Conference

Bioenergy - II: Fuels and Chemicals from Renewable Resources

HISTORICAL AND RECENT DEVELOPMENTS IN THE ENZYMATIC HYDROLYSIS OF BRAZILIAN FEEDSTOCKS FOR ETHANOL PRODUCTION

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Main Crops in Brazil

Brasil: $851 \times 10^6$ hectares

- Ceará: $14,6 \times 10^6$ ha
- Paraíba: $5,7 \times 10^6$ ha
- Paraná: $20,0 \times 10^6$ ha

- Soya: 21,5
- Corn: 12,3
- Sugar cane: 5,6
Sugarcane Biomass

Sugarcane Plantation

Mechanized harvest

Non mechanized harvest
(bagasse)

(bagasse plus straw)
Sugarcane Biomass Residues

Straw and/or bagasse to produce ethanol:

- More ethanol per sugarcane planted area
- Increase and intensification of ethanol production
SUGARCANE BIOMASS

Cellulose
~37%

→ C₆ Sugars for ETHANOL (Renewable liquid fuel)

Hemicellulose
~28%

→ C₅ Sugars for BIO-REFINERIES or ENZYMES PRODUCTION

Lignin
~21%

→ Poly aromatic hydrophobic structure (Renewable Solid Fuel)
ENZYMATIC Saccharification

Buffer treated corn stover

Enzyme treated corn stover

Acknowledgement  NREL - USA
Ethanol and energy production

Sugar Cane: 1 Ton

- Bagasse: 300 kg
  + Ethanol: 80L
  - Electric energy: 50 KWh

- Bagasse: 300 kg
  + Ethanol: 80L
  - Lignin: 100 kg
  - Ethanol: 30L
  - Electric energy: 24 KWh
World ethanol production

Brazilian government target: to substitute 10% world's demand on gasoline

Fonte: US Energy Information Administration, F. O. Licht, Novozymes estimates
The Projects started parallel to the implementation of the Proálcool Program, in the 1970 and lasted until 1985.

- Leading Institutions: (i) Instituto Nacional de Tecnologia – INT/MCT and UFRJ, Rio de Janeiro (ii) Fundação de Tecnologia Industrial – Lorena, SP
- Other institutions: CODETEC / Campinas; FUEM / Maringá; IPT / São Paulo; UFC / Fortaleza; UFPR / Curitiba, amongst others
The main areas of the Biomass Ethanol Technology were studied using different biomass sources (e.g. sugarcane bagasse, eucalyptus, switchgrass or elephant grass).

Steam pretreatment was developed at pilot scale by FTI and also a Scholler-Madison process based on concentrated sulphuric acid to hydrolyze the biomass.

This process was used to build, in the 1980s, an industrial-scale plant, with the capacity to produce 30,000 L ethanol per day, by the company Coque e Álcool da Madeira S/A (COALBRA) in Uberlândia.
The majority of the processes used acid hydrolysis and were discontinued.

The company DEDINI, Piracicaba, continues the study based on the acid - organosolv process.

Enzymes to hydrolyze biomass (cellulases) were produced at industrial scale (10 m³) by the company BIOBRAS, MG - 1980.

Enzymes availability allowed several institutions (INT/MCT, UFC, IPT, FUEM, UCS) to study enzymatic hydrolysis.
BRAZILIAN RESEARCH ON BIOMASS ETHANOL

MEETINGS AND SCIENTIFIC PRODUCTION

➢ The majority of the results were presented and published at the “SEMINÁRIO DE HIDRÓLISE ENZIMÁTICA DE BIOMASSA” – SHEB, that were organized by the “Fundação Universidade Estadual de Maringá”.

