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## HPLC mapping of second generation ethanol production with lignocelluloses wastes and diluted sulfuric hydrolysis

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**ABSTRACT.** Wood wastes are potential material for second generation ethanol production within the concept of residual forest bio-refinery. Current paper reports on ethanol production employing an HPLC method for monitoring the chemical content dispersed in the hydrolysate liquor after fermented. The proton-exchange technique was the analytical method employed. Twelve types of wood chips were used as biomass, including *Hymenolobium petraeum*, *Tabebuia cassinoides*, *Myroxylon peruiferum*, *Nectandra lanceolata*, *Ocotea catharinensis*, *Cedrelina catenaeformis*, *Cedrela fissilis* Vell., *Ocotea porosa*, *Laurus nobilis*, *Balfourodendron riedelianum*, *Pinus Elliotti* and *Brosimum* spp. The influence of diluted sulfuric hydrolysis on the yeast *Saccharomyces cerevisiae* during the fermentation assay was also investigated. Standard compounds mapped in the analysis comprised fructose, lactic acid, acetic acid, glycerol, glucose and ethanol. The yeast showed ethanol productivity between 0.75 and 1.91 g L<sup>-1</sup> h<sup>-1</sup>, respectively, without the addition of supplementary nutrients or detoxification. The use of these materials for the bioconversion of cellulose into ethanol has been proved. Current analysis contributes towards the production of biofuels by wastes recovery and by process monitoring and optimization.

**Keywords:** alternate fuels, biomass, bioconversion, monitoring, hydrolyzed liquor, yeast.

## HPLC mapeando a produção de etanol de segunda geração utilizando resíduos lignocelulósicos e hidrólise sulfúrica diluída

**RESUMO.** Resíduos de madeira são considerados materiais potenciais para a produção de etanol de segunda geração em um conceito de biorrefinaria residual florestal. Este artigo apresenta a produção de etanol, utilizando um método de HPLC para monitorar o conteúdo químico disperso no licor hidrolisado após fermentado. O método analítico empregado foi a técnica de troca de prótons. Foram utilizados doze tipos diferentes de resíduos de madeira como biomassa, incluindo: *Hymenolobium petraeum*, *Tabebuia cassinoides*, *Myroxylon peruiferum*, *Nectandra lanceolata*, *Ocotea catharinensis*, *Cedrelina catenaeformis*, *Cedrela fissilis* Vell., *Ocotea porosa*, *Laurus nobilis*, *Balfourodendron riedelianum*, *Pinus Elliotti* and *Brosimum* spp. A influência da hidrólise com ácido sulfúrico diluído sobre a levedura *Saccharomyces cerevisiae* durante o ensaio de fermentação também foi investigada. Os compostos padrões mapeados na análise foram: frutose, ácido láctico, ácido acético, glicose, glicerol e etanol. A levedura mostrou produtividades de etanol entre 0,75 a 1,91 g L<sup>-1</sup> h<sup>-1</sup>, respectivamente, sem a adição de nutrientes suplementares ou desintoxicação. Foi comprovada a possibilidade do uso desses materiais para a bioconversão da celulose em etanol. Este trabalho contribui para a produção de biocombustíveis através da recuperação de resíduos, monitoramento e otimização do processo.

**Palavras-chave:** combustíveis alternativos, biomassa, bioconversão, monitoramento, licor hidrolisado, levedura.

### Introduction

Concerns on the depletion of fossil fuel resources and climate changes attributed to CO<sub>2</sub> emissions give rise to strong global interest in renewable and carbon-neutral energy sources, as well as the production of chemical feedstock from vegetal sources (DOHERTY et al., 2011; ZHU; PAN, 2010). Biomass, one of the more important renewable energy sources, offers many advantages over petroleum-based fuels. The

environment, economy and consumers are greatly benefitted when employing several types of biofuels. Moreover, they are biodegradable and contribute towards the planet's sustainability (DEMIRBAS et al., 2008, 2009, 2011).

