Research and development aiming at the integrated exploitation of sugarcane bagasse for the biotechnological production of lignocellulosic ethanol

André Ferraz

Joint presentation for five BIOEN-FAPESP projects

1. Adriane MF Milagres and André Ferraz (EEL-USP/Lorena)
2. Aprígio A Curvelo (IQ-USP/São Carlos)
3. Celina L Duarte (IPEN-CNEN/São Paulo)
4. Marco AP Lima and Jayr Amorim Filho (IFGW-UNICAMP; CTBE-CNPEM/Campinas)
5. Sílvio S Silva, Maria GA Felipe (EEL-USP/Lorena) and Maria J Bell (UFJF/Juiz de Fora)
Overall focus of the five projects

- To understand the origins of the recalcitrance in the cell walls of sugar cane;
- To propose proper methods for characterization of the sugar cane lignocellulose;
- To develop basic knowledge on the pretreatment of this material to overcome the recalcitrance and to prepare C-5 hydrolysates;
- To propose adequate methods for conversion of the lignin and polysaccharide fractions into useful chemicals by using biological, chemical, physical or combined processes
1. Topochemistry, porosity and chemical composition determining successful enzymatic saccharification of sugarcane bagasse (08/56256-5)

Adriane MF Milagres, André Ferraz Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, SP, Brasil

Objectives

- To prepare sugarcane bagasse models with different lignin contents based on chemical delignification or to evaluate experimental hybrids with contrasting lignin contents;

- To study the lignin distribution in the cell walls of these samples based on UV-microspectrophotometry;

- To evaluate the digestibility of the sugarcane samples with commercial hemicellulases and cellulases;

- Produce, characterize and apply novel enzymes for lignin, hemicellulose and cellulose degradation
Publications based on the project results


Fernanda M Mendes, Germano Siqueira, Walter Carvalho, André Ferraz; Adriane MF Milagres. ENZYMATIC HYDROLYSIS OF CHEMITHERMOMECHANICALLY PRETREATED SUGARCANE BAGASSE AND SAMPLES WITH REDUCED INITIAL LIGNIN CONTENT. *Biotechnology Progress*, v. 27, p. 395-401, 2011.

Fernando Masarin, Daniela B Gurpilhares, David CF Baffa, Márcio HP Barbosa, Walter Carvalho, André Ferraz, Adriane MF Milagres. CHEMICAL COMPOSITION AND ENZYMATIC DIGESTIBILITY OF SUGARCANE CLONES SELECTED FOR VARIED LIGNIN CONTENT. *Biotechnology for Biofuels*, v. 4, p. 55, 2011.
Topochemical distribution of lignin

Rind fibers
Abs\(_{280 \text{ nm}}\) = 0.40

Pith fibers
Abs\(_{280 \text{ nm}}\) = 0.39

Similar lignin contents
Correlation of enzymatic hydrolysis efficiency of the rind with the lignin contents and UV-microspectrophotometric data.

Why different cells of sugar cane present varied recalcitrance?
Enzymatic hydrolysis of alkaline-sulfite pretreated sugar cane bagasse

- Untreated
- Alkali-treated + RMP
- Alkaline-sulfite treated + RMP
- RMP treated
- Untreated

Cellulose conversion (%)

Hydrolysis time (h)

Cellulose conversion (%)

Lignin or hemicellulose removal during pretreatment (%)

- hemicellulose
- lignin
Enzymatic hydrolysis of different regions dissected from the sugar cane internodes from hybrids with varied lignin contents.

Cellulose conversion after 72h of enzymatic hydrolysis (%)

Available cellulose/area occupied by vascular bundles (arbitrary units)

= cellulose / (lignin + hemicellulose)

\[
y = -258.06x^2 + 294.09x + 2.1927
\]

\[
R^2 = 0.9604
\]

Cellulose conversion after 72h of enzymatic hydrolysis (%)
2. Development of analytical methodologies and organosolv delignification process applied to bagasse and straw from sugarcane (PITE, Oxiteno/Fapesp)

Antonio Aprígio S Curvelo (Instituto de Química USP/São Carlos)

Objectives

- Development of standard methods for quantitative chemical characterization of sugarcane bagasse and straw;
- Isolation and characterization of sugarcane lignin;
- Organosolv delignification of sugarcane bagasse and straw;
- Acid hydrolysis of cellulose and hemicelluloses from raw and pre-extracted bagasse and straw by means of sub and/or supercritical fluids;
- Development of analytical methodologies for on line monitoring the delignification and hydrolysis processes.
Publications based on the project results


Mass balances for the integrated organosolv processing of sugar cane bagasse
Efficient solubilization of the hemicellulosic fraction using mineral acids and supercritical fluids

Table 5  Concentrations of xylose (X), xylan (Xyn) and total xylan (TXyn) in the spent liquor and composition (wt%) of spent liquor<sup>a</sup>

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>t (min)</th>
<th>LSR (g g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>X (g L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Xyn</th>
<th>TXyn</th>
<th>G (wt%)</th>
<th>X</th>
<th>A</th>
<th>AAc</th>
<th>Gln</th>
<th>Xyn</th>
<th>Ara</th>
<th>HMF</th>
<th>F</th>
<th>Xyn&lt;sup&gt;b&lt;/sup&gt;</th>
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</table>

<sup>a</sup>G: glucose, X: xylose, A: arabinose, AAc: acetic acid, Gln: glucan, Xyn: xylan, Ara: arabinano, HMF: 5-hydroxymethylfurfural, F: furfural and TXyn: total xylan = 0.88 X + Xyn. (n.d: no detected) <sup>b</sup>Xyn*: g equiv. total dissolved xylan/100 g xylan in initial bagasse.
Efficient acid hydrolysis of the cellulosic fraction using diluted sulfuric acid at high temperatures
3. Pretreatment of sugarcane bagasse to acid or enzymatic hydrolysis applying the advanced oxidation process by ionizing radiation to ethanol biofuel production