➢ FTI results were filled as TECHNICAL NOTES

➢ Since 1993, the Brazilian Seminar on Enzyme Technology (ENZITEC), that takes place every two years allowed the progress in the area of Industrial Enzymes, including BIOMASS DEGRADING ENZYMES. The 2008 Edition (www.enzitec.com) was attended by 500 participants.
BRAZILIAN RESEARCH ON BIOMASS ETHANOL

RECENT PROJECTS ON BIOMASS ETHANOL - I

PRODUÇÃO DE CELULASES, PEROXIDASES E XILANASES

(IQ/UFRJ, 2001 – 2005)

- Bilateral collaboration CNPq/MCT - Brasil and GRICES (Gabinete de Relações Internacionais da Ciência e do Ensino Superior), Portugal

- INSTITUTIONS: IQ/UFRJ e Depto. de Biotecnologia / Instituto Nacional de Engenharia e Tecnologia Industrial (INETI) Portugal

- COORDINATORS: Elba Bon (UFRJ) and Maria Teresa A. Collaço (INETI)

- OUTCOME: (i) Technology development and specialists training at international level (ii) Revival of the research on BIOMASS ENZYMES PRODUCTION
BRAZILIAN RESEARCH ON BIOMASS ETHANOL

RECENT PROJECTS ON BIOMASS ETHANOL - II
YEAST AND GREEN CHEMISTRY (IQ/UFRJ, 2003 – 2007)

Bilateral collaboration IQ/UFRJ (Brazil) – LUND UNIVERSITY (Sweden)

- COORDINATORS: Elba Bon (UFRJ), Bärbel Hahn-Hägerdal (LU)

- INSTITUTIONS: UFRJ, INT, UnB and UFPE - International missions and research projects involved 20 Brazilian and Swedish students and researchers

- FINANTIAL SUPPORT (R$1milhão) - The Swedish Foundation for International Cooperation in Research and Higher Education (STINT)

- OUTCOME

  - Technology development and specialists training at international level

  - Revival of the research on Biomass Ethanol
BRAZILIAN RESEARCH ON BIOMASS ETHANOL

RECENT PROJECTS ON BIOMASS ETHANOL - III

BIOETANOL Project

2006 – 2009

Financial Suporte: MCT / FINEP

RESEARCH NETWORK to develop in Brazil the technology for the conversion of the sugarcane biomass (bagasse and straw) into fuel ethanol using enzymatic hydrolysis.

• 15 Universities and Research Centers

• More than 150 researchers
RECENT PROJECTS ON BIOMASS ETHANOL – III

BIOETANOL PROJECT
ETHANOL PRODUCTION VIA ENZYMATIC HYDROLYSIS OF SUGAR-CANE BAGASSE AND STRAW
Ministry of Science and Technology

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ETHANOL FROM BIOMASS

Bagasse or Straw

Pre-treatment

Enzymatic Hydrolysis

Enzyme

Lignin (Solid Residue)

Pentoses (hemicellulose)
Bio-Refineries/Enzyme Production

Sucrose Juice or Molasses

Ethanol Fermentation

Ethanol

Yeast

Distillation

Stillage
Biomass Ethanol - Process Overview

Pre-treatment
- Steam explosion
- Milling

Sugarcane biomass → Harvest → Pre-treatment → Enzymatic hydrolysis → Fermentation → Destilation → Ethanol

Enzyme Production

Trichoderma reesei RUT C30 and Aspergillus awamori
Bioetanol Project - Main Research Areas

- Development of biomass pre-treatment processes for sugar cane bagasse and straw
- Raw and pre-treated biomass characterization
- Cellulases / xylanases production
- Enzymatic hydrolysis
- Sugars syrups characterization
- Ethanol fermentation (C$_6$)
- C$_5$ sugars and lignin uses
- Energy optimisation
- Effluents and water
Bioetanol Project

Enzymes Production - *Principles*

- Enzyme cost contribution and effectiveness depends on the biomass source and pre-treatment conditions
- Development of “tailored made” ENZYME BLENDS for sugarcane biomass
- Use of crude “cellulase/xylanase /accessory enzymes” preparations
- “In house” production to reduce cost
Bioethanol Project
Enzymes Production - Topics

- Screening of fungi and streptomyces strains
- Fungi genetic improvement
- Genes cloning and expression (*Pichia pastoris*)
- Enzymes chemical and biochemical characterization
- Enzymes production using solid state and submerged fermentation
- Sophorose production
- Enzymes production scale up
- No increase in land use
- No competition with food production
- Bioetanol from sugarcane biomass residue (bagasse)
- Using non-acidic, like milling, steam explosion and new acidic pretreatments
- Enzymatic hydrolysis
- Blends of the biomass syrups with sugarcane sucrose juice or molasses
- Traditional ethanol fermentation
Brazil has 365 sugar/ethanol producing units from which 240 produce both sugar and ethanol, 109 produce only ethanol and 15 produce only sugar.