The production of second generation ethanol from biomass is actually one method to reduce the consumption of crude oil and environmental pollution (BALAT et al., 2008, BALAT, 2011). Brazil

is one of the most important producers of ethanol, especially from sugarcane (HARTEMINK, 2008). On the one hand, diluted acid hydrolysis is one of the most popular methods for converting celluloses into ethanol (KRISHNA et al., 2001; XIE et al., 2011; WEN et al., 2010). On the other hand, a great variety of degrading compounds are released during production (CARRASCO et al., 2010), most of which contain inhibitory activities which may affect the process and result in reduced yields and efficiency of biochemical conversion. Therefore, an efficient analytical approach is increasingly needed to quantify these compounds for a better understanding of their roles in the bioconversion process (XIE et al., 2011).

Great efforts have been taken to analyze the degradation products in biomass hydrolysate, with varying degrees of success. High performance liquid chromatography (HPLC) is a method frequently used in hydrolysis liquor analysis, although gas chromatography (GC), coupled to flame ionization or mass spectrometry detection, was also successful in identifying a large variety of organic degrading products (KARAGÖZ et al., 2004; KLINKE et al., 2002).

The deployment of GC methodologies in quantitative studies has been impaired by inherent complexities of derived samples of unknown composition. Liquid chromatography (LC) methods, employing post-column UV or refractive index detection, have historically been jeopardized from incomplete resolution of its analysis. As a result, LC analysis of degrading products in hydrolysate samples has typically employed multiple chromatographic modes and several detection strategies, whereas the choice of utilization mainly depends on the analytical class (CHEN et al., 2006; 2009; LUO et al., 2002; PERSSON et al., 2002; ZEAMANN; BOBLETER, 1993).

Current assay demonstrates the production of second generation ethanol, utilizing several wood chips as biomass, for the bioconversion of cellulose into glucose; a sulfuric hydrolysis treatment was performed

and the yeast *Saccharomyces cerevisiae* was used in the fermentation assays. An HPLC method employing the proton-exchange technique was used in the monitoring the process to map the compounds dispersed in the hydrolysate liquor.

## Material and methods

All chemicals from analytical grade were purchased from commercial sources. Tests were performed in the Laboratory of the Department of Industrial Technology, Universidade Estadual de Santa Catarina (Udesc), and in the Laboratory of the Department of Chemistry, Universidade Regional de Joinville (Univille).

Samples were collected in wood transformation plants and furniture industries in the southern Brazilian states of Santa Catarina and Paraná. The material analyzed hailed from twelve different wood species including: *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.

During hydrolysis and fermentation assays, at least one experimental condition was duplicated for each set of experiments, ensuring the consistence and accuracy of results. After collected, the samples were cataloged, packed in containers (5 kg) and stored for acclimatization in laboratory at 20°C during one week. Samples were then milled with a 0.75 mm screen centrifugal mill. Only samples that passed through a 0.6 mm sieve mesh were selected for tests (Tyler system). Klason lignin was determined following guidelines by the Technical Association of the Pulp and Paper Industry (TAPPI, 2002) T-222. Holocellulose content (cellulose + hemi-celluloses) was determined by (TAPPI, 2009) T-203. The determination of density was based on (TAPPI, 2001) T-258. Standards T-264 (TAPPI, 2007) and T-257 (TAPPI, 2012) were also consulted for the tests. Table 1 shows some characteristics of the samples studied.

**Table 1.** Physical and chemical properties of the wood species analyzed.

Hard/Softwood	Name of Species	Chemical (%)			Physical (g cm <sup>-3</sup> )
		Cellulose	Hemicellulose	Lignin	Density
Hardwood	<i>Hymenolobium petraeum</i>	42.2	27.2	28.4	0.67
Hardwood	<i>Myroxylon peruiferum</i>	41.1	25.4	27.3	0.61
Hardwood	<i>Tabebuia cassinoides</i>	44.2	29.4	25.6	0.99
Softwood	<i>Nectandra lanceolata</i>	45.4	30.1	23.6	0.50
Hardwood	<i>Ocotea catharinensis</i>	44.7	27.5	27.7	0.62
Hardwood	<i>Cedrelinga catenaeformis</i>	40.6	29.5	27.2	0.50
Hardwood	<i>Cedrela fissilis</i> Vell.	40.4	28.1	29.7	0.47
Hardwood	<i>Ocotea porosa</i>	43.8	26.9	30.2	0.66
Softwood	<i>Laurus nobilis</i>	46.7	32.4	20.1	0.44
Softwood	<i>Balfourodendron riedelianum</i>	45.1	26.6	22.2	0.69
Softwood	<i>Pinus elliotti</i>	45.3	30.5	22.9	0.48
Hardwood	<i>Brosimum</i> spp.	44.1	26.5	26.2	0.54

