.83	1.52	*n.d	*n.d	23.95	*n.d
10	*Balfouro dendron*	34.09	2.27	16.00	0.06	*n.d	*n.d	23.84	*n.d	*n.d	*n.d	21.15	0.77
11	*Pinus*	33.80	2.30	16.01	0.15	*n.d	*n.d	23.94	*n.d	*n.d	*n.d	20.82	3.79
12	*Brosimum*	33.81	1.91	16.16	0.09	*n.d	*n.d	23.97	*n.d	*n.d	*n.d	20.85	1.16

Table 5. Statistic treatment with the mean values of standard error and deviation

One-Sample Statistics				
Sample	**N°**	**Mean**	**Std. Deviation**	**Std. Error Mean**
Hymenolobium	4	1,5600	,01633	,00816
Myroxylon	4	2,5550	,02082	,01041
Tabebuia	4	1,8175	,01708	,00854
Nectandra	4	2,7250	,02380	,01190
Ochotea	4	2,1775	,02500	,01250
Cedrelinga	4	2,9850	,01291	,00645
Cedrela	4	2,2300	,01826	,00913
Ocotea porosa	4	,7600	,01633	,00816
Laurus nobilis	4	2,2200	,02160	,01080
Balfourodendron	4	2,2375	,02986	,01493
Pinus Elliotti	4	2,315	,0265	,0132
Brosimum	4	1,9175	,01708	,00854

Faced with the analysis showed (Table 5) it is evidenced that the standard error obtained, based on the error between the averages of the samples is not significant in relation to the test results.

Table 6. Statistic treatment with the probability distribution inside confidence interval of 95%.

One-Sample Test						
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Hymenolobium	191,060	3	0,000	1,56000	1,5340	1,5860
Myroxylon	245,476	3	0,000	2,55500	2,5219	2,5881
Tabebuia	212,844	3	0,000	1,81750	1,7903	1,8447
Nectandra	228,946	3	0,000	2,72500	2,6871	2,7629
Ochotea	174,200	3	0,000	2,17750	2,1377	2,2173
Cedrelinga	462,434	3	0,000	2,98500	2,9645	3,0055
Cedrela	244,284	3	0,000	2,23000	2,2009	2,2591
Ocotea porosa	93,081	3	0,000	,76000	,7340	,7860
Laurus nobilis	205,532	3	0,000	2,22000	2,1856	2,2544
Balfourodendron	149,862	3	0,000	2,23750	2,1900	2,2850
Pinus Elliotti	174,998	3	0,000	2,3150	2,273	2,357
Brosimum	224,555	3	0,000	1,91750	1,8903	1,9447

Based on the statistic analysis (Table 6), the probability distribution for all species and consequently their samples is two-sided or regular. Being based by significance levels disregarded by the analysis, since these must be ≤ 0.05 to attend the requirement inside the confidence interval of 95%.

Besides ethanol, glycerol is regarded as the most important component from the quantitative viewpoint. However, glycerol reduces the positive effect of nutrients, resulting in minor hydrolysis yields (Tengborg et al., 2001). In a biomass-to-ethanol process a reduction in cellulose conversion, as well as an accumulation of glycerol was observed with increased recirculation of the process stream. In this work, concentrations of glycerol were identified only using Hymenolobium petraeum 0.264 g/L specimen.

Usin sugar cane bagasse, the maximum ethanol volumetric productivities of 0.29-0.30 g/L.h were achieved, varying between 2 g/L and 4 g/L in the first 8 hours of fermentation (Santos et al., 2010b). However in this work, ethanol was produced in the ranges: *Cedrelinga catenaeformis* 3.00 g/L; followed by *Nectandra lanceolata* 2.71 g/L; *Myroxylon peruiferum* 2.55 g/L; *Balfourodendron riedelianum* 2.27 g/L; *Pinus elliotti* 2.30 g/L; *Cedrela fissilis Vell.* 2.25 g/L; *Laurus nobilis* 2.22 g/L; *Ocotea catharinensis* 2.18 g/L; *Brosimum spp.* 1.91 g/L; *Tabebuia cassiniodes* 1.81 g/L; *Hymenolobium petraeum* 1.56 g/L and *Ocotea porosa* 0.76 g/L. Softwoods showed similar ethanol production than hardwoods. The (Fig. 6) shows the chemical composition of diluted nitric hydrolysate liquor after fermented.