It is forecast that 41 new distilleries will be built before 2010.

Ethanol production around 27 billion L.

Ethanol and sugar industry produces 84 million tonnes of dry bagasse.

Sugarcane straw (leaves) will be also available.
- 88% bagasse used for co-generation (heat and electricity)
- New boilers will decrease bagasse use and increase surplus
- The use of straw for biomass ethanol or as fuel in the boilers will increase biomass availability
- 12% present surplus bagasse (10 million tons)
- Production of additional 2.4 billion L of ethanol
Bagasse production and availability/year (kg)

<table>
<thead>
<tr>
<th>Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sugarcane production</td>
<td>600.000.000.000</td>
</tr>
<tr>
<td>Total bagasse production (28% of sugarcane - 50% moisture)</td>
<td>168.100.000.000</td>
</tr>
<tr>
<td>Total dry bagasse production</td>
<td>84.000.000.000</td>
</tr>
<tr>
<td>Surplus bagasse (12% dry bagasse)</td>
<td>10.000.000.000</td>
</tr>
</tbody>
</table>

Theoretical ethanol yield from sugarcane biomass cellulose

| 1 Kg of bagasse:              | 0.24 L          |
| 10.000.000.000 Kg of bagasse: | 2.400.000.000 L |
RESULTS

Sugarcane bagasse and wood enzymatic hydrolysis using the ENZITEC enzyme blend
BIOMASS

Sugarcane Bagasse

Steam treated Sugarcane Bagasse
BIOMASS RAW MATERIALS: SUGARCANE BAGASSE AND MILLED Eucalyptus

Substrates: Sugarcane Bagasse and Milled Eucalyptus respectively
Milling pretreatment: Brazil – Japan Collaboration on Biomass Ethanol

MILLING DOES NOT

- USE WATER, HIGH TEMPERATURE OR PRESSURE
- ALTER THE BIOMASS COMPONENTS CHEMICALLY (ADVANTAGE FOR BIOREFINERY)
- GENERATE INHIBITORS FOR THE HYDROLYSIS AND FERMENTATION STEPS
- GENERATE POLLUTANT WATER STREAMS AND SALT
- MAY BE EASIER TO SCALE UP IN COMPARISON TO STEAM EXPLOSION
- IT IS SAFER TO OPERATE

- ENERGY CONSUMPTION AND MAINTENANCE ARE THE SENSITIVE ASPECTS
Sugarcane biomass hydrolysis using the ENZITEC enzyme blend
HYDROLYSIS EXPERIMENTS (STBA – 53% cellulose)

• 130 g/L of treated sugarcane bagasse

• ENZITEC blend – 10 FPU/g

• Sodium citrate buffer pH 4.8

• Temperature - 50ºC

• Agitation - 200 rpm
BIOMASS HYDROLYSIS RESULTS

Biomass load – 25 g/L
MILLED EUCALYPTUS – HYDROLYSIS RESULTS

- Eucalyptus particle size: 25 to 100 micrometer
- Eucalyptus cellulose content: 42%
- Hydrolysis: ENZYTEC enzyme blend (15 FPU/g), 50°C, 200 rpm
- Hydrolysis yields (48h): 25 g/L – 85%, 50 g/L – 84%, 75 g/L - 83%, 100 g/L – 61%.
STBA HYDROLYSIS RESULTS

- STBA + ENZITEC Enzyme
- Glucose Syrup
  - 60 g/L (87% Yield)
- Ethanol fermentation
- Lignin
  - Solid hydrophobic fuel
Biomass Syrups Ethanol fermentation – industrial
Saccharomyces cerevisiae strain

Ethanol quantification
RECENT PROJECTS ON BIOMASS ETHANOL – IV

PROJECT
SECOND GENERATION ETHANOL PRODUCTION
FROM SUGARCANE BIOMASS

FINANTIAL SUPPORT - MCT / FINEP

Elba P.S. Bon - IQ/UFRJ

Industry partner: Dedini S/A Indústrias de Base
Membrane Bioreactor

- Biomass hydrolysates lignin separation
- Sugars syrups concentration
- Enzyme recovery

Prof. Suely Freitas – Chemical Engineering School – UFRJ

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Membrane Bioreactor
THANK YOU!