### Biomass treatment

Samples underwent a 2% ( $\text{H}_2\text{SO}_4$  v v<sup>-1</sup>) sulfuric hydrolysis and maintained during 2h in a water bath at  $120 \pm 5^\circ\text{C}$  to partially remove the lignin fractions, so the yeast had an easier access to the cellulose. An additional step of partial delignification was performed with alkaline treatment 1.0% ( $\text{NaOH}$  v v<sup>-1</sup>) maintained at  $120 \pm 5^\circ\text{C}$  during 30 min. (VASQUEZ et al., 2007, ZHANG et al., 2010) to correct treatment's pH from 2 to 5.5. The hydrolysis assay was performed in steps following procedures established by the National Renewable Energy Laboratory (NREL). The cellulose solid phase was separated with a hydraulic press and the content was filter by applying 2 ton pressure over an area of  $200\text{ cm}^2$  (MAEDA et al., 2011).

### Microorganism culture

Strains of *Saccharomyces cerevisiae* were available from the microbiological culture collection of the Laboratory of Chemistry at Udesc. All materials were previously sterilized using a steam autoclave at  $120 \pm 5^\circ\text{C}$ . For seed culture, strain was grown in an incubator by the agar-malt method consisting of malt ( $5\text{ g L}^{-1}$ ), yeast ( $5\text{ g L}^{-1}$ ) extract, peptone ( $5\text{ g L}^{-1}$ ), agar ( $20\text{ g L}^{-1}$ ) and distilled water (1L), supplemented with ( $1\text{ g L}^{-1}$ ) glucose in a flask. Prior to their use as inocula for fermentations, the culture was aerobically propagated utilizing 200 mL Erlenmeyer flasks. After incubation, the strains were grown overnight in a regulate climate environment ( $30^\circ\text{C}$ ), stirred at 200 rpm, and placed inside a shaking bath until the concentration reached approximately 3% (w w<sup>-1</sup> per liter). They were then separated by centrifugation, albeit monitored by optical density measurements (OD-600 nm, Agilent UV-visible Spectroscopy system).

### Preparation of inocula

After hydrolysis, 250 g of each processed biomass were separately fermented in 200 mL Erlenmeyer flasks. Samples were inoculated with 3% of the microorganism colony formed and the solution was completed with 50 mL of distilled water. During the fermentation assay, samples were stored in anaerobic conditions ( $30^\circ\text{C}$ ) in a regulated climate environment during 8 hours, after which HPLC analysis was performed.

### Analytical method

Analyses were performed with High Performance Liquid Chromatography (Merck-Hitachi) model (D-7000 IF), with refractive index (RI) detector and single column (Transgenomic ICE-ION/300). The analytical method employed

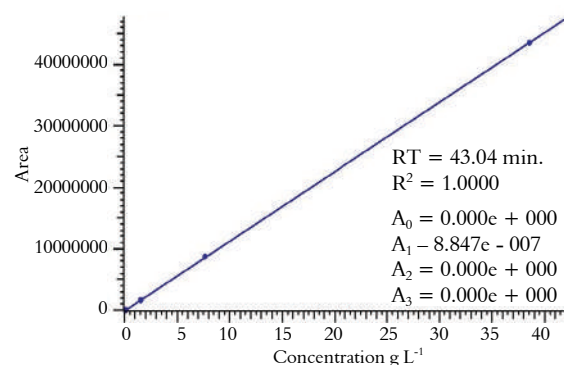
was the proton-exchange technique, with ultra-pure water as mobile phase and 8.5 mM of sulfuric acid as eluent (isocratic).