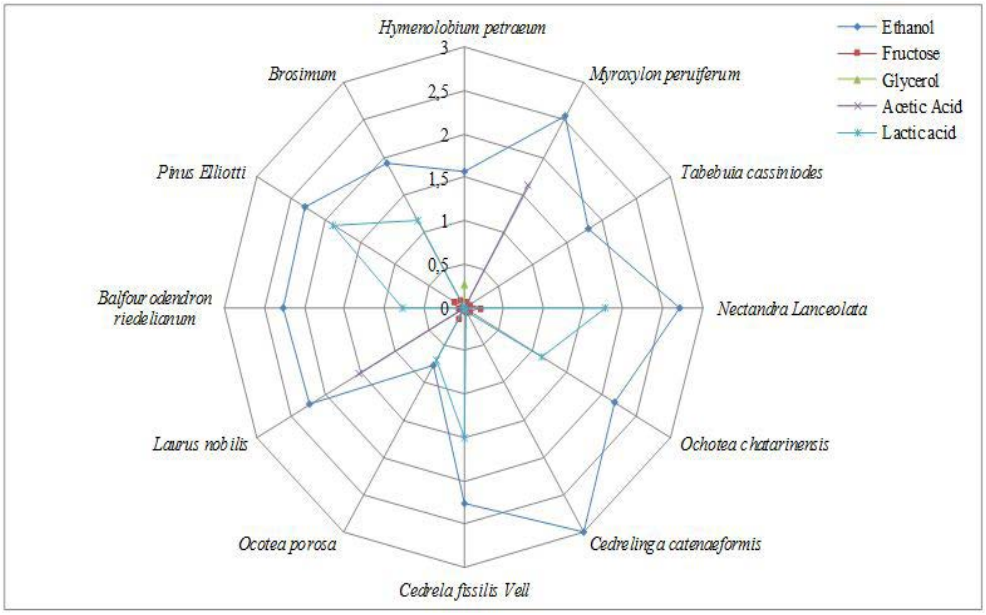

Figure 6. Mapped standard compounds dispersion in hydrolysate liquor per sample.

Actually, these wood residues are commonly used for heat generation by burning them in boilers to produce steam, so this work contributes in attempt to reduce the spent of raw materials. This optimizes the process of cellulose-to-ethanol production by monitoring hydrolysis and fermentation, by using proton-exchange HPLC technique.

Data obtained from the companies managers, indicates that possibly exists more than 4153 furniture and wood processes/transformation factories in the researched region. Also, studies made in the areas of samples collection indicated that at least 30% of each m^3 are estimated to produce wood chips in the regions of Santa Catarina and Paraná states, located at south Brazil. A volume in produced wood chips an amount almost incalculable of these promising biomass for energetic purposes.

CONCLUSIONS

The method used in this paper demonstrates to be a useful analytical for monitoring the ethanol production using wood chips, through mapping compounds in fermented liquor. HPLC employing proton-exchange technique evidenced to be rapid and precise for analysis. Calibrations for all target compounds were satisfactory despite of the complex matrix content present in nitric hydrolysate liquor. Also, good spectrum separations among chromatography curves were obtained. All analyzed species demonstrated the possibility of utilization in cellulose-to-ethanol conversion. Glucose, fructose, acetic acid, lactic acid, and glycerol were standard compounds mapped. *Saccharomyces cerevisiae* produced ethanol in the range of 3.00 g/L using *Cedrelinga catenaeformis* and 0.76 g/L/h using *Ocotea porosa*, respectively after 8h fermentation essay. All wood residues demonstrated cellulosic ethanol production, demonstrating that these are feasible for use as biomass instead its usual discard or use, burned in boilers for steam generation. It´s also becomes possible to utilize the lignin fractions of these biomasses for this purpose.

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