Celina Lopes Duarte (IPEN-São Paulo)

Objectives

- To study chemical and ultrastructural changes occurring in sugar cane bagasse after pretreatment;

- To characterize the soluble sugars fraction released after the pretreatment;

- To combine the pretreatment and the fermentation technologies in order to reach minimal sub-products formation

- To evaluate the economical viability of the processes
Publications based on the project results


Effect of irradiation intensity on the chemical composition of sugar cane bagasse components
Enzymatic conversion of the pretreated materials

Enzyme complex of *Trichoderma reesei*, Celluclast 1.5 L, supplied by Novozymes (Bagsvaerd, Denmark), 5FPU/g-cellulase and Beta-glycosidase 0.5% (p/p)
Combined pretreatment using electron beam and thermal/acid processes – Solubilization of the bagasse components
4. Processing of sugarcane cellulose employing atmospheric pressure plasmas (08/58034-0)

Marco Aurélio Pinheiro Lima (IFGW-UNICAMP) and Jayr de Amorim Filho (CTBE – CNPEM / Campinas)

Objectives

- To develop atmospheric-pressure plasmas for the pretreatment of biomass;
- To scale-up the plasmas at the Brazilian Bioethanol Science and Technology Laboratory (CTBE);
- To develop theoretical studies on the interaction of the plasma electrons with lignocellulosic materials: (1) low-energy electron scattering from α-glucose and β-glucose monomers and dimmers to elucidate the differences in resonant processes responsible for the breakdown of the respective (α1→4) and (β1→4) linkages;
- To evaluate the role of water in the process
Publications based on the project results


The plasma-microwave equipment was set up and used to treat sugar cane bagasse that was previously subjected to a hydrothermal pretreatment. The treatment step was followed by optical and mass spectrometry to identify the radicals formed during the reaction.
5. Research and development aiming at the integrated exploitation of sugarcane bagasse for the biotechnological production of lignocellulosic ethanol (2008/57926-4)

Silvio Silvério da Silva and Maria GA Felipe (EEL/USP – Lorena – SP)
Maria José Valenzuela Bell (UFJF – Juiz de Fora – MG)

Objectives

- Pretreatment of sugarcane bagasse for releasing of pentoses
- Screening of new microorganisms able for utilization of the C5-sugars as carbon sources;
- Isolation of new yeasts for the bioprocessing of the sugar cane fractions;
- Comparison of different strategies for sugarcane bagasse hydrolysate detoxification;
- Fermentation of the C5-sugars by new xylose-fermenting selected strains;
- Development of new analytical methods based on spectroscopic principles for characterization of sugarcane bagasse, hydrolysates and ethanol produced by fermentation process;
Publications based on the project results

AD Ferreira, SI Mussatto, RM Cadete, CA Rosa, SS Silva. ETHANOL PRODUCTION BY A NEW PENTOSE-FERMENTING YEAST STRAIN, SCHEFFERSOMYCES STIPITIS UFMG-IMH 43.2, ISOLATED FROM THE BRAZILIAN FOREST, *Yeast* 28 (7), 547-554, 2011


Strain screening and use of selected strains for xylose utilization

224 yeast strains were isolated
32 yeast strains were selected

<table>
<thead>
<tr>
<th>Yeast species</th>
<th>Yeast strains</th>
<th>Sugars consumption (%)</th>
<th>Cells (g/L)</th>
<th>Ye_{p/x} (g/g)</th>
<th>Qp (g/L·h)</th>
<th>η (%)</th>
<th>Ethanol concentration (g/L)</th>
<th>Ye_{p/x} (g/g)</th>
<th>Xylitol concentration (g/L)</th>
<th>Fermentation time (h)</th>
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<td>0.05</td>
<td>31.6</td>
<td>4.6</td>
<td>–</td>
<td>–</td>
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<td>3.4</td>
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<td>–</td>
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Table 3. Ethanol yield [Ye_{p/x} (g/g)], xylitol yield [Ye_{p/x} (g/g)], ethanol productivity [Qp (g/L·h)], fermentation efficiency [η (%)], sugar (D-xylose and glucose) consumption (%), cell concentration (g/L), ethanol concentration (g/L) and xylitol concentration (g/L) in sugarcane bagasse hemicellulosic hydrolysate assays.
Time course of xylose consumption and ethanol production by *Scheffersomyces stipitis* selected strain

**Figure 1.** Time evolution of xylose and glucose consumption and biomass and ethanol production by *Scheffersomyces stipitis* UFMG-IMH 43.2 when cultivated in sugarcane bagasse hemicellulosic hydrolysate (conditions of assay 8; Table 1)
Joint presentation for five BIOEN-FAPESP projects

1. Adriane MF Milagres and André Ferraz (EEL-USP/Lorena) (topochemistry, porosity and saccharification)

2. Aprígio A Curvelo (IQ-USP/São Carlos) (analytical procedures, organosolv and acid hydrolysis)

3. Celina L Duarte (IPEN-CNEN/São Paulo) (ionizing radiation-AOP-pretreatment)

4. Marco AP Lima and Jayr Amorim Filho (IFGW-UNICAMP; CTBE-CNPEM/Campinas) (plasma pretreatment)

5. Sílvio S Silva, Maria GA Felipe (EEL-USP/Lorena) and Maria J Bell (UFJF/Juiz de Fora) (C-5 fermentation and process integration)