### Technical data

Acquisition method: acid lactic-ion 300; column type: RP18; pump A type: L-7100; solvent A: HAc 1%; solvent B:  $\text{H}_2\text{SO}_4$  8.5 mM; solvent C: methanol; solvent D: can; method description: acid lactic determination using column (Transgenomic Ice-Ion 300), chromatography type: HPLC channel: 2, peak quantification: area, calculation method: EXT-STD. Additional parameters employed in HPLC analyses comprised injection volume  $20\text{ }\mu\text{L}$ ; column-temperature  $30^\circ\text{C}$ . Samples were injected using an auto-sampler with injection volume of  $0.25\text{ }\mu\text{L min}^{-1}$ .

### Equipment calibration

Calibration of HPLC was used to determine with precision the concentrations of analytcs. The calibration curves were from series 4663 (glycerol), and series 4731, for the other target compounds. Figure 1 shows the calibration curve for ethanol.



**Figure 1.** Retention times (RT) for the ethanol compound and calibration calculus of the equipment by equation (1):

$$y = (1/a) \cdot x$$

where: y = surface area, x = concentration of standard compounds is the straight slope, a = angular coefficient.

The Table 2 shows the retention times for the compounds analyzed and the isocratic eluent used.

**Table 2.** Retention times (RT) and isocratic eluent.

Compounds	Isocratic eluent ( $\text{H}_2\text{SO}_4$ )	
	Retention Time (RT)	Mobile phase
identification	(min.)	(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

It must be underscored that the acceptability criteria for the acknowledgement of an individual component in validation studies employing high-purity reference samples, required retention times for a given analytic within  $\pm 2\%$  average for each respective standard used to construct the calibration curve for that analytic (CHENG et al., 2010).

### Quantifying target compounds

For the quantification of compounds, approximately 5 mL of hydrolysate fermented liquor from each sample was stored at 2°C during 30 min. until chromatography analysis. Samples were diluted (1:1 v v<sup>-1</sup>) with ultra-pure water and filtered with a 0.45- $\mu$ m Millipore membrane (VWR Scientific, Suwanee, GA, USA). Samples were then transferred to a vial (auto-sampler vial specific for chromatography) and placed in the machine carrousel.

### HPLC analytical performance

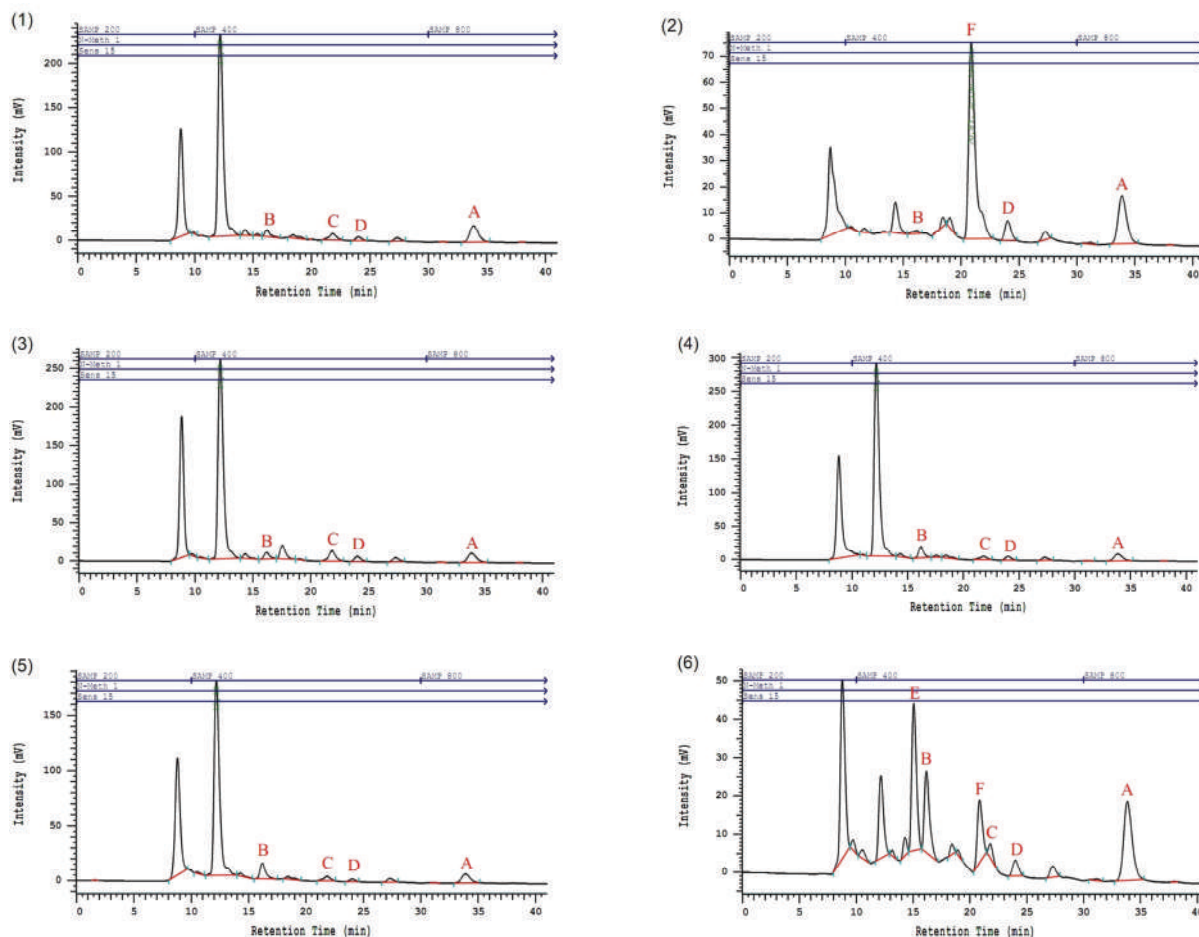
The equipment mapped the chemical compounds dispersed in the hydrolysate liquor in a peak

quantification area mode. The final concentration of ethanol after 8 hours of fermentation assay is given in g L<sup>-1</sup> h<sup>-1</sup>. Figures 2 and 3 and Tables 3 and 4 demonstrated the main results of current research.

### Results and discussion

All target compounds remained within the calibration range. The identification of compounds was satisfactory and they appeared at peaks with good resolution between the analytics. Differences in resolutions among the compounds always respect the interval of  $\pm 2\%$  for retention times, as expected. The successfully mapped target compounds were glucose, fructose, lactic acid, acetic acid, glycerol and ethanol.

HPLC's capacity to separate the curves and still maintain linear peaks demonstrated its efficiency. Indeed, the temperature of the column remained equal to 30°C. In evaluating the target compounds, a total time of 40 min. was needed, using flow injection of 0.5 mL min.<sup>-1</sup>, which remained equal until the end of the analysis.



**Figure 2.** Chromatogram: (1) *Hymenolobium petraeum*; (2) *Myroxylon peruiferum*; (3) *Tabebuia cassinoides*; (4) *Nectandra lanceolata*; (5) *Ocotea catharinensis*; (6) *Cedrelinga catenaeformis*. Identification of compounds: (A) Ethanol; (B) Fructose; (C) Glycerol; (D) Acetic acid; (E) Glucose; (F) Lactic acid. Transgenomic column (ICE-ION 300); flow rate 0.5 mL min.<sup>-1</sup>; mobile phase 8.0 mM H<sub>2</sub>SO<sub>4</sub>.

Glucose fractions which were not consumed by the microorganism during the initial 8h of the fermentation assay were detected using *Cedrelinga catenaeformis* (0.8356 g L<sup>-1</sup>) and *Laurus nobilis* (0.0148 g L<sup>-1</sup>). The above evidenced positive glucose consumption among the samples.

Moreover, the ability of the microorganism to survive in an environment fixed with pH 5.5 was also demonstrated.

Studying robust cellulosic ethanol production (SPORL), utilizing wood chips of pretreated lodge-pole pine and an adapted strain of *S. cerevisiae*, Tian et al. (2010) produced ethanol ranging between 0.81 and 2.0 g L<sup>-1</sup> h<sup>-1</sup>, over 4 and 24 hours in the fermentation assay, respectively, in the un-detoxified run.

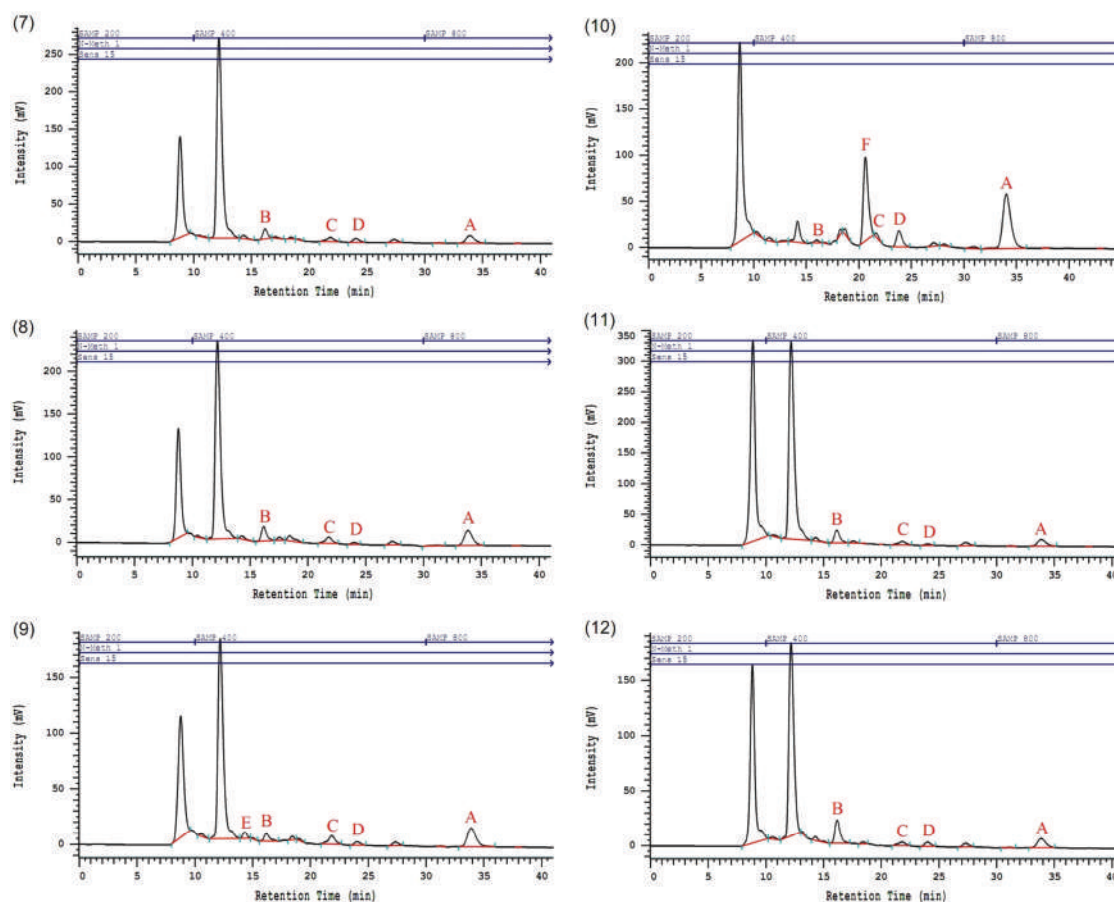
When Brandberg et al. (2004) used wood chips and HPLC to analyze the hydrolysate liquor, the researchers proved that the more viable strains were able to consume nearly 2.0 g of glucose per gram of biomass during the first 8h in the fermentation assay, with ethanol production rates

ranging between 0.1 and to 0.5 g L<sup>-1</sup> h<sup>-1</sup> during the same period.

According to Brandberg et al. (2004), even for the most metabolically active strains, the colony-forming capacity decreased by at least two orders of magnitude over the initial 8h. Actually the inhibitory effect of the diluted acid on biomass seemed to be directly linked to the reproductive ability of the microorganism, or biosynthesis, rather than to its catabolic activity.

It is highly relevant to emphasize that several researchers, such as Chen et al. (2009, 2010), Matías et al. (2011) and others, have spent efforts in substantial contributions towards HPLC analytical techniques by monitoring the compounds dispersed in hydrolysate liquor and thereby optimizing the process.

Fructose fractions were detected among all samples, with results varying between 0.012 g L<sup>-1</sup>, using *Cedrela fissilis* Vell, and 0.15 g L<sup>-1</sup>, using *Balfourodendron riedelianum*. The reminiscent presence of this carbohydrate indicates that fructose was consumed at a lower rate than glucose during the initial hours of the fermentation assay.



**Figure 3.** Chromatogram: (7) *Cedrela fissilis* Vell; (8) *Ocotea porosa*; (9) *Laurus nobilis*; (10) *Balfourodendron riedelianum*; (11) *Pinus elliotti*; (12) *Brosimum* spp. Identification of compounds: (A) Ethanol; (B) Fructose; (C) Glycerol; (D) Acetic acid; (E) Glucose; (F) Lactic acid. Transgenomic column (ICE-ION 300); flow rate 0.5 mL min<sup>-1</sup>; mobile phase 8.0 mM H<sub>2</sub>SO<sub>4</sub>.



**Table 3.** Quantitative results of mapped standard compounds.

Wood species	A - Ethanol		B - Fructose		C - Glycerol		D - Acetic acid		F - Lactic acid	
	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>
1 <i>Hymenolobium petraeum</i>	35.85	1.71	*n.d	*n.d	*n.d	*n.d	23.93	*n.d	20.82	0.89
2 <i>Myroxylon peruiferum</i>	33.88	1.69	*n.d	*n.d	*n.d	*n.d	23.94	*n.d	20.83	0.68
3 <i>Tabebuia cassiniodes</i>	33.87	1.11	*n.d	*n.d	*n.d	*n.d	23.90	*n.d	20.84	0.99
4 <i>Nectandra lanceolata</i>	33.85	0.95	16.05	0.03	*n.d	*n.d	23.91	*n.d	20.81	0.58
5 <i>Ochotea chatarinensis</i>	34.87	0.75	*n.d	*n.d	*n.d	*n.d	23.94	*n.d	20.83	0.77
6 <i>Cedrelinga catenaeformis</i>	33.84	1.91	*n.d	*n.d	*n.d	*n.d	23.91	*n.d	20.81	0.53

<sup>a</sup>(min.): retention times, <sup>b</sup>(g h<sup>-1</sup> L<sup>-1</sup>): concentration, \*n.d: not detected.

**Table 4.** Quantitative results of mapped standard compounds.

Wood species	A - Ethanol		B - Fructose		C - Glycerol		D - Acetic acid		F - Lactic acid	
	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>	(min.) <sup>a</sup>	(g L <sup>-1</sup> h <sup>-1</sup> ) <sup>b</sup>
7 <i>Cedrela fissilis Vell</i>	33.88	0.93	16.11	0.01	*n.d	*n.d	23.95	*n.d	20.84	0.75
8 <i>Ocotea porosa</i>	33.83	1.59	*n.d	*n.d	21.79	0.23	23.93	*n.d	20.82	0.29
9 <i>Laurus nobilis</i>	33.88	1.59	16.08	0.02	*n.d	*n.d	23.93	*n.d	20.83	1.38
10 <i>Balfourodendron riedelianum</i>	34.01	1.60	15.90	0.15	21.78	*n.d	23.83	*n.d	20.64	1.64
11 <i>Pinus elliotti</i>	33.85	1.03	*n.d	*n.d	*n.d	*n.d	23.91	*n.d	20.81	0.69
12 <i>Brosimum</i>	33.84	0.77	16.17	0.02	*n.d	*n.d	23.92	*n.d	20.81	0.84

<sup>a</sup> (min.): retention times, <sup>b</sup> (g h<sup>-1</sup> L<sup>-1</sup>): concentration, \*n.d: not detected.

According to Costa et al. (2008), as a result of the catabolic activities of *S. cerevisiae*, lactic acid was produced during fermentations and consequently ethanol yields were reduced. In current research lactic acid production was detected in *Ocotea porosa* (0.293 g L<sup>-1</sup> h<sup>-1</sup>) and *Balfourodendron riedelianum* (1.644 g L<sup>-1</sup> h<sup>-1</sup>).

It should be underscored that in the evaluation of the production of ethanol on a large scale, all parameters must be completely free from competing source organisms, due to the fact that the whole production could be enormous advantageous, with the possibility to utilize antibiotics, although extra costs are required in the process.

A study conducted by Moreira et al. (2008) demonstrated that the production of microbial lactic acid was present in almost several fermentation tanks of sugarcane in ethanol conversion plants. The same author reported that lactic acid was produced, ranging between 6.84 and 3.48, Mol L<sup>-1</sup>, thereupon contributing with significant diminutions in the total amounts of ethanol produced.

In current study, fractions of acetic acid were also registered among the chromatograms, albeit in small quantities, without ethanol production being affected.

In a previous research, glycerol reduced nutrient effects, thereby reducing hydrolysis and ethanol yields (TENGBORG et al., 2001). Indeed Stenberg et al. (1998), studying biomass-to-ethanol conversions, reported that reductions in cellulose conversion and glycerol accumulation were also observed, with increased recirculation of the process flow.

This compound normally appears as a byproduct of fermentations when utilizing yeasts, as result of the catabolic activities of the microorganism, together with carbonic gas (CO<sub>2</sub>), certain alcohols,

and pyruvic and succinic acids. However, from the quantitative viewpoint, only the most important component is glycerol. However, glycerol fractions were only detected in *Ocotea porosa* 0.23 g L<sup>-1</sup>.

#### Ethanol productivity

Cellulosic ethanol was produced with varying results: *Myroxylon peruiferum* (1.69 g L<sup>-1</sup> h<sup>-1</sup>); *Tabebuia cassiniodes* (1.11 g L<sup>-1</sup> h<sup>-1</sup>); *Balfourodendron riedelianum* (1.60 g L<sup>-1</sup> h<sup>-1</sup>); *Cedrela fissilis Vell.* (0.93 g L<sup>-1</sup> h<sup>-1</sup>); *Cedrelinga catenaeformis* (1.91 g L<sup>-1</sup> h<sup>-1</sup>); *Brosimum* (0.77 g L<sup>-1</sup> h<sup>-1</sup>); *Ocotea catharinensis* (0.75 g L<sup>-1</sup> h<sup>-1</sup>); *Pinus elliotti* (1.03 g L<sup>-1</sup> h<sup>-1</sup>); *Ocotea porosa* (1.59 g L<sup>-1</sup> h<sup>-1</sup>); *Laurus nobilis* (1.59 g L<sup>-1</sup> h<sup>-1</sup>); *Hymenolobium petraeum* (1.71 g L<sup>-1</sup> h<sup>-1</sup>); and *Nectandra lanceolata* (0.95 g L<sup>-1</sup> h<sup>-1</sup>).

Zhu et al. (2011) found similar results when studying Eucalyptus sludge, sulfuric hydrolysis and enzymatic saccharifications and registered ethanol concentrations ranging between 1.88 and 36.42 g L<sup>-1</sup>, during the initial 120h of the fermentation assay.

Current analysis showed that the highest quantities of ethanol were produced by *Cedrelinga catenaeformis*, *Ocotea porosa*, *Balfourodendron riedelianum* and *Laurus nobilis*. These samples actually revealed a potential for their employment at high scale biomass in ethanol plants.

From the technical viewpoint, current assay successfully demonstrated the possibility to produce second generation ethanol, utilizing diluted sulfuric hydrolysis and several lignocellulosic materials. In the case of ethanol productivity, the softwoods showed similar productivity to hardwoods. Research also contributes towards the reduction of these raw materials by employing them as fuel. These promising results demonstrated that bioconversion was efficient even without the need of detoxifications or nutrient supplementation.

## Conclusion

Current investigation validated the bioconversion of cellulose into ethanol by monitoring the hydrolysate liquor with subsequent fermentation. The ethanol productivity ranged between 1.91 and 0.75 g L<sup>-1</sup> h<sup>-1</sup> after 8h of fermentation assay. The yeast showed resistance and positive carbohydrates consumption, coupled to low biosynthesis of degrading compounds. The HPLC proton-exchange technique proved to be a quick, sensitive and precise method to analyze the hydrolysate liquor even after fermented, therefore demonstrating good resolution among the analytics. Furthermore, the risk of degradation of the target compounds under analysis seemed to be low when such technique is used. Calibrations and recoveries for all standard compounds were satisfactory, despite the complex matrix content of the hydrolysate.

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